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# Sex-Specific Distributions of Leopard Sharks (*Triakis semifasciata*) In an Estuarine Environment

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SEX-SPECIFIC DISTRIBUTIONS OF LEOPARD SHARKS  
(*TRIAKIS SEMIFASCIATA*) IN AN ESTUARINE ENVIRONMENT

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Andrea L. Launer

December 2014

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The Designated Thesis Committee Approves the Thesis Titled

SEX-SPECIFIC DISTRIBUTIONS OF LEOPARD SHARKS  
(*TRIAKIS SEMIFASCIATA*) IN AN ESTUARINE ENVIRONMENT

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## ABSTRACT

### SEX-SPECIFIC DISTRIBUTIONS OF LEOPARD SHARKS (*TRIAKIS SEMIFASCIATA*) IN AN ESTUARINE ENVIRONMENT

By Andrea L. Launer

Estuaries are used seasonally by many elasmobranchs for reproduction and as nurseries. The character, location, and availability of elasmobranch habitats in estuaries are often altered by anthropogenic activities and environmental changes. These habitat changes may have different effects on groups within a shark population because sex-specific segregations are common for many elasmobranchs. Leopard Sharks (*Triakis semifasciata*) are important estuarine predators in California and exhibit sexual segregations. Movements of 13 Leopard Sharks (male n=6, female n=7) tagged with coded acoustic transmitters were monitored from March 14, 2013 to March 9, 2014 using moored receivers in Elkhorn Slough, CA. Movement patterns were compared by region within Elkhorn Slough and related to environmental variables. Tagged individuals of both sexes spent the majority of time in the middle section of Elkhorn Slough, though females were more frequently present early in the day and uncommonly recorded after noon; males consistently used the middle section of Elkhorn Slough throughout the day. The monthly number of hours male Leopard Sharks were detected in the study site was positively correlated with water temperature and female Leopard Shark detected hours were negatively correlated with dissolved oxygen concentration. Differences in frequency of detection at temperatures ranging from 13°-16° C were dependent on sex and region of Elkhorn Slough.

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Surveying three habitat types nearly every month for multiple days would have been impossible on my own. In total, volunteers helped me conduct 60 days of sampling, 133 hours of fishing time, and catch 122 elasmobranchs. Divers and boat drivers were also essential to this project to be able to monitor shark movement for 361 days. A few individuals stood out above the rest: Molly Fredle, Melinda Wheelock, Melissa Nehmens, Elizabeth Ramsey, Ashley Wheeler, Catherine Drake, and Anne Tagini. Your time and

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## **Introduction**

Estuaries are ecologically important interfaces of marine, freshwater, and terrestrial systems (Beck et al. 2001). Productivity of estuaries is among the greatest of any ecosystem in the world; they support great secondary production (Allen et al. 2006). Great productivity in estuaries is a result of the interaction among tidal flows, nutrients, and a diversity of primary producers (Schelske and Odum 1961). The high diversity of primary producers in estuaries allows photosynthesis to remain great year round, as seasonality of production by individual species is masked by their combined production (Schelske and Odum 1961). Estuarine habitat characteristics also vary through time as freshwater inputs, marine connections, and tidal flows fluctuate on daily, monthly, and seasonal timescales (Gillanders et al. 2011). Seasonal differences in environmental conditions, such as salinity and temperature, greatly influence the fish assemblage of estuaries (Yoklavich et al. 1991; Allen et al. 2006).

Estuaries contain a diverse assemblage of species due to habitat heterogeneity (e.g., deep channels, tidal flats, tidal creeks, seagrass beds). Additionally, abiotic factors such as temperature and salinity vary along gradients within estuaries based on proximity to the ocean and sources of freshwater inputs (Vinagre and Costa 2014). These factors influence food web dynamics along this continuum as interactions among species are affected by the physical tolerances of prey species compared with their predators (Vinagre and Costa 2014). Great habitat heterogeneity and productivity make estuaries important for resident, migrant, and seasonal species (Beck et al. 2001).

Estuarine species can be greatly affected by anthropogenic factors such as agricultural runoff, dredging, and alterations to tidal prisms. The majority of threats to the health and habitat quality of estuaries stem from environmental responses to habitat degradation and nutrient inputs (Kennish 2002). Dense human populations have led to increased pressures at the interface between upland habitats and estuaries. Expansion of populations, industry, and use of estuaries as harbors has greatly contributed to changes in estuaries (Cronin 1967). For example, dredging in an Australian estuary re-suspended sediments that contained heavy metals, pesticides, and other contaminants, resulting in decreased recruitment of infaunal species (Knott et al. 2009). Alterations to estuaries from human activities can change the distribution of nutrients and organisms within the system due to hydrology or water quality (Cronin 1967; Broenkow and Breaker 2005). Decreases in water quality may lead to changes in the locations and amounts of critical habitat (areas determined essential to one or more life stages of a species) for species that reside in estuaries. In addition, physical alterations to habitats and changes in environmental factors are predicted to accelerate as global climate change alters habitat distribution and availability in estuarine environments (Scavia et al. 2002; Gillanders et al. 2011), making estuaries even more susceptible to natural disturbances (Kennish 2002). Natural disturbances can intensify natural processes that have been altered by anthropogenic factors.

Eutrophication of estuaries stands to be a greater prospective threat than other human caused alterations (Kennish 2002). Nitrate added to the environment will likely be exacerbated by climate change, leading to eutrophication in estuaries (Scavia et al.



2002), often leading to the creation of hypoxic or anoxic conditions. Lab experiments with juvenile estuarine fishes, Pinfish (*Lagodon rhomboids*) and Atlantic Croaker (*Micropogonias undulates*), revealed hypoxic conditions caused both species to actively seek other habitats (Froeschke and Stunz 2012). When oxygen concentration levels were greater, both species selected other habitats based on a variety of other factors, such as prey availability or substrate type. Environmental conditions can cause species to change habitat preferences in estuaries.

Estuaries provide habitats for teleost and elasmobranch species (Blaber and Blaber 1980; Beck et al. 2001; Allen et al. 2006). Several shark species use estuaries seasonally, and site fidelity to estuaries has been documented for many species, some over multiple years. In Australia, Spottail Sharks (*Carcharhinus sorrah*) exhibit long-term site fidelity, which is likely the result of localized experience with habitat and prey resources (Knip et al. 2012b). Presence of Carcharhinid and Sphyrnid shark species varies seasonally in Australian estuaries, likely based on prey abundance and timing of biological processes (Simpfendorfer and Milward 1993). Bonnethead Sharks (*Sphyrna tiburo*) were abundant in Florida estuaries but were not permanent residents (Heupel et al. 2006b). Whereas these species range across a large spectrum of habitats, use of estuaries during some portion of their life cycle is typical and there may be variability in use by different sexes.

Sex-specific segregation is common for many shark species and this behavior has been attributed to many factors (Springer 1967; Sims 2005; Wearmouth and Sims 2008). The sex ratio of Gray Smoothhounds (*Mustelus californicus*) differed between seasons in

southern California: males were most abundant in spring and females were most abundant in summer (Espinoza et al. 2011). Photoperiod, a proxy for season, and sea surface temperature were significant in predicting the abundance of Leopard Sharks (*Triakis semifasciata*) in southern California (Nosal et al. 2014). In addition, spatial segregation of the sexes has been shown to correlate with water temperature. Adult females often occupied shallow (Wetherbee et al. 1997; Knip et al. 2012a) or warmer water likely to increase metabolic rates and reproductive processes (Hight and Lowe 2007). Conversely, males were often distributed in deeper, cooler areas where conditions are less stressful (Ebert and Ebert 2005; Hight and Lowe 2007). The distribution and abundance of the sexes of a species may relate to many different factors.

Several strategies have been proposed as to why sharks segregate by sex. Females may choose habitat where they can remain inactive to compensate for increased metabolic costs due to elevated water temperature (Hight and Lowe 2007; Wearmouth and Sims 2008). Segregation also can relate to a social aspect of the population as females avoid males to limit mating attempts because aggressive male courtship behavior can cause females to expend large amounts of energy which would otherwise be devoted to egg production (Wearmouth and Sims 2008). Another strategy for sex-specific spatial segregation involves reducing risk of conspecific predation on offspring. Males of the species are distributed based on prey abundance and females segregate to reduce cannibalism of pups by males, thereby increasing survivorship of offspring (Wearmouth and Sims 2008). Factors influencing sexual segregation in estuaries may vary based on biological functions.

Shark species commonly use estuaries as nursery habitats. Density and site fidelity of neonate and juvenile sharks are greater in nursery habitats than other available habitats (Heupel et al. 2007), which results in increased survival of neonate and juvenile sharks (Simpfendorfer and Milward 1993; Heupel et al. 2007). Bull Sharks (*Carcharhinus leucas*) partition habitats within estuaries by size, and as individuals grow, they begin to inhabit more open systems such as bays (Simpfendorfer et al. 2005). There also is habitat overlap between young of the year and juvenile Bull Sharks (Froeschke et al. 2010). Diets of Sevengill Sharks (*Notorynchus cepedianus*) differed by size class, which would minimize intraspecific competition for resources (Ebert 2002). Pigeye Sharks (*Carcharhinus amboinensis*) in Australia shift depth and habitats used based on ontogeny (Knip et al. 2011). Changes in use of habitat by size class likely stems from differences in caloric requirements and influence of environmental factors, such as water depth and tidal cycle (Knip et al. 2011). Species may utilize different habitats in estuaries during stages of their ontogeny, which may increase their adaptability to changes in the habitat and environment. This is known as the portfolio effect (Yates et al. 2012). Shark species may also have localized areas of great population density in specific habitats within estuaries, making them particularly susceptible to depletion (Heupel et al. 2006b; Nosal et al. 2014). Estuaries also may be shared by several species as primary and secondary nurseries, reducing inter- and intraspecific predation on pups and juveniles (Simpfendorfer and Milward 1993). Survival is increased because nursery habitats may possess a combination of abundant food resources, warmer water, and

reduced predation (Heupel et al. 2007). Reasons for preference of particular nursery habitat by a species is diverse.

Selection of nursery habitats by shark species may be based on environmental factors and the energetic costs to endure stressful conditions, such as changes in water temperature or salinity (Simpfendorfer et al. 2005; Heupel and Simpfendorfer 2008), even when conditions are not ideal (Heupel and Hueter 2002). Blacktip Shark (*Carcharhinus limbatus*) abundance in a nursery area was not correlated with prey abundance or salinity, but likely a response of habitat preference based on temperature or avoidance of larger predators (Heupel and Hueter 2002). Juvenile Bull Sharks remained in the estuarine habitat longer than conspecific adults, potentially to reduce predation risk (Simpfendorfer et al. 2005), while taking advantage of increased prey in estuaries (Heupel and Simpfendorfer 2008). Size specific segregations may also relate to differences in habitat and prey preferences at different life stages (Springer 1967).

Elkhorn Slough is an estuary used by several elasmobranch species located along the central coast of California, USA. It is the largest wetland south of San Francisco Bay (Yoklavich et al. 1992; Carlisle et al. 2007) and directly connects to Monterey Bay. The hydrography of Elkhorn Slough changed dramatically in the last 200 years, primarily as a result of human activities (Lindquist 1998; Broenkow and Breaker 2005). In 1946, jetties were constructed at the mouth of Elkhorn Slough, thereby changing it from a shallow, depositional embayment to an erosional system of marine and tidally-influenced habitats. This switch resulted in an increase in the tidal prism, which created narrow, deep channels (Lindquist 1998), thus increasing the rate of change in erosion (Reyes 2009).

Between 1988 and 1993, Malzone (1999) observed a 55% increase in water volume and greater loss of sediment in Elkhorn Slough.

As with other estuaries, habitats within Elkhorn Slough are susceptible to alteration. Changes from environmental factors that will likely alter habitat are a result of modification of the hydrology in estuaries (Gillanders et al. 2011). Tidal flat habitats are particularly susceptible to erosion as they require replenishment of fine grain sediments and cannot maintain themselves in the presence of stronger currents and wave action (Largier et al. 2010). Great abundance and diversity of invertebrate fauna occur in tidal flat habitats (Browning et al. 1972). Tidal creeks are highly productive areas in Elkhorn Slough. Between 1980 and 2003, tidal creeks throughout much of Elkhorn Slough greatly increased in their widths (van Dyke and Wasson 2005). Lindquist (1998) found that more eroded tidal creeks contained a species assemblage and diversity that resembled the main channel, in contrast to the diversity of less eroded tidal creeks. Habitat alteration and loss as a result of erosion could alter the abundance of resident fauna (Lindquist 1998; Carlisle et al. 2007), distribution of higher trophic levels (Lindquist 1998), and disrupt the function of nursery habitat (Yoklavich et al. 1991; Lindquist 1998).

Leopard Sharks reside in Elkhorn Slough from as early as March to as late as December (Ackerman 1971; Carlisle 2006) and have exhibited sexual segregation in other areas. Female Leopard Sharks in Elkhorn Slough used habitat within Elkhorn Slough National Estuarine Research Reserve (ESNERR) throughout the year and extensively during the fall (Carlisle and Starr 2009). In La Jolla, CA, large aggregations

(up to 125 individuals) of female Leopard Sharks occurred adjacent to the head of a submarine canyon (Nosal et al. 2013). A large aggregation in La Jolla was the first documented mating of Leopard Sharks the wild (Smith 2005). Hight and Lowe (2007) documented female Leopard Sharks aggregating in warmer areas near the Catalina Islands.

Female Leopard Sharks reach sexual maturity at ages between 10 and 15 years and males mature between 7 and 13 years of age (Kusher et al. 1992). Females produce yolk-sac viviparous pups annually in the early spring. Cailliet (1992) reported that female Leopard Sharks do not likely reproduce until 17 years of age and fecundity increases with size in this species (Ebert and Ebert 2005). In Humboldt Bay, habitats utilized by neonate and adult Leopard Sharks overlap. This pattern may occur because adults often switch prey after pupping, which may maximize food for neonates (Ebert and Ebert 2005) or be a response to postpartum changes in dietary requirements. There are significant overlaps in diets among sizes classes (Kao 2000), though diets of Leopard Sharks have changed over time (Talent 1976) in Elkhorn Slough.

Despite extensive studies of Leopard Sharks in Elkhorn Slough, no data exist on sex-specific habitat use in this estuary. Sexual segregation of Leopard Sharks was documented in several studies in southern California (e.g., Hight and Lowe 2007; Nosal et al. 2013; Nosal et al. 2014); however, patterns of behavior in salinity mixed estuaries that occur in northern California may be different. This poses an important gap in our understanding of Leopard Sharks as portions of the population may use different habitats for critical biological functions (e.g., breeding, pupping, foraging; Knip et al. 2012a) and

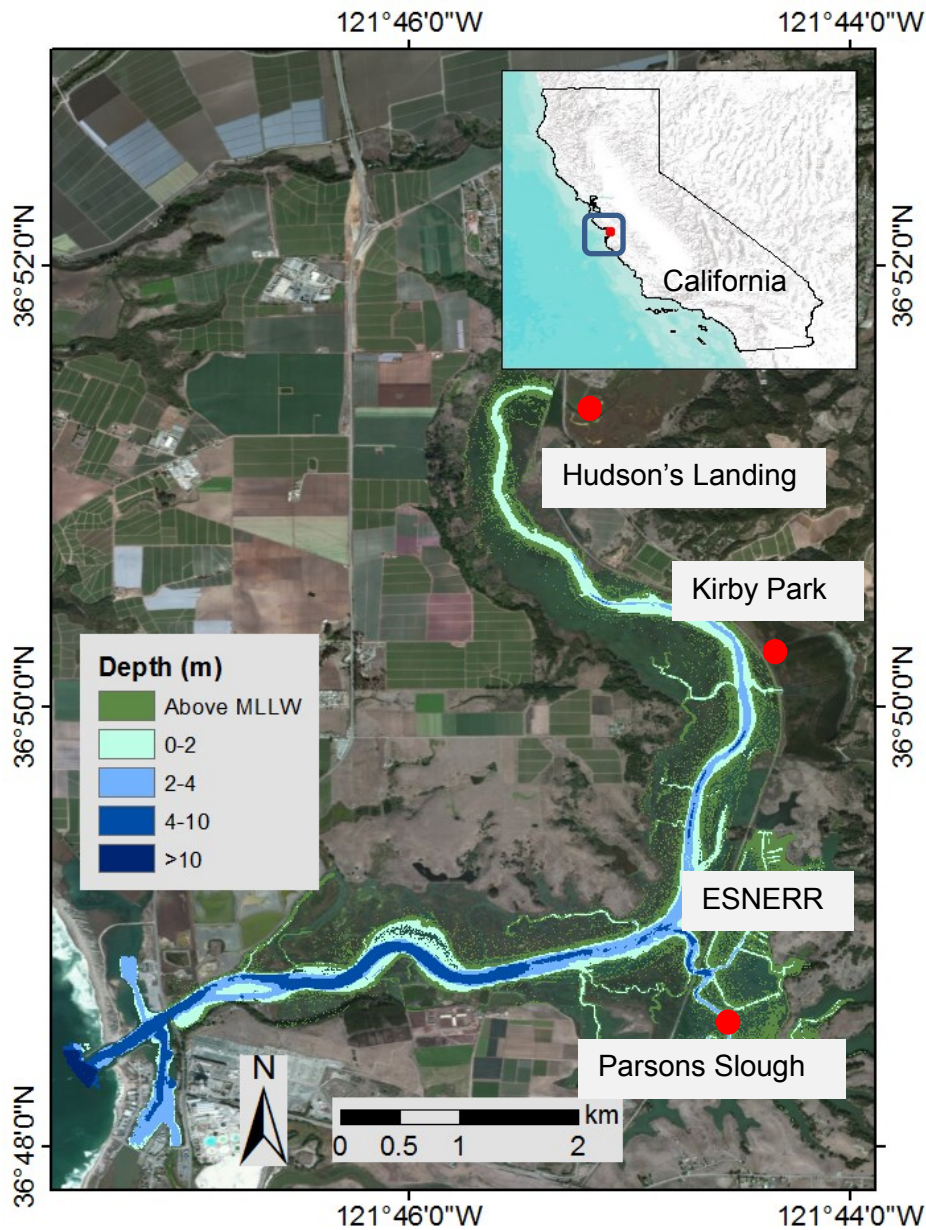
shark species to have low fecundity and delayed sexual maturity (Smith et al. 1998; Walker 1998). The specific objectives of this study were to: (1) determine residence time of adult male and female Leopard Sharks in different estuarine habitats; and (2) determine sex-distribution of Leopard Sharks in different estuarine habitats. Based on previous findings, I hypothesized that adult females would be more abundant in tidal creeks and tidal flats where water temperature would be warmer than channel habitats. Additionally, I hypothesized that adult male Leopard Sharks spent time in channel habitats that were still highly productive but not as physically stressful and energetically costly to endure.

## **Methods**

### **Study Site**

Approximately 9 m deep at the mouth, Elkhorn Slough becomes shallower along its 7 km stretch (CSUMB Seafloor Mapping Lab; Fig. 1). Within Elkhorn Slough, several habitats are available (e.g., main channel, tidal creek, and tidal flat) but differ from one another in physical characteristics (e.g., temperature, turbidity, tidal influence) and species composition (Barry et al. 1996). Depth at low tides is a factor that easily distinguishes habitats from one another. Main channel habitats are generally deeper than 4 m at low tide and as deep as 9 m at the mouth of Elkhorn Slough. The main channel covers approximately 142 ha, which is spread out along the length of Elkhorn Slough (Lindquist 1998). This is the main thoroughfare in Elkhorn Slough as animals would need to use this habitat to reach adjacent tidal creek or tidal flat habitats. Channel habitats are tidally influenced for the most part and are flushed more frequently than tidally restricted habitats.





**Fig. 1** Map of the study site, Elkhorn Slough, CA. The lower portion of Elkhorn Slough varies between depths of 4 to 9 m. However, the water depth throughout the majority of Elkhorn Slough is shallower than 4 m at lower low tide. The blue rectangle on the extent map indicates Monterey Bay, with the red dot indicating the location of Elkhorn Slough. GIS map layers (and sources): USA shapefile (Esri: Redlands, CA), California shapefile (Esri), Imagery basemap (Esri), and bathymetry (CSUMB Seafloor Mapping Lab).

Tidal flat and tidal creek habitats are similar to one another as they both consist of mud/clay to medium or fine sands (Browning et al. 1972); however, they differ in depth at high tide and exposure at low tide. Tidal flat habitat depths range from 0 to 4 m and tidal creeks rarely exceed 2 m deep. Tidal creek and tidal flat habitats have relatively equal area (1,000 ha) and similar distributions (Lindquist 1998). Tidal creeks are found in the middle of Elkhorn Slough. These areas have little connectivity to the channel at lower tides, though they are not completely closed off. As the tide floods, the water rises above the shallow mouths, making the habitats available to sharks until the tide falls again. Tidal flat habitat occurs in the southeast corner within Elkhorn Slough National Estuarine Research Reserve (ESNERR), along the channel in the middle section of Elkhorn Slough, and above Kirby Park. Tidal flats are characterized by gradual sides, often with depths less than a few meters at high tide.

The Lower region of Elkhorn Slough is nearest to Monterey Bay and has the shortest residence time (the amount of time water spends in the system) of all of the regions. The region is comprised primarily of channel habitat with sloping sides, some eelgrass habitats and depths of 5 to 9 m at lower low tide in most areas. The current is particularly strong in this region after flood and ebb tides.

The Fork region is comprised of a few different habitats. Whereas the majority of habitat covered by this region is similar to channel habitats in the Lower region, there are narrow corridors that lead to shallow tidal flat habitats in ESNERR. Although there are some pools that are always available (5-8 m depth), the majority of habitats in the Fork region are only available to sharks at high tides. The water in these shallow areas can be

much warmer than those in the Lower region. ESNERR is an area within Elkhorn Slough that is a breeding and nursery ground for Leopard Sharks (Ackerman 1971; Barry 1983; Talent 1985; Yoklavich et al. 1991). In 2011, a sill was installed adjacent to Parsons Slough (Fig. 1) that reduces the amount of tidal exchange with ESNERR but causes strong currents after flood and ebb tides at the entrance.

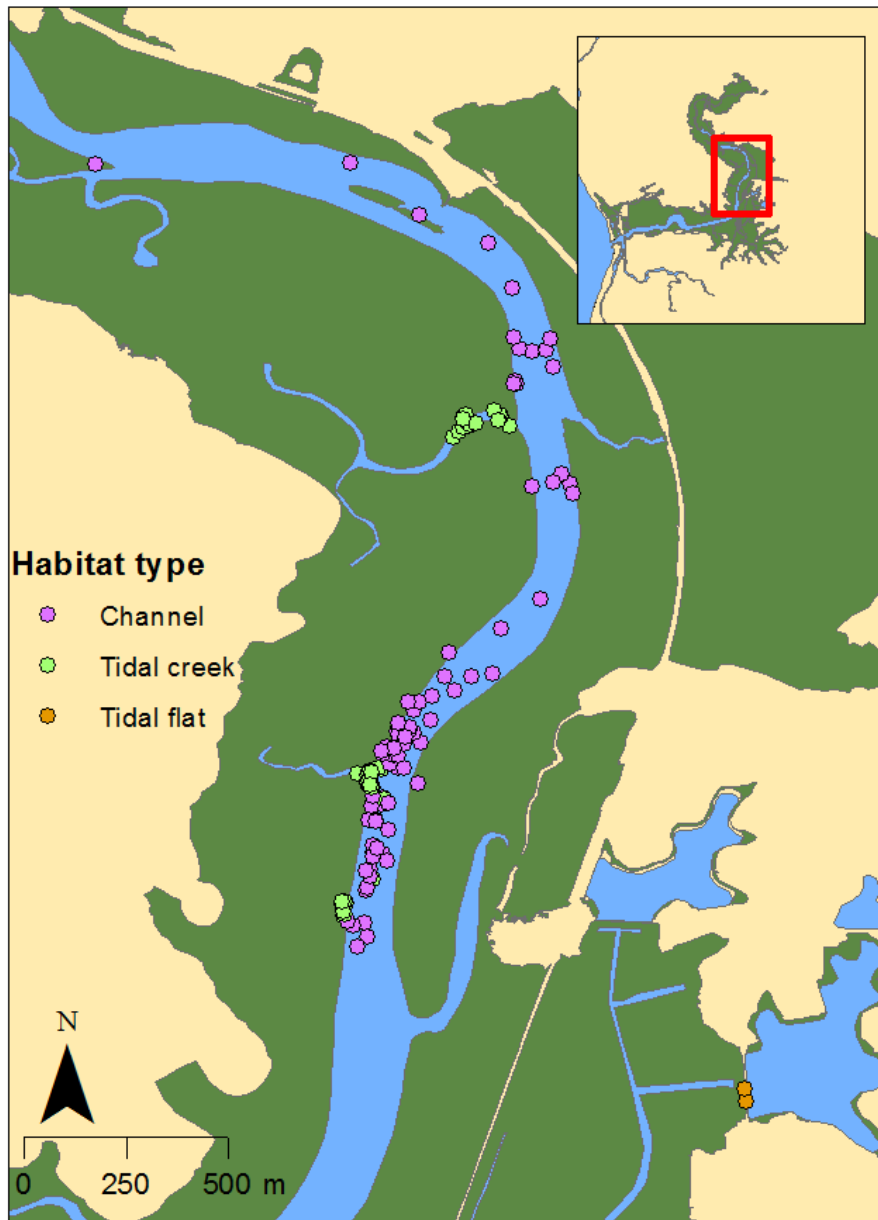
The channels in the Mid region of Elkhorn Slough are between 2 and 5 m deep. This region contains channel, tidal flat, and tidal creek habitats. There are several shallow expanses of tidal flats less than 2 m depth at high tide in this region. Tidal creeks wind back into wetlands with shallow sections connecting adjacent tidal creeks in some cases.

The Upper region of Elkhorn Slough lies just above Kirby Park leading to Hudson's Landing and the end of Elkhorn Slough. Depths in the channel range between 2 and 4 m and adjacent sloping areas are shallower than 2 m. It is difficult to reach some of these areas via boat even at higher tides due to the narrow and shallow channel.

#### Habitat-specific estimates of relative abundance

In order to estimate relative abundance of Leopard Sharks in the different habitat types within Elkhorn Slough, elasmobranchs were collected using tanded gillnets at several sites within Elkhorn Slough (Fig. 2). The sites were sampled during flooding or ebbing high tide, when Leopard Sharks move in and out of tidal habitats (Carlisle and Starr 2010). Sites within each habitat type were haphazardly chosen and were influenced

by occurrence of recreational activities (e.g., kayaks, hunters, canoes), winds, and distribution of marine mammals. For tidal flat habitats, gillnets were set in deeper areas within ESNERR that sharks would have to pass through to reach shallow tidal flats. Gillnets were set consistently in one location over the course of the study to sample tidal flat habitat therefore sampling locations, but not sampling efforts, were accurately represented in Fig. 2. Similarly, tidal creek sites were sampled by placing the gillnet across the mouth of the tidal creek and subsequent sets on different sampling days were located within meters of each other. Sites above Kirby Park were rarely sampled as catch rates in those areas were not as high as those in the Fork or Mid region, though another MLML graduate student caught several elasmobranchs in that area (K. van Hees, Moss Landing Marine Laboratories, pers. comm). Sampling occurred monthly from March 2013 to March 2014 over a period of 3-6 days per month, with the exception of September and January when no nets were set in any of the habitat types.



**Fig. 2** Locations of gillnet sets in Elkhorn Slough. Nets were set in three habitat types: channel (n=93), tidal creek (n=93), and tidal flat (n=100). Locations of gillnets sampling tidal creek and tidal flat habitats were within meters of one another in their respective habitats, therefore sample locations, but not sample sizes were not accurately represented by symbols on the map. GIS map layers (and sources): California shapefile (Esri), channel shapefile (CSUMB Seafloor Mapping Lab), and wetland shapefile (CSUMB Seafloor Mapping Lab).

The gillnet used to catch sharks measured 20 m long by 2 m high with a single 12.7 cm diamond stretch mesh panel. Each gillnet set was allowed to soak for a minimum 20 min period before retrieval. Duration of the set was recorded to determine fishing effort and was dependent on the number of animals caught, the time required to collect data (e.g., time to identify species, sex, and total length), and release elasmobranchs. Sex was assessed externally by the presence or absence of claspers. Total length of sharks was measured to the nearest cm. Captured elasmobranchs were removed from the net and put in a large bin with fresh seawater while data were collected. Elasmobranchs were subsequently released at the site they were captured. During release, Leopard Sharks were held behind their pectoral fins and moved slowly through the water to aid in seawater passing over their gills. When sharks became responsive and showed to be under their own power, they were released. Most releases lasted fewer than one or two minutes. Species caught included Leopard Shark (n=96), Bat Ray (*Myliobatis californica*: n=15), Thornback Ray (*Platyrrhinoidis triseriata*: n=9), Gray Smoothhound (n=1), and Round Stingray (*Urobatis halleri*: n=1).

### Shark Tagging

In order to record movements in Elkhorn Slough, Leopard Sharks captured in good condition and of appropriate size were surgically implanted with VEMCO Limited acoustic tags (Halifax, NS, Canada: models V9 and V13). VEMCO (2012) recommended that an implanted tag was less than 2% of the total body mass of the tagged

fish. Model V9 tags weighed 6.2 g in air and V13 tags weighed 12 g in air; therefore, the minimum mass of a Leopard Shark suitable for an acoustic tag was 0.1 kg based on the mass of the larger acoustic tag (Table 1; VEMCO 2013a; VEMCO 2013b). Generally, Leopard Sharks smaller than 0.1 kg would not have been caught in gillnets used in this study because they were small enough to swim through the mesh without entanglement. The approximate battery lives of the tags used in this study were 425 d and 1,195 d, but tag life is reduced for tags that sit on the shelf in the lab for an extended period of time (VEMCO 2013a). The tags used in this study were at minimum two years old. Despite this, tags were expected to transmit for a minimum of 6 months, which proved to be sufficient time for this study.

**Table 1** Specifications of acoustic tags used in this study (VEMCO 2013a; VEMCO 2013b).

Model	Quantity	Transmit Interval (s)	Estimated life (d)	Diameter (mm)	Length (mm)	Mass in air (g)
V9P-2L	10	60-180	425	9	46	6.2
V13P-1L	3	60-180	425	13	45	12
V13P-1L	8	90-220	1195	13	45	12

Surgical implantation of acoustic tags occurred on shore at the site of capture or aboard a Boston whaler. Leopard Sharks were inverted and placed in a wooden V-board with seawater pumping over the shark's gills. This technique induced tonic immobility, causing immediate relaxation that minimized thrashing and reduced stress to the animal as a result of handling (Gruber and Zlotkin 1982; Watsky and Gruber 1990; Henningsen

1994). This method has been commonly used for acoustic tag implantation in sharks (e.g., Holland et al. 1999; Lowe et al. 2006; Brooks et al. 2011; Espinoza et al. 2011).

To prevent the introduction of septic germs into the shark's body, each tag was dipped in an antiseptic (povidone-iodine, Betadine, Purdue Products: Stamford, CT), a practice used in surgeries (Lister 1967). Antiseptics sterilize non-living objects, as opposed to antibiotics that kill bacteria in a living body. Antibiotics were not recommended for use as their use may lead to creating antibiotic resistant bacteria in animals and their habitats (Mulcahy 2010).

Each tag was inserted into the selected shark's peritoneal cavity via a small incision (approximately 2 cm long) made with a scalpel soaked in isopropyl alcohol. Incisions were closed using absorbable sutures (Ethicon PDSII monofilament absorbable sutures, Ethicon, Inc.: Somerville, NJ). After the surgery was completed, the shark was righted and an external dart tag was inserted into its dorsal fin musculature for external identification. Upon revival, the shark was released at the same site it was captured. Tags were implanted in a total of 21 sharks (male n=11, female n=10) following practices that were in accordance with San José State University IACUC Protocol 983.



## Acoustic Monitoring

Signals from tagged sharks were monitored using nine moored acoustic receivers deployed throughout Elkhorn Slough from March 14, 2013 to March 9, 2014. VEMCO Receivers (Models VR2W and VR3) recorded signals from tagged sharks in four regions of Elkhorn Slough: Lower, Fork, Mid, and Upper (Fig. 3). Grouping receivers allowed for better spatial resolution of detection of tagged animals (Heupel et al. 2006a). Using the assistance of SCUBA divers, moorings were visited every 4-6 months to retrieve a receiver and replace it with another receiver to maintain continuous coverage of the study site. Additionally, two of the receivers (station 1&2) contained an underwater acoustic modem (Model VR3) that allowed data downloads to occur from a boat using a surface modem, which permitted data to be downloaded more frequently. One of these receivers at station 1 lapsed in coverage from 4-18 November 2013 due to a failure of the mooring, though station 2, the closest in proximity, was fully active during this time. When VR3 receivers at station 1 and 2 were removed in March, they were replaced with VR2 receivers which were monitored until 9 June 2014 to extend the observation of sharks returning to the estuary for a second season.



**Fig. 3** Map of the study site split into regions using 9 moored acoustic receivers. Regions of Elkhorn Slough for the purposes of this study were signified by receiver color (Lower: green, Fork: orange, Mid: red, Upper: purple) and labels signify receiver numbers. Yellow markers signified water quality stations, MBARI LOBO L01 and ESNERR South Marsh, that recorded the environmental variables used in analyses. GIS map layers (and sources): California shapefile (Esri), wetland shapefile (CSUMB Seafloor Mapping Lab), and bathymetry (CSUMB Seafloor Mapping Lab).

The detection ranges of receivers were tested in three regions of Elkhorn Slough. A receiver was attached to an anchored line with a surface buoy and a GPS location was recorded. The boat was then moved 20 m distance away from the buoy's coordinates, measured using a handheld GPS. A coded acoustic tag was secured in a mesh goodie bag and tied off to a cleat on the bow of the boat. The bag was lowered into the water to a depth of 1 m, and held there for approximately 15 minutes while the boat driver maintained a 20 m distance from the receiver. This method was repeated at distances of 40 and 60 m with 3 to 4 replicates per distance. Performing range testing at distances greater than 60 m would have proved difficult from a Boston whaler at some locations due to shallow depths across the width of Elkhorn Slough.

Receiver numbers 1 and 2 were located in the Lower region of Elkhorn Slough. Receivers 3, 4, and 4.5 were located in the mid portion of Elkhorn Slough, an area termed the Fork region in this study. Station 3 is a "fork" in Elkhorn Slough, where animals can continue along its length, or follow narrow corridors past stations 4 and 4.5 into ESNERR, which contains the majority of tidal flat habitat of Elkhorn Slough. Receivers 5 and 6 covered the channel of the Mid region of Elkhorn Slough, though the width of the channel was less than that of the Lower region. Finally, receivers 7 and 8 were located in the channel of the Upper region, though the channel was narrow and shallow at this location.

## Environmental Data

In order to determine relationships between Leopard Shark movements and water conditions, environmental data were obtained from existing moorings. Environmental variables used in analyses included temperature, salinity, dissolved oxygen, and nitrate concentrations. Water temperature may influence distribution of Leopard Sharks based on physiological processes and stress caused to the animal. Sharks control the salinity within their body through the process of osmoregulation, requiring additional energy to be devoted to the process under hypo- and hyper-saline conditions. Lowered levels of dissolved oxygen can lead to hypoxia. Additionally, nitrate concentrations in Elkhorn Slough are elevated beyond levels in Monterey Bay based on runoff from nearby agriculture. Algal blooms caused by elevated nitrate levels can rapidly reduce the amount of dissolved oxygen concentration of water.

Data used for environmental analyses were obtained from the Monterey Bay Aquarium Research Institute's (MBARI) Land/Ocean Biogeochemical Observatory (LOBO) located on the L01 mooring in Elkhorn Slough (Fig. 3). A suite of environmental variables were recorded hourly and data are publically available on their website ([www.mbari.org/lobo/loboviz.htm](http://www.mbari.org/lobo/loboviz.htm)). Dissolved oxygen was recorded in millimolar ( $\mu\text{M}$ ) and converted to milligrams per liter (mg/L) by multiplying by 0.032. The L01 mooring was located approximately 100 m from the station 1 receiver.

An additional set of environmental data was downloaded from a water quality station near the South Marsh, located within ESNERR (Fig. 3). This station is monitored

as part of the National Estuarine Research Reserve System's Centralized Data Management Office. These data also are available to the public via a website ([cdmo.baruch.sc.edu/get/landing.cfm](http://cdmo.baruch.sc.edu/get/landing.cfm)). For the purposes of this study, the variables used in analyses included temperature, salinity, and dissolved oxygen concentration. Environmental parameters were recorded every 15 minutes, however, only measurements taken on the hour were used in analyses.

### Data Analyses

Leopard Shark relative abundance (catch per unit effort: CPUE) was calculated by dividing the total number of Leopard Sharks caught each month by the cumulative time of gillnet sets. Transforming the data did not result in equal variance and normal distribution of residuals, so non-parametric statistics were used. Monthly catch rates were grouped by season to increase sample size and reduce variability. To account for differences in sample size by season, fall and winter months were combined. Catch rates, measured in sharks per net hour, were compared by season and habitat type using the Kruskal-Wallis test. Significant results were further investigated using Dunn's test for post hoc analysis. To compare differences in sex by season and habitat type, the total number of male and female sharks was tallied and compared using Chi-squared goodness of fit test.

Receiving ranges in three regions (Lower, Fork, and Mid) were compared by calculating the number of observed detections divided by the number of expected

detections for the monitoring time period. Expected detections were calculated based on elapsed time divided by the mean transmit interval of the tag (e.g., V13P transmitted between 90 s and 220 s, where the mean of these values was 155 s). This created a proportion, though values could range above 1 if tags were transmitting on the lower end of the transmit interval (e.g., a tag reporting every 90 s when the mean transmit interval was 155 s).

Acoustic signals from multiple tags could collide and result in an erroneous recording of one of the coded numbers of the tags used in the study, though the tagged shark was not present at that time. To prevent this, presence in the study area was only confirmed if two detections from an individual shark's tag were recorded in a day to reduce the number of false positives. The number of days present also was influenced by the date the individual was tagged so data were normalized to a percentage of days before further analyses. Residency of sharks in Elkhorn Slough was calculated by dividing the number of days present by the number of days between the shark being tagged and the last day of the study (9 March 2014) inclusive. Values of residency, in percent study days, ranged from 0% to 100%. It also can be thought of as the proportion of days present relative to the possible days the shark could be observed in the study site. Residency of sharks also was compared by sex using a Mann-Whitney U-test.

Frequency and duration of exits from the study site were calculated for each individual shark. Exits were identified when more than a 24-h period elapsed between detections that were not on adjacent calendar days. Length of absence was calculated by

subtracting time between detections, measured in days. These values were compared by sex using a Mann-Whitney U-test.

Time at which tag detections were recorded on receivers was rounded to the nearest hour. These detections were considered to be in an hour bin, with 24 hour bins occurring in a day, to estimate time spent in the study area. For example, all detections occurring from 2130 to 2229 would be assigned to the 2200 hour bin. These detections were then categorized by the region of Elkhorn Slough where the detection was recorded (Lower, Fork, Mid, or Upper) and assigned a value of 1 hr per hour bin. An hour of time was assigned to each region when an individual shark was detected at least once on any of the receivers in that region, regardless of the number of detections recorded for an individual shark during that hour. For the purposes of this study, hereafter “hours” refers to hour bins as explained. The total number of hours a shark was present in the study per month was calculated by individual shark and normalized by the number of days per month to calculate mean daily detected hours. These procedures were repeated using only detections from station 4.5, to represent Leopard Shark use of ESNERR, due to its reputation of being a nursery ground.

To evaluate diel differences in patterns of movement, hour bins were categorized by time of day (Night: 0000-0500, Morning: 0600-1100, Afternoon: 1200-1700, Evening: 1800-2300). Acoustic data were recorded in Coordinated Universal Time (UTC) and hour bins were converted to Pacific Standard Time (PST: UTC -7:00) to coordinate with diel differences (ignoring daylight savings) for this analysis only. The percentage of time sharks were detected in each region was calculated by individual shark and compared

using a cluster analysis. In a Multidimensional Scaling (MDS) plot, data were plotted in a two dimensional space. Samples that plotted near one another are more similar in the observed variables than samples that are plotted farther apart, which are dissimilar. Additionally, the proportion of total time sharks were detected in each region was calculated by sex and normalized by the sample size of each sex to determine diel difference by sex in each of the regions.

Patterns in the number of hours sharks were detected were compared to determine any differences among individual sharks. The number of hours a shark was detected was calculated by region and divided by total number of hours detected in the study site to find the percentage of time spent by region for each individual shark. Data were then compared using a cluster analysis and plotted using MDS.

Monthly mean values of environmental factors (temperature, dissolved oxygen, salinity, and nitrate concentration) from 14 March 2013 to 28 February 2014 were calculated. Salinity and nitrate concentration were not greatly variable throughout the study period thus were not compared with acoustic monitoring data or included in further analyses. Temperature and dissolved oxygen concentration were compared with mean daily hours detected of all sharks and also sharks separated by sex. Correlations were determined between monthly means of daily hours detected with temperature and dissolved oxygen concentration separately.

Temperatures from the L01 mooring were related to shark tag detections based on corresponding hour bins. Water temperature at the L01 mooring at the time of of detection in the Lower region was compared by sex using a Mann-Whitney U-test. This



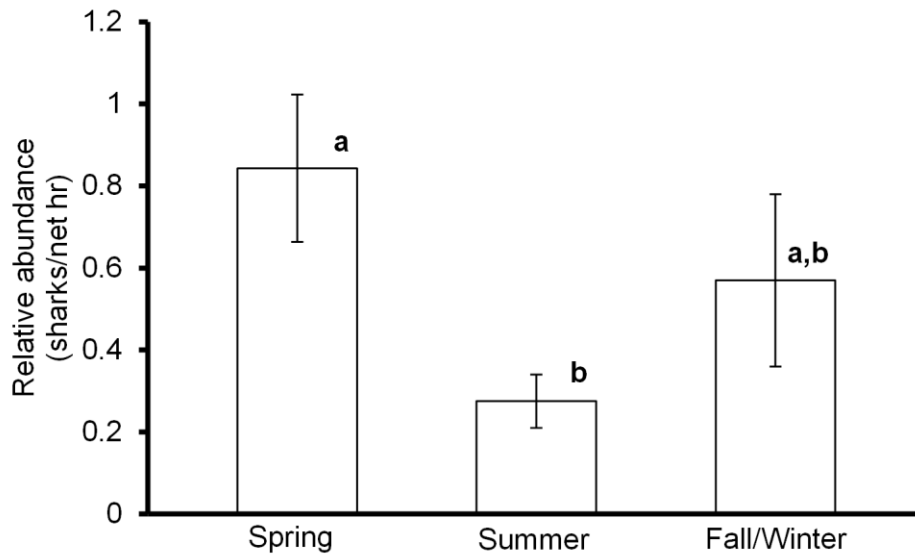
procedure was repeated using water temperature from the South Marsh station and detections from station 4.5. Temperature at time of detection was compared by sex for each individual region (Lower, Fork, Mid, and Upper). Those values were then rounded to the nearest degree and tallied by region to determine sex-specific differences. The total number of shark hours detected for males and females were separated and divided by the total number of hours detected in the study. Proportion of total shark hours was compared by region and sex. The total number of hours Leopard Sharks were present at individual temperatures between 13°C and 16°C was tallied by sex at each of the four regions in the study. These data were tested using Chi-squared test of independence for each region to determine if factors of temperature and sex were independent in each of the regions of Elkhorn Slough. The Marascuillo procedure was used to examine post hoc relationships for significant results.

Previously identified departures and arrivals of individual sharks and corresponding temperatures from the L01 mooring were explored to determine if patterns in environmental factors were drivers for sharks leaving and reentering Elkhorn Slough. Data were analyzed using a Mann-Whitney U-test. Differences in dissolved oxygen between departures and arrivals also were tested with these same procedures. These data were then tested with a Mann-Whitney U-test to determine sex related differences.

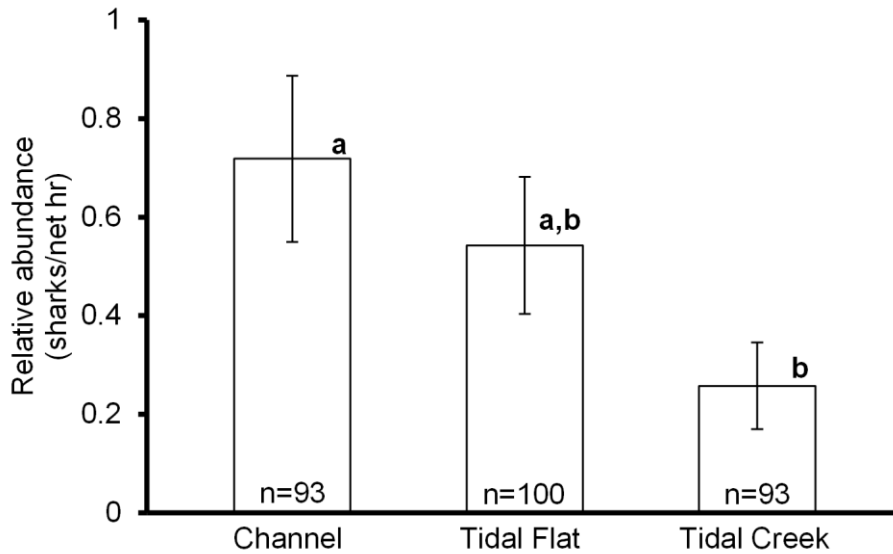
## Results

### Habitat-specific estimates of relative abundance

CPUE of sharks were 0-1.2 sharks/net hr monthly from March 2013 through February 2014. Catch rates were greatest in the spring and fall/winter and lowest in the summer. Mean catch rate in spring was 0.84 sharks/net hr  $\pm$  0.18 (SE), 0.28 sharks/net hr  $\pm$  0.07 (SE) in summer, and 0.57 sharks/net hr  $\pm$  0.21 (SE) in fall/winter (Fig. 4). Leopard Shark abundance was significantly different by season (Kruskal-Wallis,  $\chi^2=8.5$ ,  $n=286$ ,  $df=2$ ,  $p=0.01$ ); however, post hoc analysis revealed only spring and summer were statistically different (Dunn's test:  $\chi^2=2.8$ ,  $p<0.01$ ; spring-fall/winter  $\chi^2= -2.1$ ,  $p=0.054$ ; summer-fall/winter  $\chi^2= 0.37$ ,  $p=1.0$ ). Grouping monthly catch rates by season was an attempt to reduce the variability in the data though differences in sample sizes were present (spring  $n=81$ , summer  $n=136$ , fall/winter  $n=69$ ). CPUE in channel habitats was the greatest, followed by tidal flat and tidal creeks (Fig. 5). Mean catch rate in the channel was 0.72 sharks/net hr  $\pm$  0.17 (SE), 0.54 sharks/net hr  $\pm$  0.13 (SE) in tidal flats, and 0.26 sharks/net hr  $\pm$  0.09 (SE) in tidal creeks. Relative abundances of Leopard Sharks were significantly different among habitat types (Kruskal-Wallis,  $\chi^2=6.97$ ,  $n=286$ ,  $df=2$ ,  $p=0.03$ ; Fig. 5). Dunn's post hoc test results indicated catch rates in tidal creeks were significantly less than in channel habitats ( $\chi^2= 2.6$ ,  $p=0.013$ ) but no other differences among groups (channel-tidal flat  $\chi^2=1.1$ ,  $p=0.41$ ; tidal flat-tidal creek  $\chi^2=1.5$ ,  $p=0.17$ ).



**Fig. 4** Relative abundance (sharks/net hr) of Leopard Sharks caught in gillnets by season. Error bars represent standard error and significant groupings ( $\alpha=0.05$ ) indicated from Dunn's test, are denoted by letter.



**Fig. 5** Relative abundance (sharks/net hr) of Leopard Sharks caught in gillnets by habitat type. Error bars represent standard error and significant groupings ( $\alpha=0.05$ ) indicated from Dunn's test, are denoted by letter.

Females were more abundant in gillnet surveys than were males. The sex ratio in Elkhorn Slough was highest at 3.2 females for every 1 male in the fall/winter season (Chi-squared,  $\chi^2=6.8$ ,  $df=1$ ,  $p<0.01$ ). The female to male sex ratios were nearly equal for spring 1.6:1 and summer 1.2:1 (spring: Chi-squared,  $\chi^2=2.3$ ,  $df=1$ ,  $p=0.13$ ; summer, Chi-squared,  $\chi^2=0.2$ ,  $df=1$ ,  $p=0.65$ ). When comparing sexes by habitat type, the number of females caught in tidal flat habitats was significantly greater than the number of males, with a sex ratio of 2.9:1 (Chi-squared,  $\chi^2=8.26$ ,  $n=35$ ,  $df=1$ ,  $p<0.01$ ; Table 3). The sex ratio for channel habitats was nearly equal at 1.2:1 and slightly greater in tidal creeks at 2:1 (channel: Chi-squared,  $\chi^2=0.38$ ,  $n=42$ ,  $df=1$ ,  $p=0.53$ ; tidal creek: Chi-squared,  $\chi^2=1.33$ ,  $n=12$ ,  $df=1$ ,  $p=0.25$ ).

**Table 2** Number of Leopard Sharks caught by season in Elkhorn Slough.

Season	Male	Female	All
Spring	17	27	44
Summer	9	11	20
Fall/Winter	6	19	25
TOTAL	32	57	89

**Table 3** Number of Leopard Sharks caught by habitat type summed across all seasons.

Habitat Type	Male	Female	All
Channel	19	23	42
Tidal Flat	9	26	35
Tidal Creek	4	8	12
TOTAL	32	57	89

### Shark Tagging

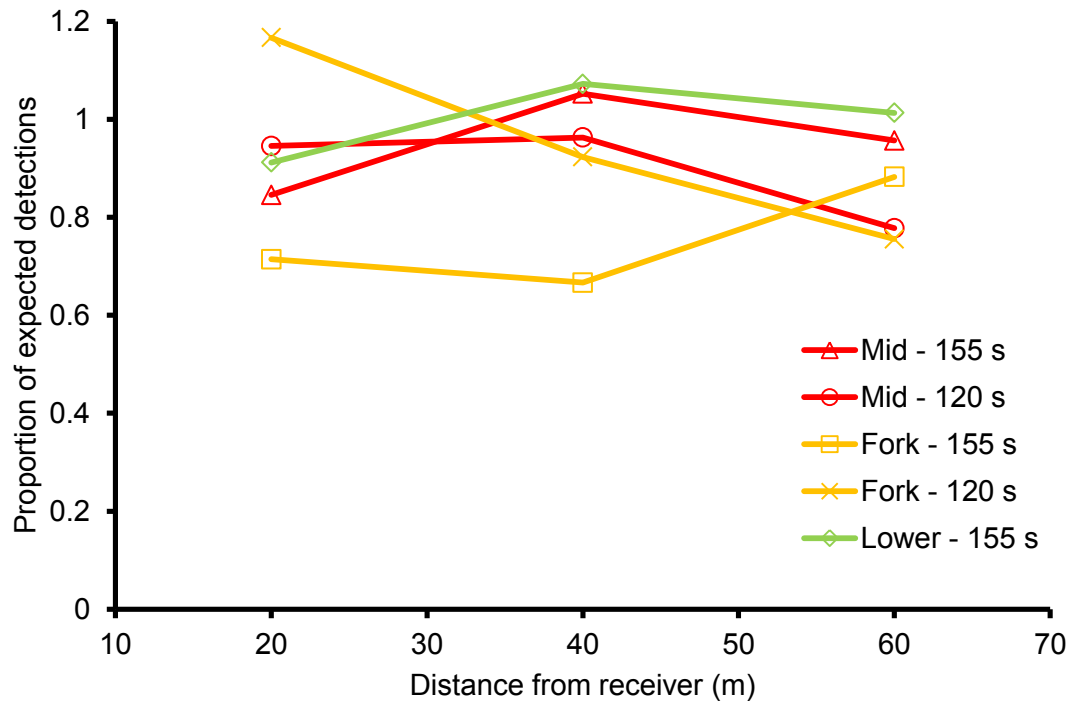
Twenty-one Leopard Sharks ranging in size from 90 cm to 142 cm total length (TL) were implanted with acoustic transmitters from March to June 2013 and tracked until 9 March 2014. Of those sharks tagged, 11 were male (113 cm to 142 cm TL) and 10 were female (90 cm to 128 cm TL; Table 4). All males were above the size at 50% maturity (100 cm TL: Kusher et al. 1992) and appeared to be sexually mature in the field based on observation of calcification of claspers. Three of the tagged females (42F, 41F, and 45F) were less than 105 cm TL, the size at 50% maturity (Kusher et al. 1992).

**Table 4** Detections of tagged Leopard Sharks in Elkhorn Slough, CA. Sex was assessed externally by presence or absence of claspers. Sharks were considered present when a minimum of two detections were recorded in a calendar day. Days elapsed was the inclusive number of days between the tag date and the last day of the study. Residency was the number of days present divided by the number of days elapsed, expressed in % d.

Tag	Sex	TL (cm)	Tag date	Last study detection date	Detections	Days present (d)	Elapsed days (d)	Residency (% d)	Returned in 2014
43M	M	120	03/14/13	04/16/13	964	27	361	8	N
42F	F	90	03/14/13	03/08/14	7,099	260	361	72	Y
213M	M	123	03/14/13	03/08/14	8,666	305	361	85	Y
206F	F	107	03/14/13	03/09/14	13,136	324	361	90	Y
216M	M	131	04/25/13	05/14/13	2,963	19	319	6	N
207F	F	117	04/25/13	06/02/13	3,045	35	319	11	N
44M	M	129	04/25/13	03/09/14	7,551	167	319	52	Y
217M	M	134	04/30/13	03/09/14	20,469	271	314	86	Y
41F	F	104	05/21/13	10/09/13	6,162	137	293	47	Y
214F	F	114	05/21/13	02/02/14	18,835	227	293	78	Y
46F	F	114	05/21/13	-	-	-	-	-	-
215M	M	132	05/23/13	03/09/14	29,334	268	291	92	Y
49F	F	112	05/28/13	10/21/13	4,718	137	286	48	N
201F	F	128	05/28/13	11/01/13	3,428	142	286	50	N
200M	M	137	05/28/13	03/09/14	16,668	250	286	87	Y
45F	F	102	05/28/13	03/09/14	9,442	265	286	93	Y
218F	F	115	05/28/13	-	-	-	-	-	-
202M	M	115	05/30/13	05/30/13	13	1	284	<1	N
17M	M	140	05/30/13	06/01/13	174	3	284	1	N
18M	M	142	05/30/13	03/09/14	18,844	224	284	79	Y
48M	M	113	06/18/13	08/14/13	1,678	52	265	20	N

## Acoustic Monitoring

Range testing of acoustic tags yielded similar results among the Lower, Fork, and Mid regions. All areas measured recorded greater than 60% of expected detections up to 60 m distance from the receiver, regardless of transmit interval or region (Fig. 6). For the majority of locations where receivers were placed, Elkhorn Slough is less than 120 m wide. Stations 1, 3, and 7 were situated in locations where Elkhorn Slough was wider than 120 m and as such, sharks could potentially swim past the receiver without being detected greater than 60% of the time. Though Elkhorn Slough is less than 120 m wide at station 2, the receiver was not placed in the middle of the channel, potentially allowing some sharks to swim by without detection. Trends in proportion of expected detections were similar in the Lower and Mid regions: the proportion of expected detections only varied slightly with increasing distance from the receiver. For the Fork region, the tag with a mean transmit interval of 120 s decreased in detectability with increasing distance from the receiver. However, the opposite trend was observed for the tag with the mean transmit interval of 155 s, as observed detections increased with increasing distance.



**Fig. 6** Range testing results in three regions of Elkhorn Slough. Proportion of expected detections was based on the mean transmit interval of the tag reporting, which could result in values greater than 1 if tags reported more frequently. Two types of tags were used with different signal delays; 60-180s (mean reporting 120 s) and 90-220s (mean reporting 155 s).

Signals from tagged sharks were recorded the greatest amount of time in the Fork region of Elkhorn Slough (Table 5). The receiver at station 3 recorded the greatest amount of detections being in a central location: it connects to the Lower and Mid regions, and ESNERR. Tagged sharks were also frequently recorded in the Mid region. Fewer detections were recorded in the Lower region of Elkhorn Slough, though the number of hours recorded was similar to both the Fork and Mid regions. Stations in the Upper region of the slough recorded the fewest number of signals and hours.



**Table 5** Summary of shark tag detections by receivers and time monitored.

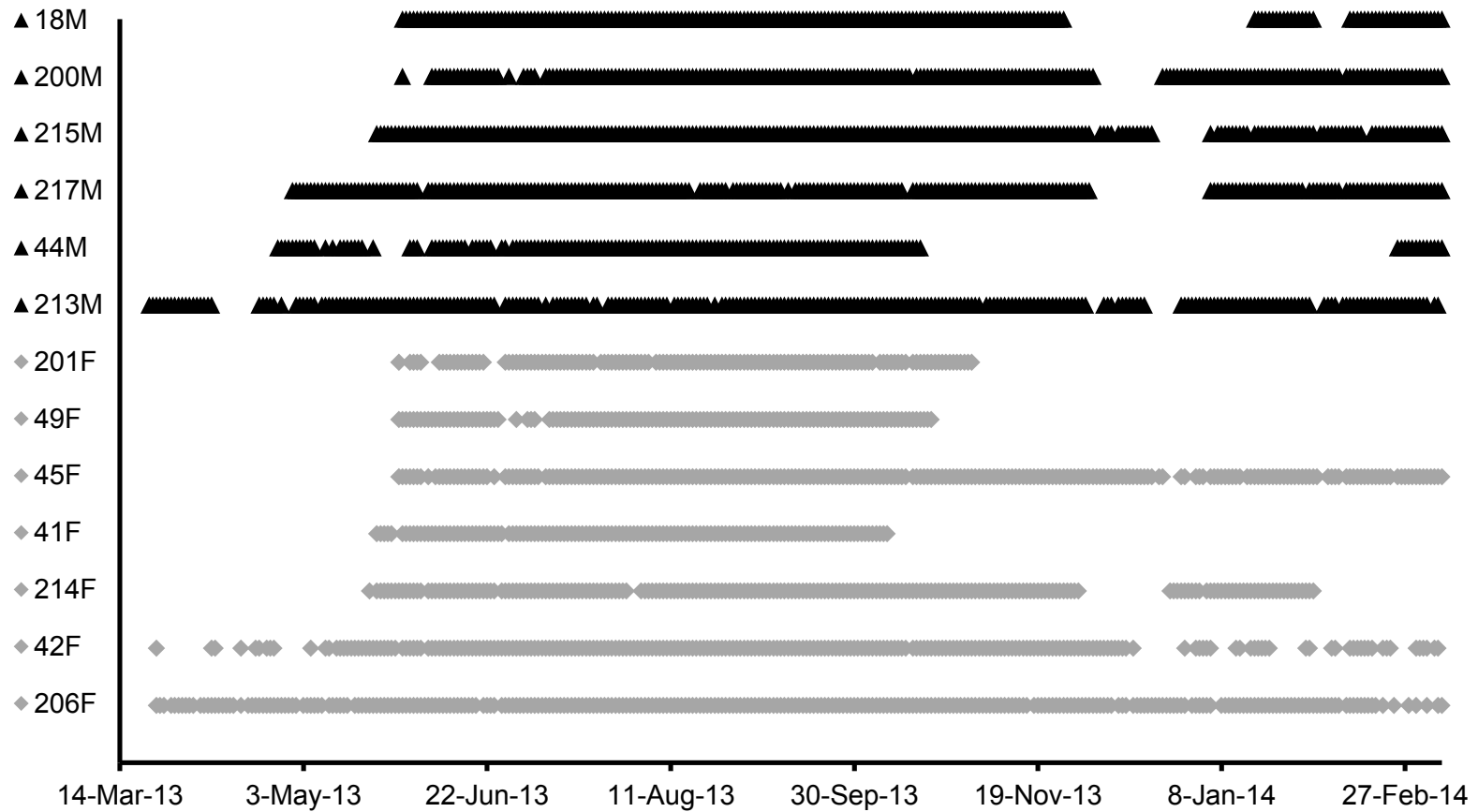
Station	Region	Detections	Hours of shark detections
1	Lower	14,730	3,076
2	Lower	9,256	2,023
3	Fork	35,901	5,893
4	Fork	27,878	2,299
4.5	Fork	24,733	4,096
5	Mid	26,591	2,941
6	Mid	27,982	2,788
7	Upper	3,182	416
8	Upper	2,938	283
TOTAL	4	173,191	23,815

Not all of the tagged sharks were frequently detected in the study site. Two tags (Shark 46F and 218F) were never detected on any receivers in Elkhorn Slough during the course of the study. An additional six tags (17M, 43M, 48M, 202M, 207F, 216M) were present fewer than 53 days (<20% of study period). Size of Leopard Sharks was not related to propensity to leave the slough (linear regression, adjusted  $r^2=0.05$ ,  $p=0.80$ ). These sharks may have vacated the study area or were removed by anglers. For these sharks, only presence and residency in the study site were calculated for Table 4. The remaining 13 sharks were comprised of 6 males (TL 123 cm to 142 cm) and 7 females (TL 90 cm to 128 cm), including all three females below the size at 50% maturity. Further analyses to determine patterns of movement for resident sharks were conducted with a sample size of 13.

The number of days a Leopard Shark was present in the study area was 137 d to 324 d, with a mean of  $229 \text{ d} \pm 17.8$  (SE: Fig. 7). Male presence in the study area ranged from 167 d to 305 d, with a mean of  $247 \text{ d} \pm 19.4$  (SE). Similarly, presence of females ranged from 137 d to 324 d, with a mean of  $213 \text{ d} \pm 28.4$  (SE). All sharks were absent for periods of time from Elkhorn Slough during the study period, though some absences were longer than others.

Leopard Sharks vacated Elkhorn Slough a total of 131 times for a mean of  $8.4 \text{ d} \pm 2.1$  (SE) per shark. The number of departures ranged from 2 to 22 per individual shark, and absences lasted between 1 d and 151 d (the upper end was from sharks that did not return to the study area after departing). No difference in duration of departure by sex was detected (Mann-Whitney U Test,  $W=2035$ ,  $n=131$ ,  $df=1$ ,  $p=0.93$ ). Seasonal absences

from Elkhorn Slough (>5 days) began occurring in October and lasted through December. Several tagged sharks (Shark 42F, 45F, 200M, 213M, 215M, 216F) vacated for only short periods of time during the end of the 2013 season. Of the 13 sharks, 11 returned (85%) for a second season in 2014. Shark 41F was last detected on 9 October 2013 and was detected in the study site the day after the study ended, 10 March 2014. This individual appeared to remain in the study site until all receivers were removed 9 June 2014.



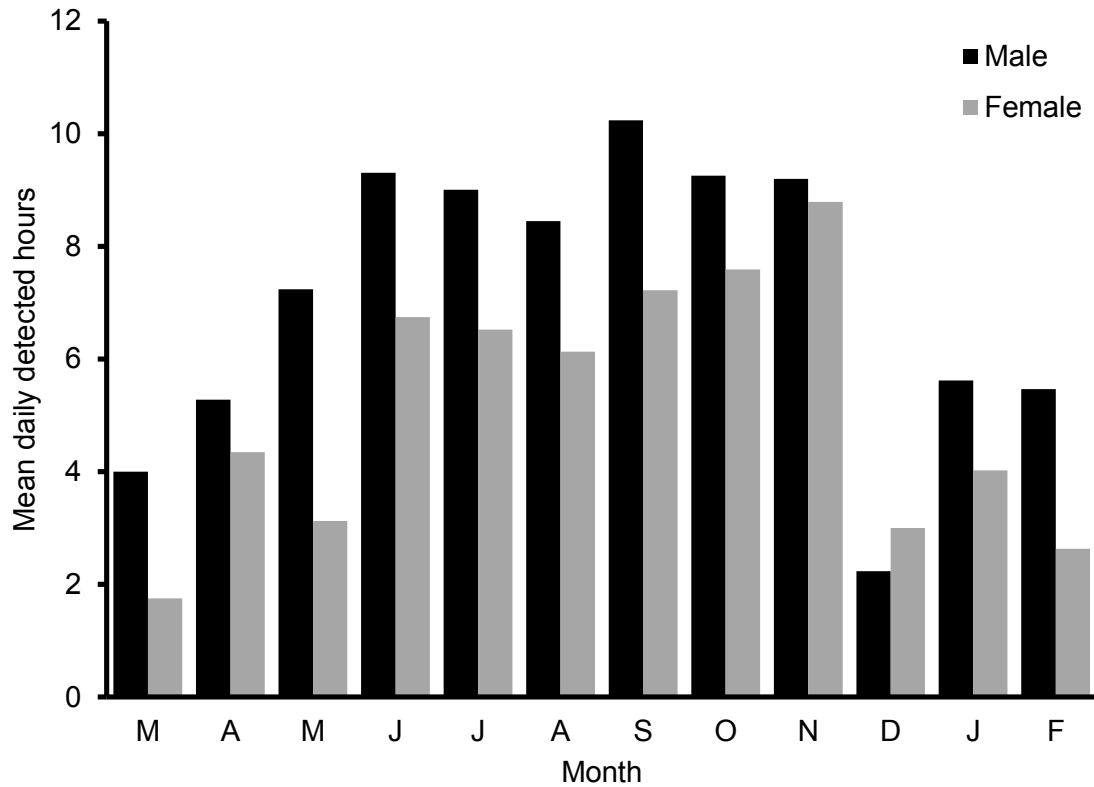
**Fig. 7** Abacus plot of signals recorded from tagged resident sharks from 14 March 2013 to 9 March 2014. Presence in the study area, represented by a symbol for each day, required a minimum of two detections per day. Males are represented by black triangles, females by gray diamonds. One receiver was absent 4-18 November 2013 due to mooring failure.

Residency of Leopard Sharks was relatively high from 47% d to 93% d during the study period (Table 4). Mean residency for all Leopard Sharks in Elkhorn Slough was 74% d  $\pm$  5 (SE). Mean residency of female Leopard Sharks was 68% d  $\pm$  8 (SE), whereas residency of males was 80% d  $\pm$  6 (SE). There was no difference in residency by sex (Mann-Whitney U Test,  $W=13$ ,  $n=13$ ,  $df=1$ ,  $p=0.29$ ).

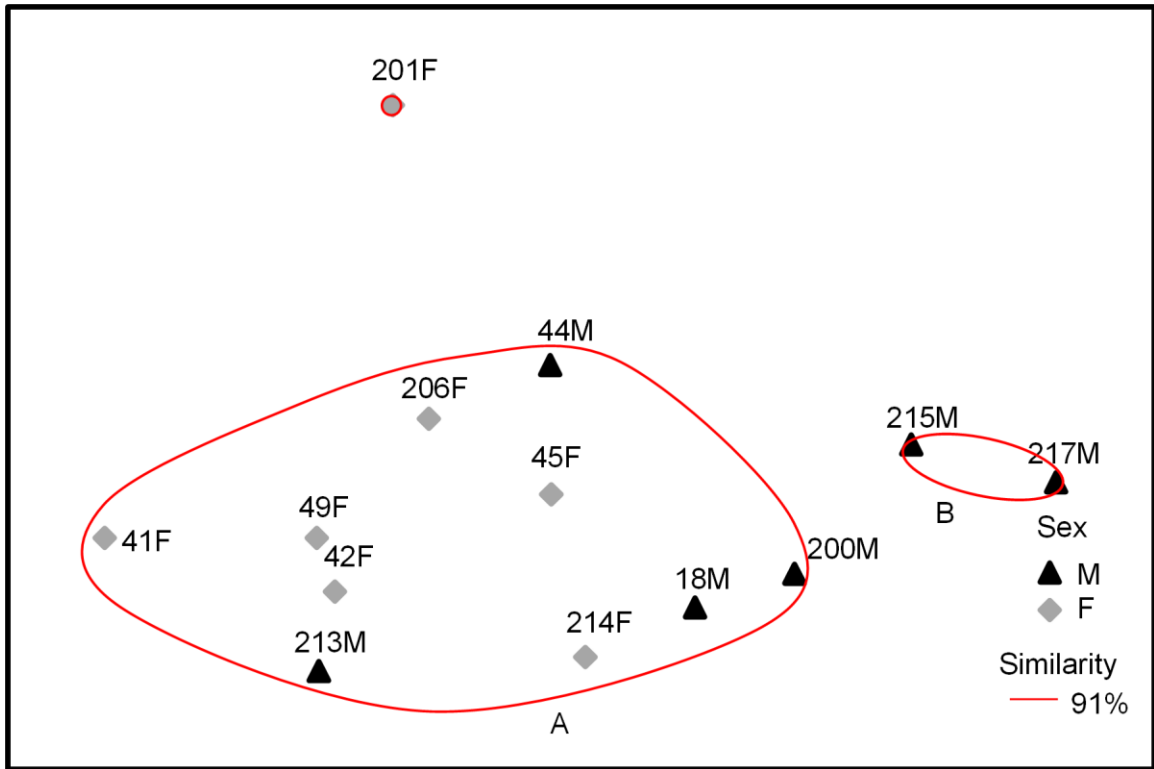
Differences in mean daily use of Elkhorn Slough by sex were observed. Males were more abundant in all months than were females, with the exception of December (Fig. 8). For June through November, males were consistently recorded 8 to 10 hr/d in the study site, whereas females were recorded between 6 and 8 hr/d during that same time. The number of signals detected by both sexes decreased greatly in December; however, the difference between the sexes was slight. November and December exhibited the most similar amount of hours sharks were detected between the sexes.

Cluster analysis of the percentage of hours each shark was detected in the study site by time of day resulted in two groups (A and B) and one female outlier (201F: Fig. 9). Group A was loosely aggregated on the MDS plot with members of both sexes. The loose aggregation indicates that there was large variability in the diel detections of these individuals. Group B was comprised of two male sharks (215M and 217M) that were plotted together, though not tightly aggregated. Individuals of Group B were detected the most out of any sharks in the study (29,334 and 20,469 detections, respectively). Additionally, these individuals were present the greatest amount of hours in the study area, though males that were within Group A were detected a similar amount of time. Sharks in Group B were recorded in the study site throughout the day, whereas tagged

sharks in Group A were recorded less frequently in the afternoon hours (Table 6). Shark 201F resembled the patterns of Group A except 38% of the tag's detections were in the night hours, compared with mean of 31% for sharks in Group A.



**Fig. 8** Mean daily detected hours of Leopard Sharks in Elkhorn Slough, separated by sex. Males were detected a greater number of hours in Elkhorn Slough throughout the year, with the exception of December. Males are represented by black bars, females by grey bars.



**Fig. 9** MDS plot of proportion of time tagged Leopard Sharks were detected in the study site by time of day, separated by statistically significant groupings at 91% similarity. Groupings revealed Group A, which is a loosely aggregated group of male and female Leopard Sharks, Group B consisting of two males (215M and 217M), and an outlier (201F). Male Leopard Sharks are represented by black triangles and females by grey diamonds.

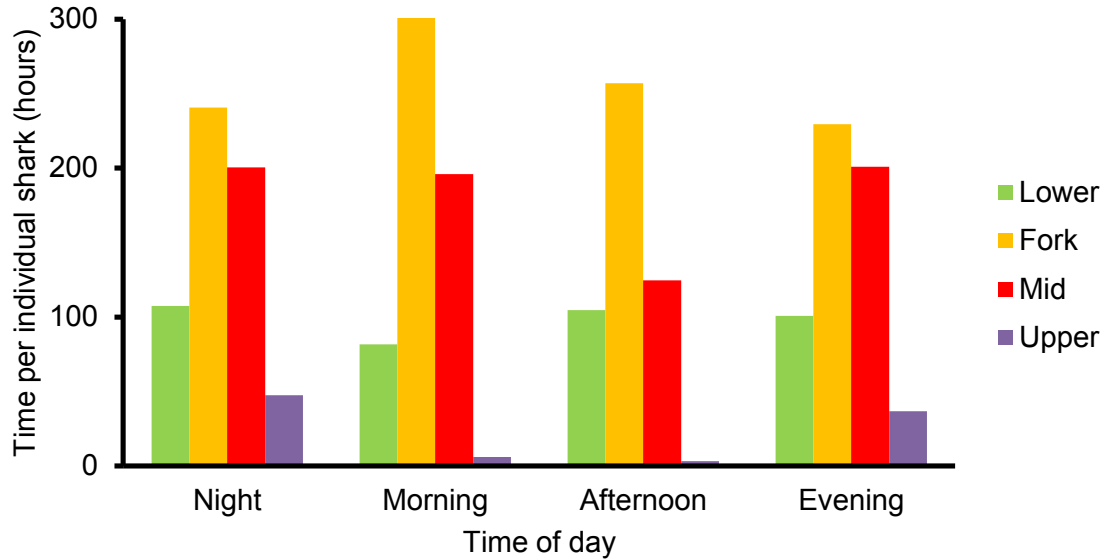
**Table 6** Percentage of time tagged Leopard Sharks were detected by time of day, separated by groupings identified by cluster analysis. Standard error was only included for group A, in parentheses, as the other groups did not have enough samples for calculation (Group A: n=10, Group B: n=2, 201F: n=1).

	Night	Morning	Afternoon	Evening
Group	0000-0500	0600-1100	1200-1700	1800-2300
A	31 ( $\pm 3$ )	29 ( $\pm 1$ )	16 ( $\pm 1$ )	24 ( $\pm 1$ )
B	24	23	26	27
201F	38	24	10	28

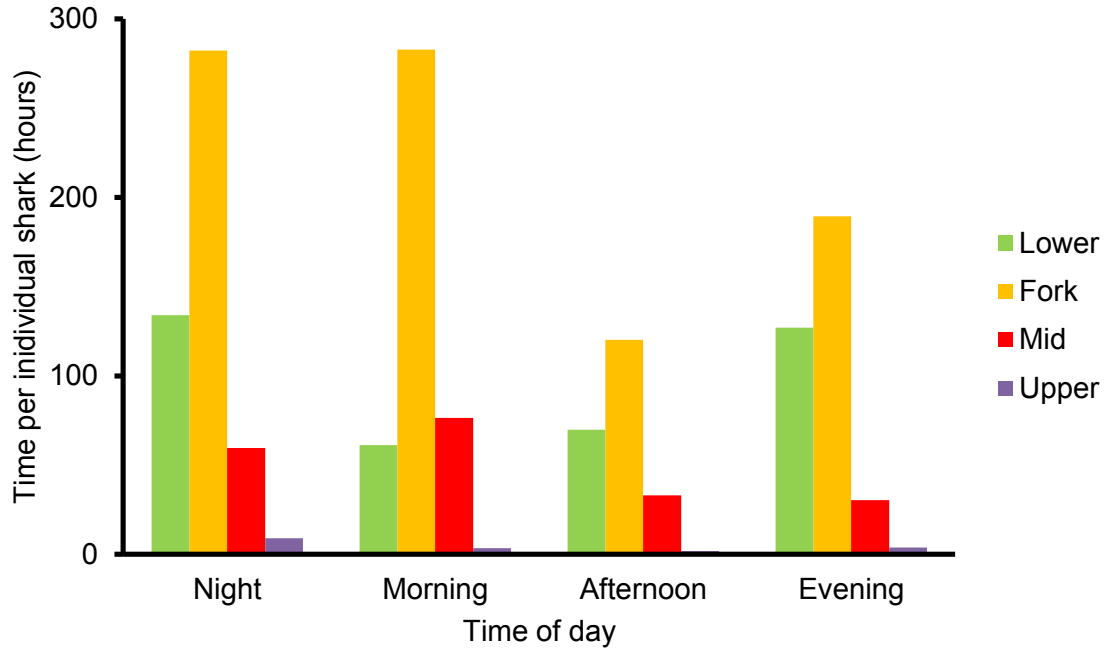
There were diel differences between sexes. In the Lower region, the pattern for both sexes was the same, use was greatest in the evening and night and decreased during the day (Fig. 10). Female use of the Fork region was greatest before noon, whereas male Leopard Sharks were consistently present throughout the day. The use of the Mid region was consistent throughout the day for both sexes, although males occurred in the area more frequently than females. Few sharks of either sex used the Upper region at any point throughout the day: the greatest use occurred by males who were detected between 36 to 48 hours in the evening and night (1800-0500).



**a** Male sharks

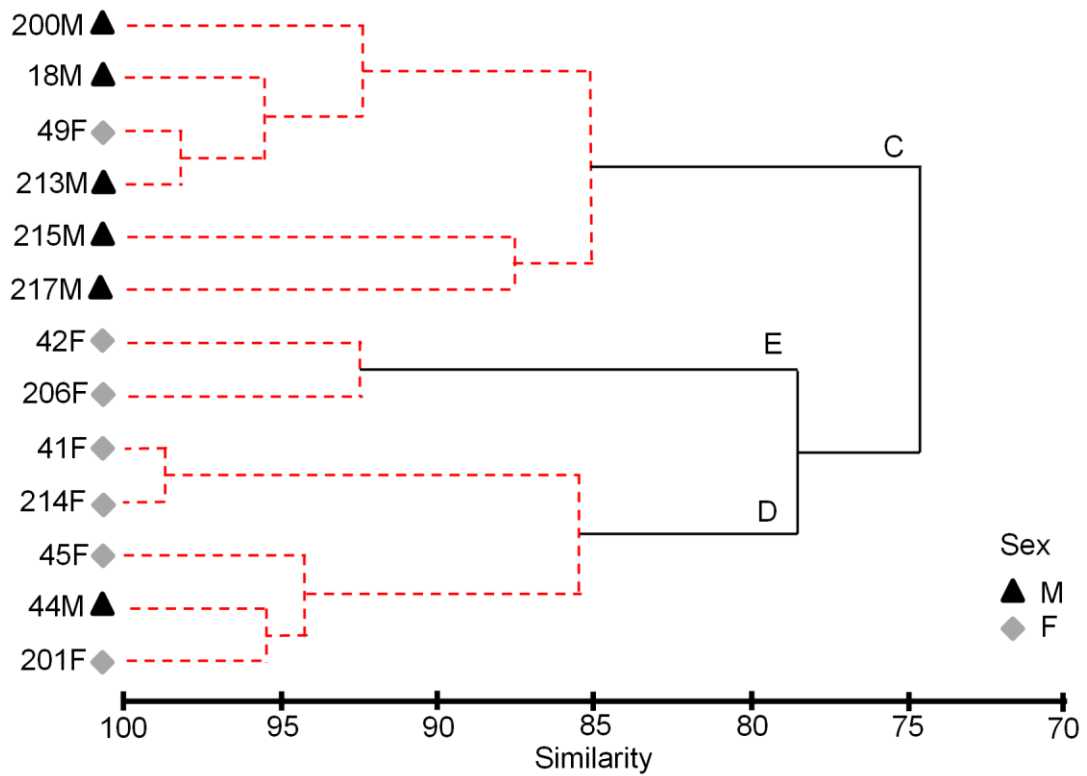


**b** Female sharks

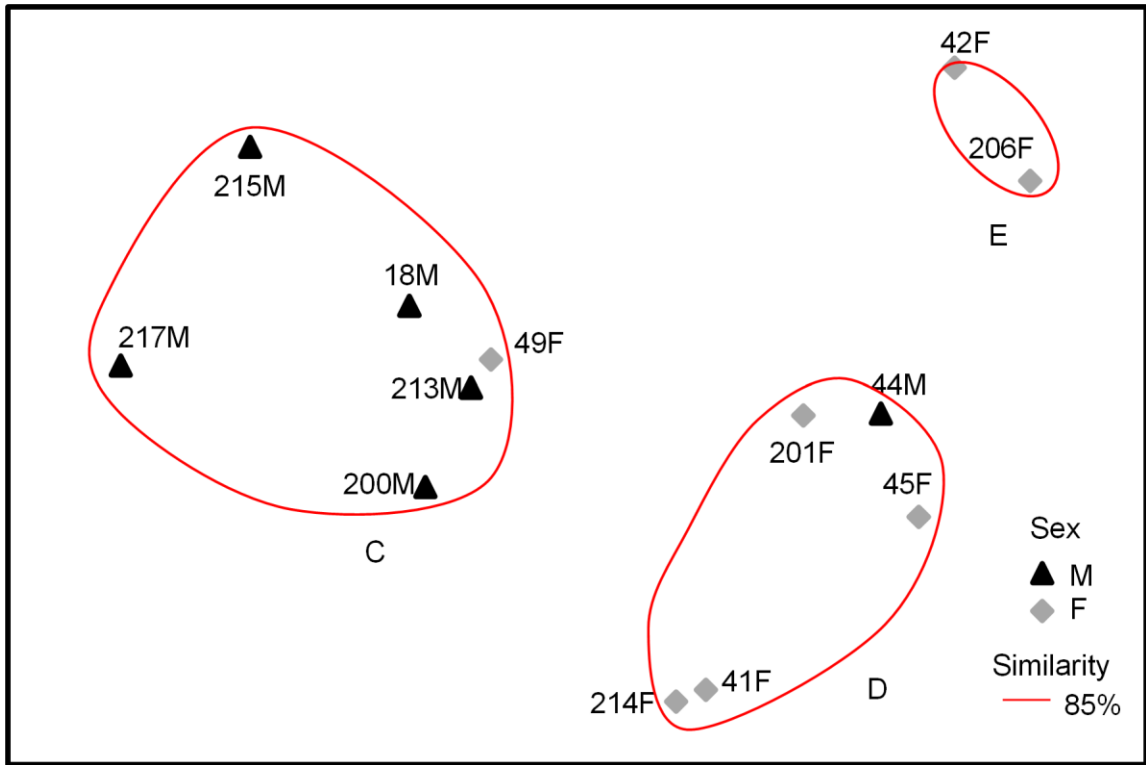


**Fig. 10** Number of hours tagged **a** male, and **b** female Leopard Sharks were recorded in each of the four regions of Elkhorn Slough throughout the day. Data were normalized based on sample size (male  $n=6$ , female  $n=7$ ).

With respect to regional differences in occurrences of Leopard Sharks, a Bray Curtis plot revealed great similarity among groups (Fig. 11). None of the groups were tightly aggregated, though some individuals within groups were closely grouped in the MDS plot (Fig. 12). Whereas all groups used the Fork region of Elkhorn Slough the most, the percent of time spent in the region differed amongst them (Table 7). Group E consisted of only females (42F, 206F). All members of the two other groups belonged to a single sex with the exception of one individual in the group. Group C composed of 5 males and 1 female and Group D was made up of 4 females and 1 male. Size did not appear to play a factor in these groupings, as smaller female Leopard Sharks occurred in both Group E and C.



**Fig. 11** Bray Curtis similarity plot of percentage of time tagged Leopard Sharks spent in each region. Groupings were statistically significant at 85% similarity, revealing three groups: C, D, and E. Male Leopard Sharks are represented by black triangles and females by grey diamonds.

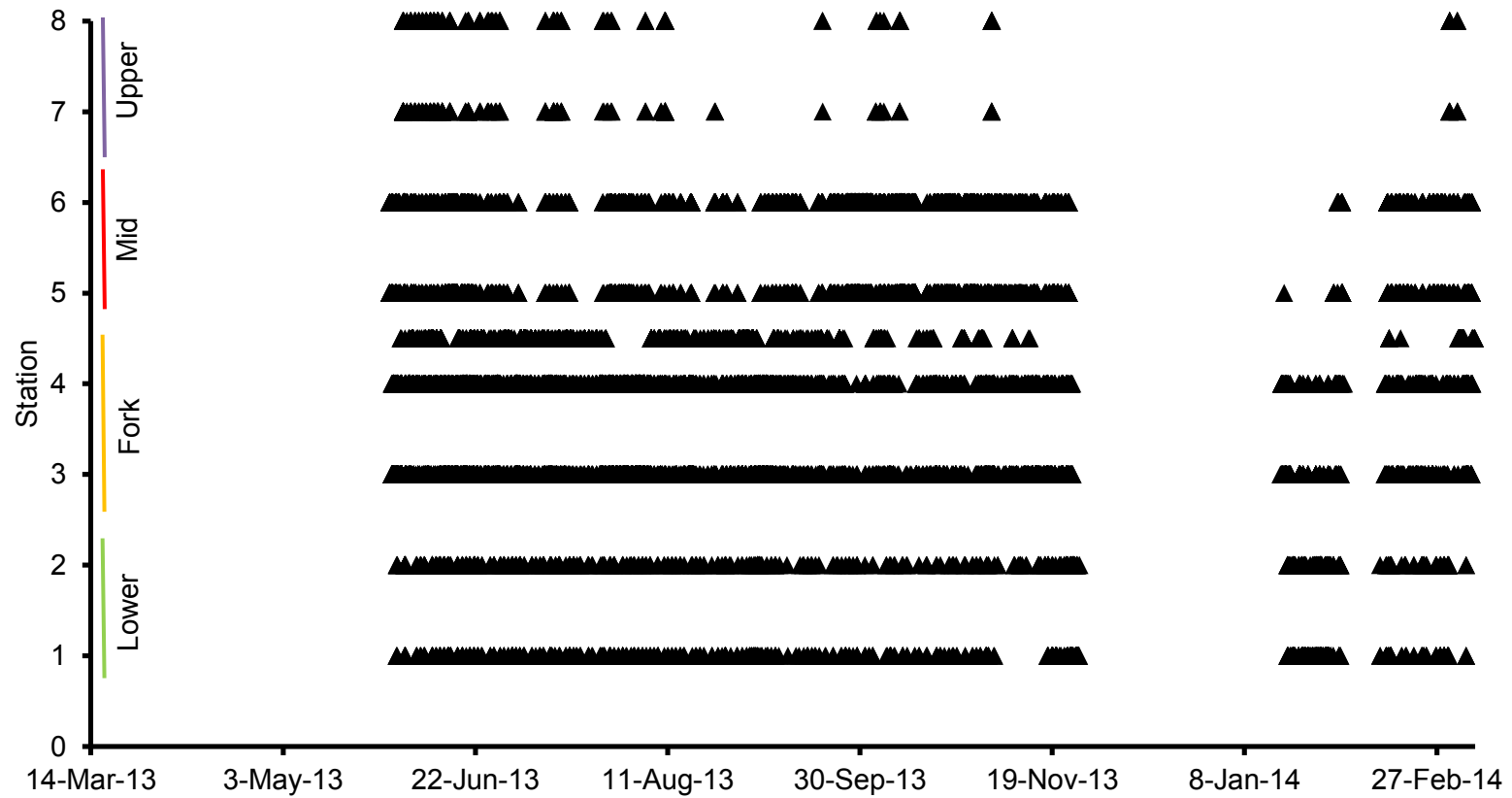


**Fig. 12** MDS plot of percentage of time tagged Leopard Sharks were detected by region. Outlines indicate groupings determined by cluster analysis. Male Leopard Sharks are represented by black triangles and females by grey diamonds.

**Table 7** Mean percentage of time spent by sharks in regions of Elkhorn Slough for groups separated using cluster analysis. Standard error was not included for group E as it contained only two sharks in the group.

Group	Lower	Fork	Mid	Upper
C	18 ( $\pm 3$ )	44 ( $\pm 2$ )	33 ( $\pm 2$ )	4.4 ( $\pm 0.8$ )
D	33 ( $\pm 3$ )	52 ( $\pm 3$ )	13 ( $\pm 1$ )	1.8 ( $\pm 0.7$ )
E	16	73	10	0.3

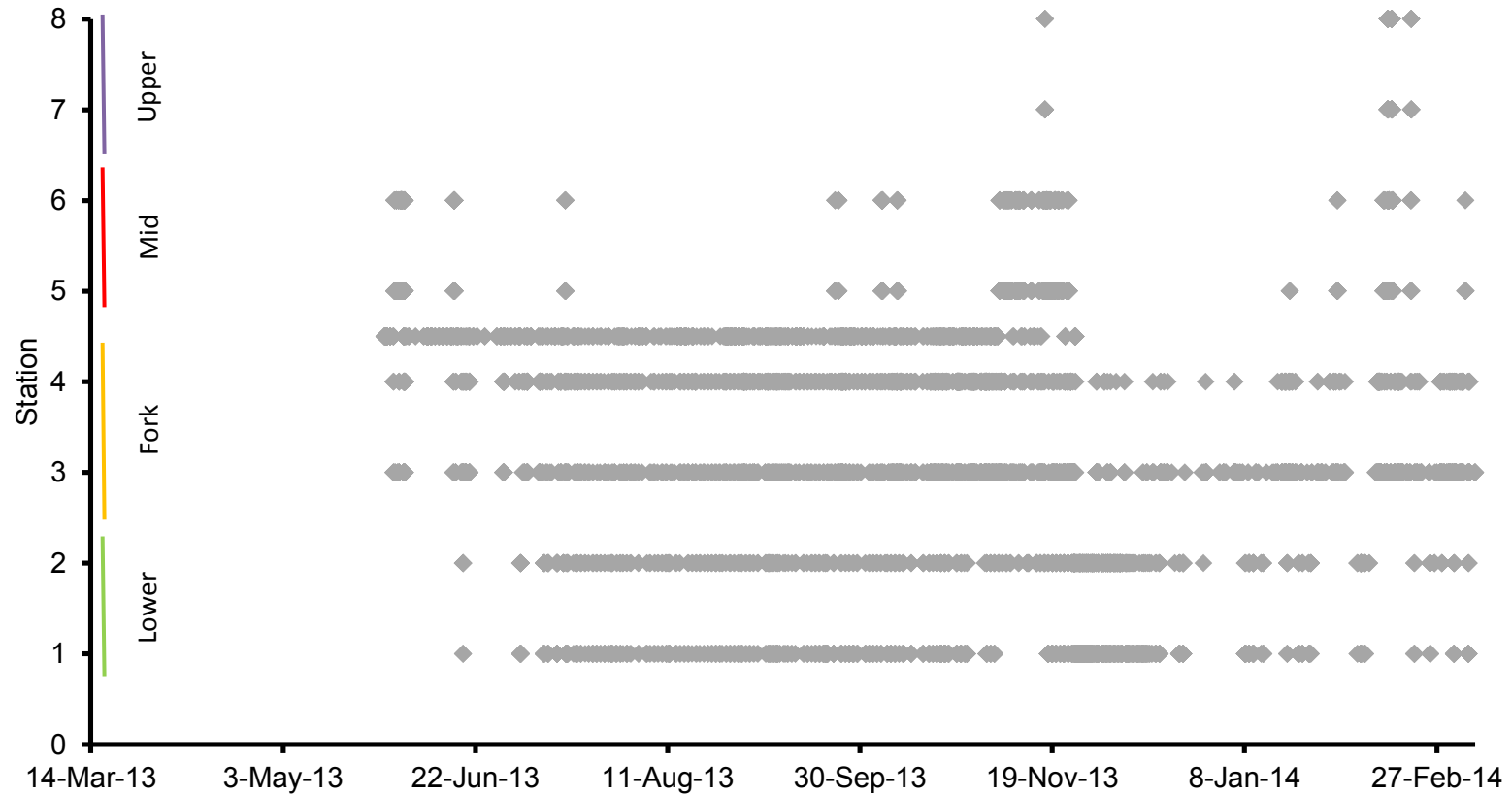
Individuals in Group C, such as Shark 18M, were characterized by spending greater percentages of time in the study site in the Fork and Mid region (Fig. 13). For every day that Shark 18M was present in the study area (a minimum of 2 detections per day), it is noted on the abacus plot with a symbol (as seen in Fig. 7). Whereas the study site was continuously covered by receivers, station 1 lapsed in coverage from 4-18 November 2013. The plot for 18M shows nearly daily detections for stations 3 and 4 (Fork Region) throughout the season. Patterns of detections in the Mid (stations 5 and 6) and Lower regions (stations 1 and 2) did not appear to differ greatly from the Fork region with the exception of more frequent absences from receivers in those regions. Group C spent a mean 18% of time detected in the Lower region, compared with a mean of 33% in the Mid region and 44% in the Fork region.



**Fig. 13** Abacus plot of detections recorded from Shark 18M, as a representative from Group C. Station 1 lapsed in coverage from 4-18 November 2013. Other gaps when symbols were not present indicate that sharks were absent from the study area.

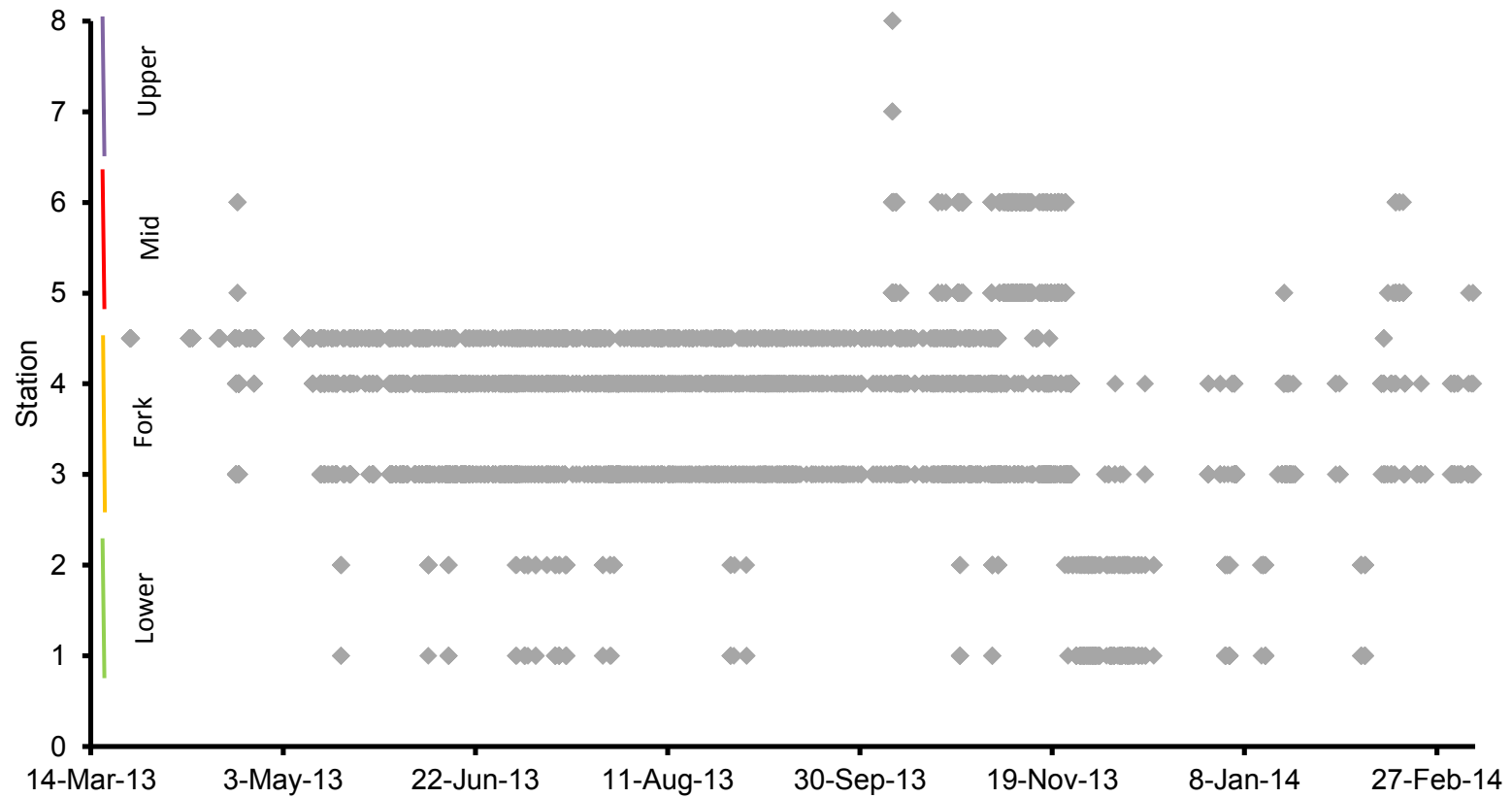
Group D was characterized by having individuals that spent a greater percentage of time in the Fork and Lower regions. Shark 45F was detected by receivers in the Lower (stations 1 and 2) and Fork regions (stations 3, 4, and 4.5) on a daily basis when present in the study site (Fig. 14). Decreased percentages of time detected in the Mid (stations 5 and 6) and Upper (stations 7 and 8) regions are obvious on this plot, with group mean use of 13% and 1.8% respectively. The mean percentage of time detected in the Fork region was 52% and 33% in the Lower region.

Sharks in Group E were both female and spent their time almost exclusively in the Fork region, consisting of 73% of total time in the study area. Shark 42F was seldom detected at other stations outside the Fork region (stations 1, 2, 5-8; Fig. 15). There were two short (3-4 week) periods where the Shark 42F was present on a daily basis in the Mid and Lower region later in the season. Sharks in the group spent a mean 16% of time in the Lower region, 10% of time in the Mid region, and 0.3% in the Upper region.



**Fig. 14** Abacus plot of detections recorded from Shark 45F, as a representative from Group D. Station 1 lapsed in coverage from 4-18 November 2013. Other gaps when symbols were not present indicate that sharks were absent from the study area.



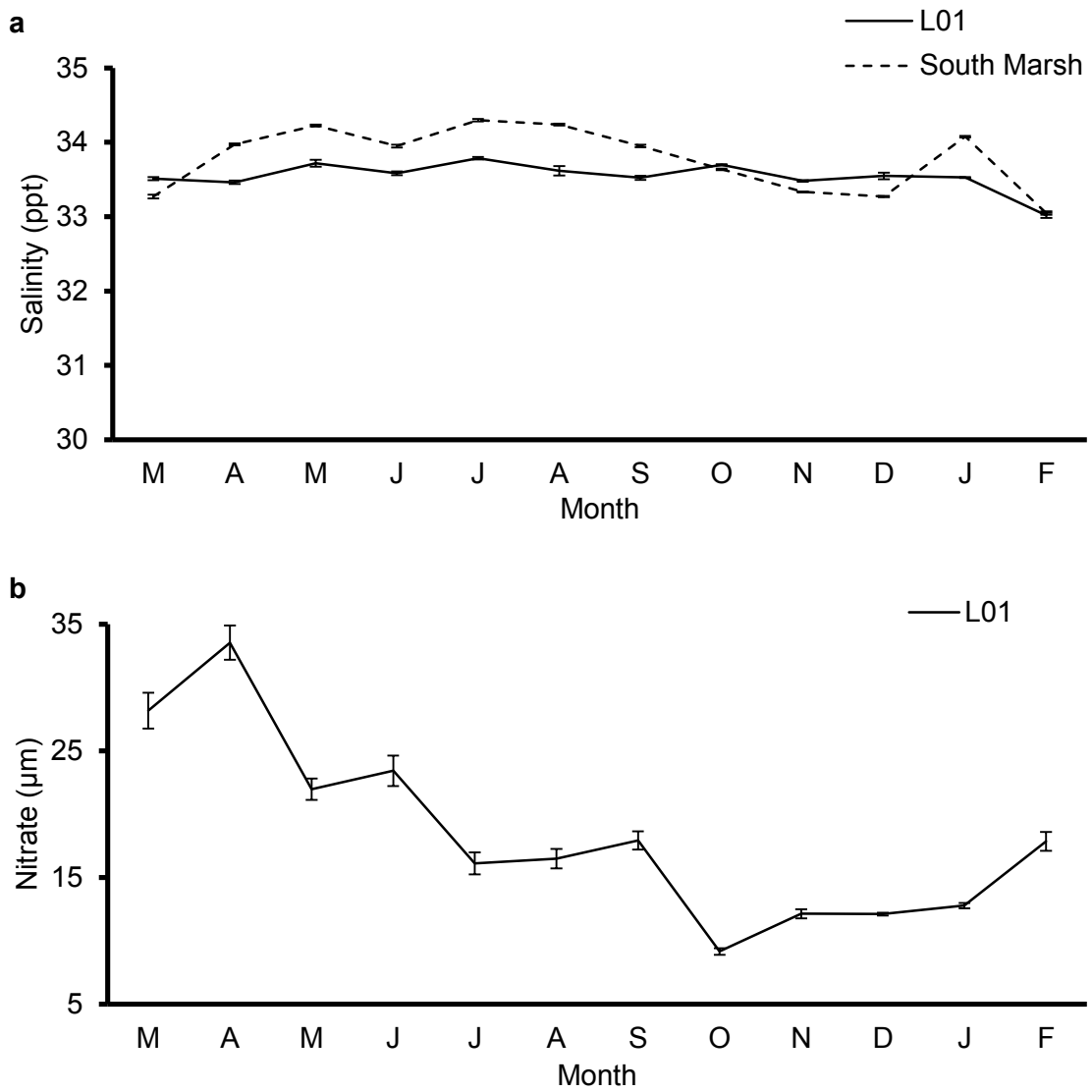


**Fig. 15** Abacus plot of detections recorded from Shark 42F, as a representative from Group E. Station 1 lapsed in coverage from 4-18 November 2013. Other gaps when symbols were not present indicate that sharks were absent from the study area.

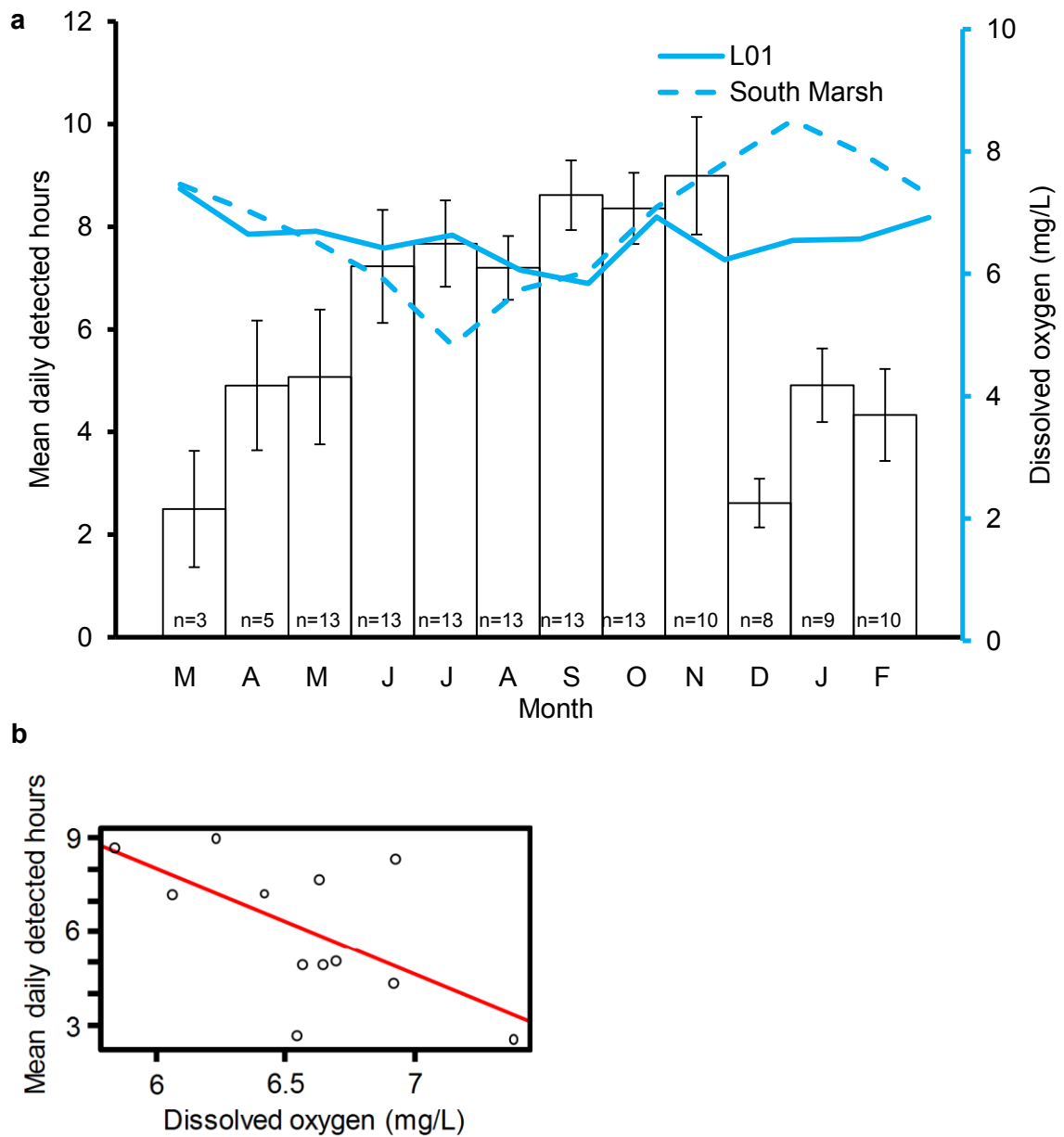
## Environmental Data

Salinity and nitrate concentration levels varied throughout the length of Elkhorn Slough. Data downloaded from the L01 mooring indicated little variability during the study period, therefore, were not used in any of the further analyses (Fig. 16). The range of salinity at the L01 mooring resembled that of Monterey Bay, and waters at L01 were not as influenced by evaporation or freshwater input as other regions of Elkhorn Slough such as the South Marsh station. Similarly, nitrate concentrations at the L01 mooring resembled levels in Monterey Bay, which were not as elevated as some areas of Elkhorn Slough that receive greater input from runoff water.

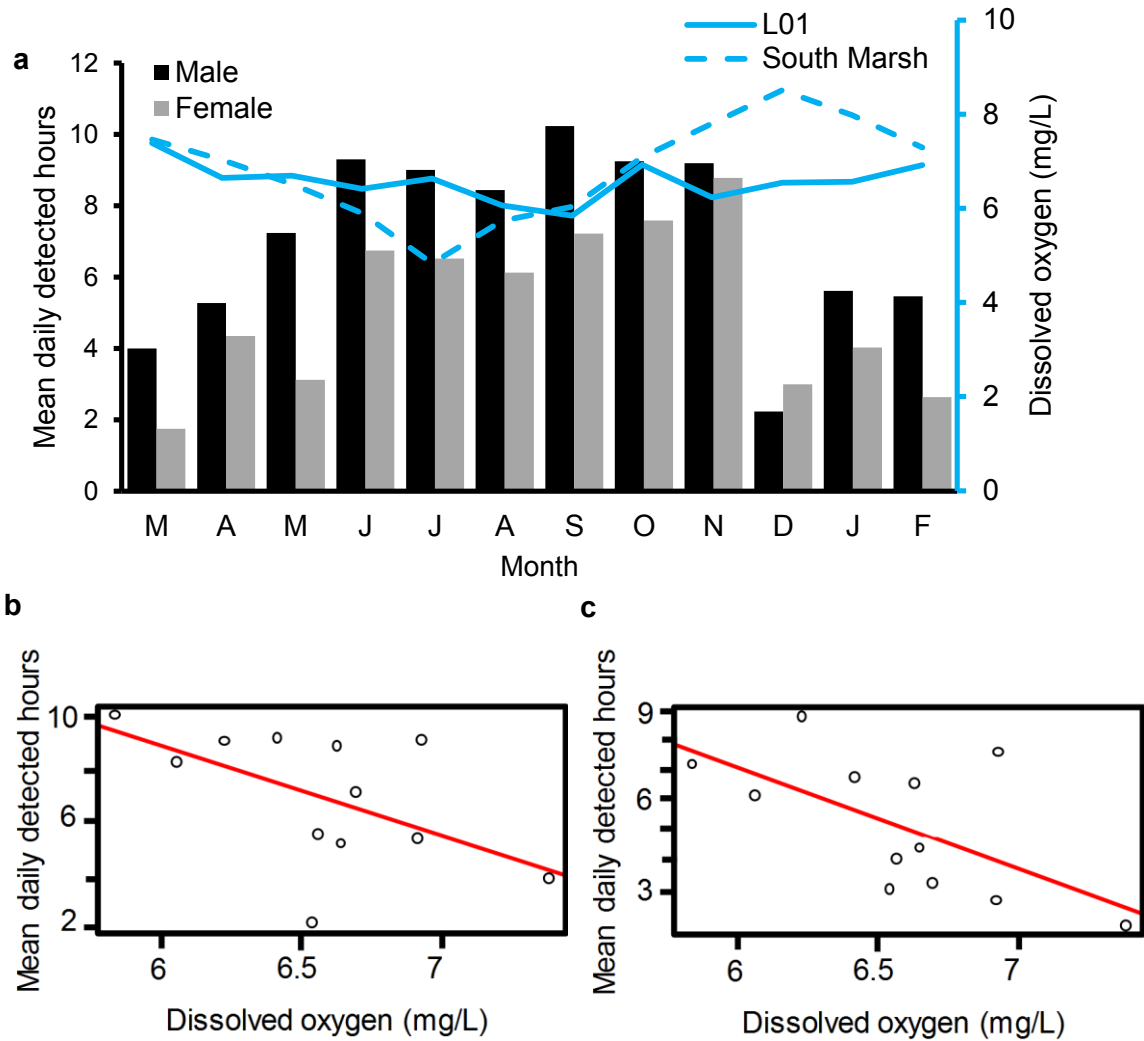
Both the mean number of hours Leopard Sharks were detected per day in Elkhorn Slough and dissolved oxygen concentrations varied seasonally (Fig. 17). Mean daily hours detected in the study area was negatively related to dissolved oxygen concentration measured at the L01 mooring (correlation,  $r = -0.61$ ,  $p = 0.03$ ). For March through May, sharks were detected less than 5 hr/d. Values of hours detected per day varied between 7 hr/d and 9 hr/d from June through November. Dissolved oxygen concentration was lowest in July at the South Marsh Station and September at the L01 mooring. Although the amount of detected hours did not seem to vary by sex, there was a negative correlation with abundance of female sharks (correlation,  $r = -0.62$ ,  $p = 0.03$ ) and no correlation with male sharks (correlation,  $r = -0.54$ ,  $p = 0.07$ ; Fig. 18).



**Fig. 16** Mean monthly **a** salinity recorded at the L01 mooring and South Marsh station, and **b** nitrate concentrations recorded at the L01 mooring from 14 March 2013 to 28 February 2014.



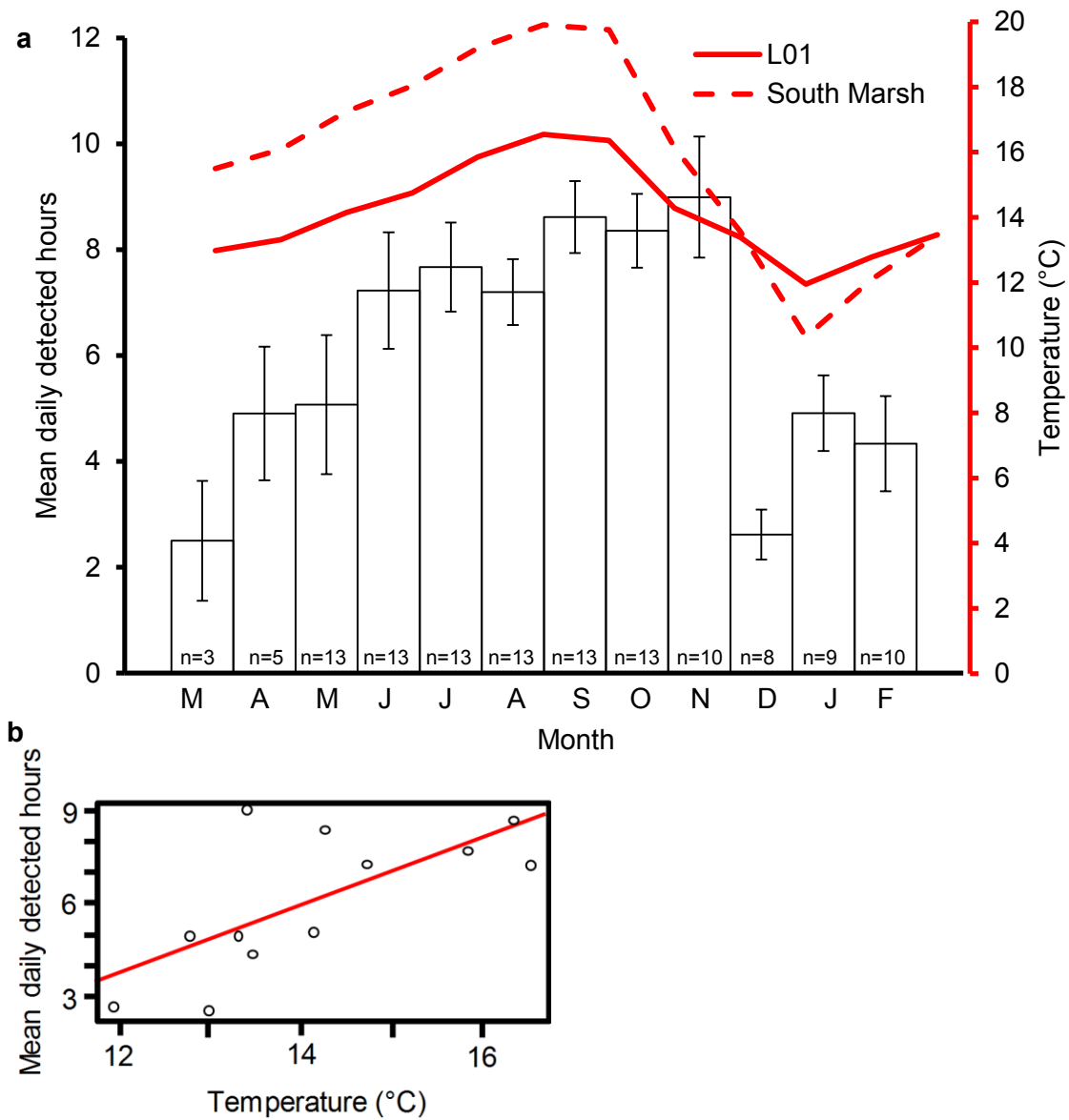
**Fig. 17 a** Mean number of hours Leopard Sharks were detected daily in the study site from March 2013 to February 2014 with mean dissolved oxygen concentration on the secondary Y axis, and **b** correlation plot. Numbers in columns denote the number of sharks present during the month. Dissolved oxygen concentrations measured at the L01 mooring were negatively correlated with Leopard Shark mean daily detected hours in Elkhorn Slough (correlation,  $r = -0.61$ ,  $p = 0.03$ ).



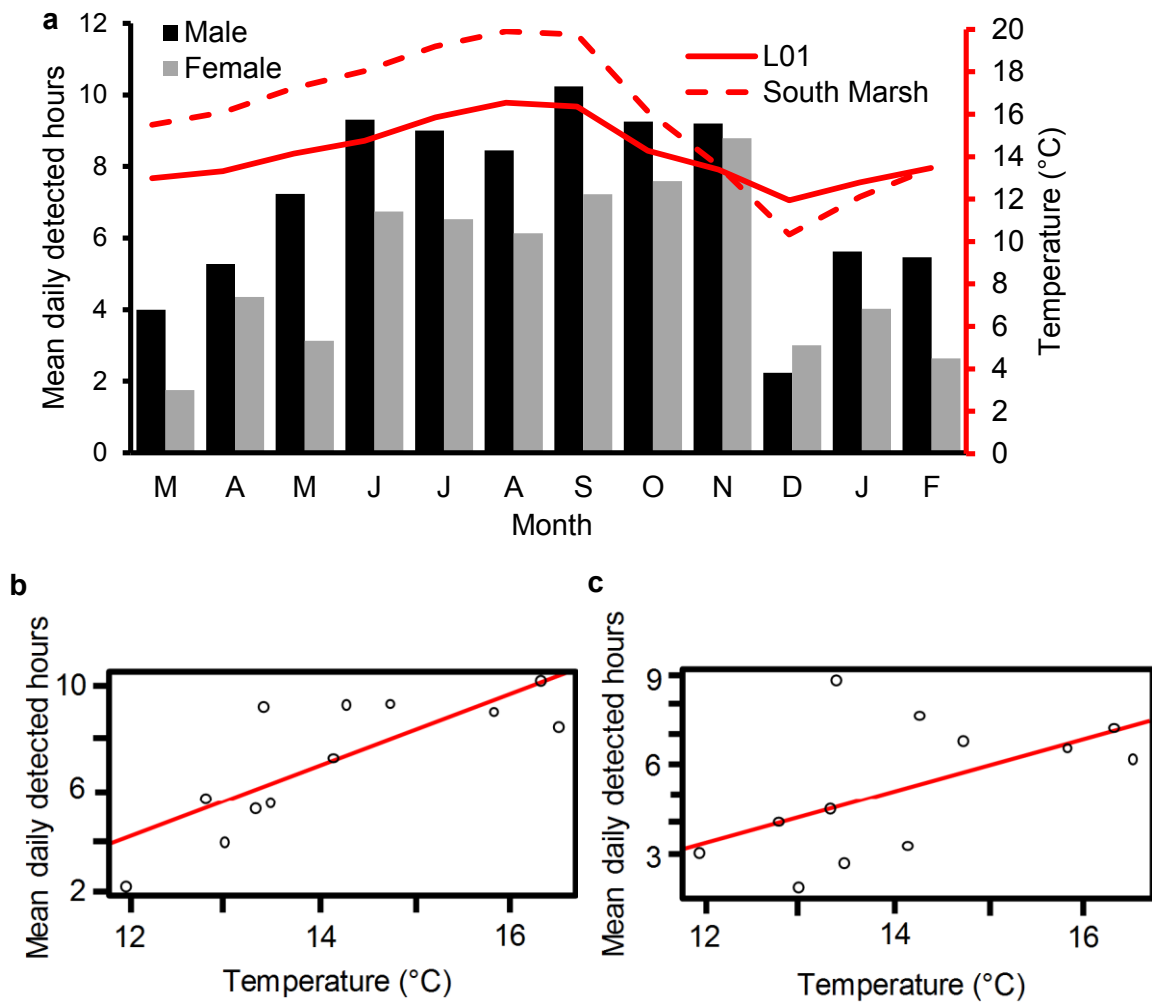
**Fig. 18** a Mean number of hours Leopard Sharks were detected daily in the study site by sex compared with mean dissolved oxygen concentration at the MBARI L01 mooring and ESNERR South Marsh station shown on the secondary Y axis, and correlation plots for **b** males, and **c** females. Abundance of females was negatively correlated with dissolved oxygen concentration (correlation,  $r = -0.62$ ,  $p = 0.03$ ), whereas no correlation existed with male sharks was observed (correlation,  $r = -0.54$ ,  $p = 0.07$ ). Males are represented by black bars, females by gray bars.

The monthly amount of hours sharks were detected in the study area was positively correlated with temperature recorded at the L01 mooring (correlation,  $r=0.69$ ,  $p=0.01$ ). Mean hours detected over the months followed the temperature curves for the most part, increasing through the summer until the peak in September and lowest in December (Fig. 19). However, the mean temperature in November was similar to April's but Leopard Sharks were detected 5 hr/d in April compared with 9 hr/d in November. Amount of time detected in the study area for male Leopard Sharks was positively correlated with mean temperature (correlation,  $r=0.79$ ,  $p<0.01$ ) though the same correlation did not exist with females (correlation,  $r=0.55$ ,  $p=0.06$ ; Fig. 20).

Similar patterns were observed with mean daily number of hours sharks were detected in ESNERR. Female Leopard Sharks were detected between 1.2 hr/d and 3.4 hr/d for the season, peaking in April. Females spent more time than males in ESNERR through the fall. Beginning in November, occurrence of males and females was minimal. When compared with the South Marsh temperature data, daily hours that male and female sharks were detected in ESNERR followed the water temperature curve increasing through the summer and decreasing in the fall and both sexes were positively correlated (male: correlation,  $r=0.84$ ,  $p<0.001$ ; female: correlation,  $r=0.76$ ,  $p<0.01$ ; Fig. 21). As the temperature increased again, male daily detected hours was greater than female Leopard Sharks. Relative abundances of both sexes at station 4.5 were negatively correlated with South Marsh station dissolved oxygen concentration (male: correlation,  $r= -0.79$ ,  $p<0.01$ ; female: correlation,  $r= -0.68$ ,  $p=0.02$ ).

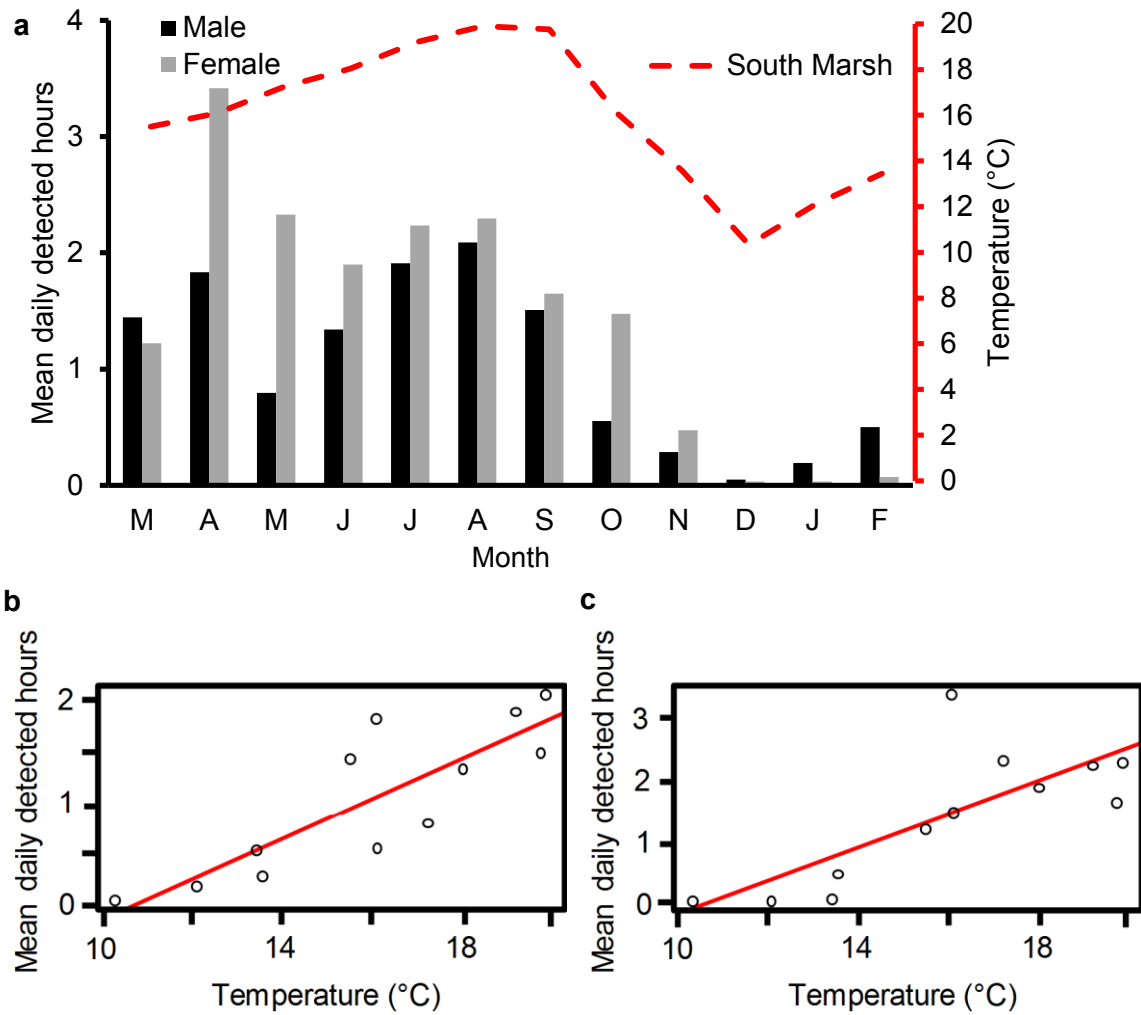


**Fig. 19** **a** Mean number of hours Leopard Sharks were detected daily in Elkhorn Slough from March 2013 to February 2014 compared with mean water temperature on the secondary Y axis, and **b** correlation plot. Numbers in columns denote the number of sharks present during the month. Mean daily detected hours was positively correlated with temperature from the L01 mooring (correlation,  $r=0.69$ ,  $p=0.01$ ).



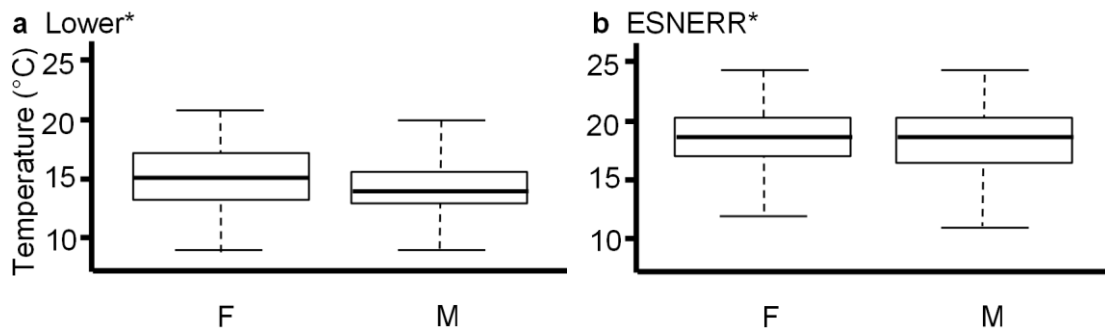
**Fig. 20 a** Mean number of hours Leopard Sharks were detected daily compared with mean water temperature at MBARI L01 mooring and ESNERR South Marsh station on the secondary Y axis, and correlation plots for **b** males, and **c** females. Abundance of males was correlated with water temperature (correlation,  $r=0.79$ ,  $p<0.01$ ), whereas no correlation existed with female sharks was observed (correlation,  $r=0.55$ ,  $p=0.06$ ).





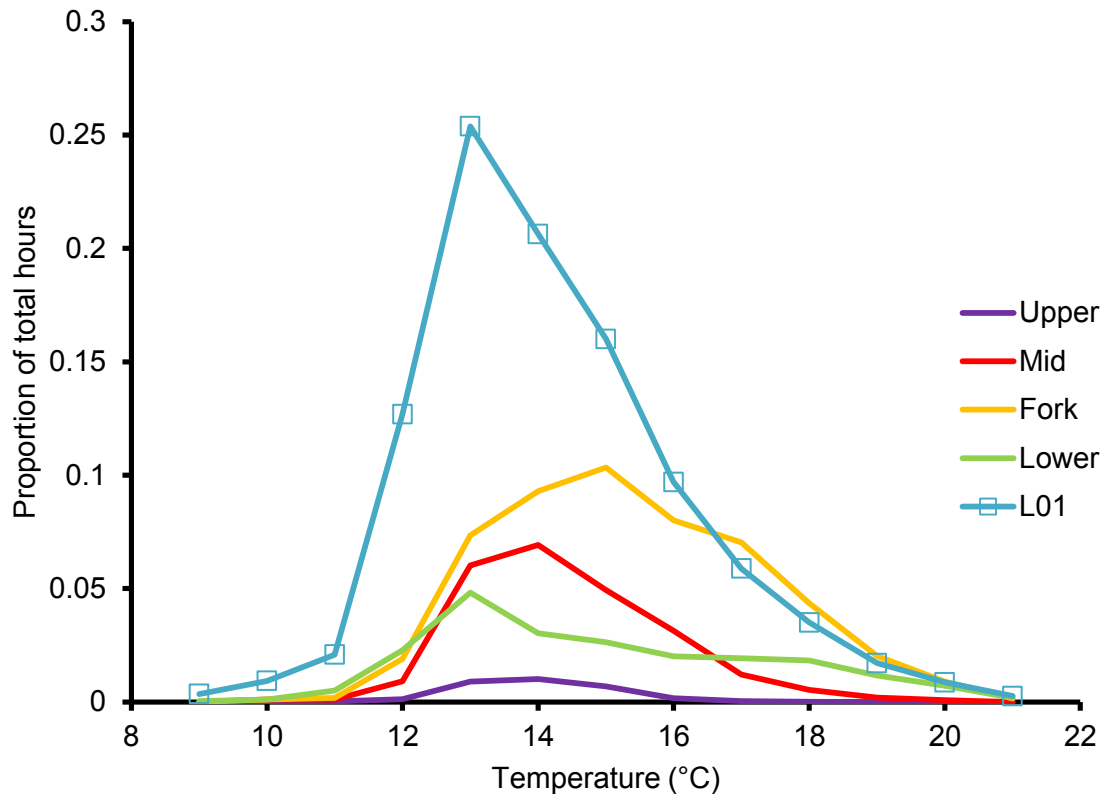
**Fig. 21** a Mean number of hours Leopard Sharks were detected daily compared with mean water temperature at ESNERR South Marsh water quality station on the secondary Y axis, and correlation plots for **b** males, and **c** females. Abundance of males and females was positively correlated with water temperature (male: correlation,  $r=0.84$ ,  $p<0.001$ ; female: correlation,  $r=0.76$ ,  $p<0.01$ ). Males are represented by black bars, females by gray bars.

Temperature recorded at water quality stations when sharks were detected was compared. Mean temperature of detection for males [ $14.5^{\circ}\text{C} \pm 0.04$  (SE)] was slightly less than females [ $15.3^{\circ}\text{C} \pm 0.04$  (SE)] when detected in the Lower region of Elkhorn Slough. There were significant differences by sex in water temperature when Leopard Sharks were detected in the Lower region (Mann-Whitney U test,  $W=3.7 \times 10^6$ ,  $n=5,012$ ,  $df=1$ ,  $p<0.01$ ; Fig. 22). In ESNERR, mean water temperature when males were detected at station 4.5 was  $18.3^{\circ}\text{C} \pm 0.06$  (SE) and  $18.5^{\circ}\text{C} \pm 0.04$  (SE) for females (Mann-Whitney U test,  $W=3.1 \times 10^6$ ,  $n=5,132$ ,  $df=1$ ,  $p=0.02$ ), though the difference is slight. Sample sizes of detections used for both comparisons were large ( $<5,000$  each) which greatly reduces variability, making slight differences of  $0.2^{\circ}\text{C}$  significantly different. Additionally, water temperatures when Leopard Sharks are detected within the constrained habitat were likely autocorrelated.



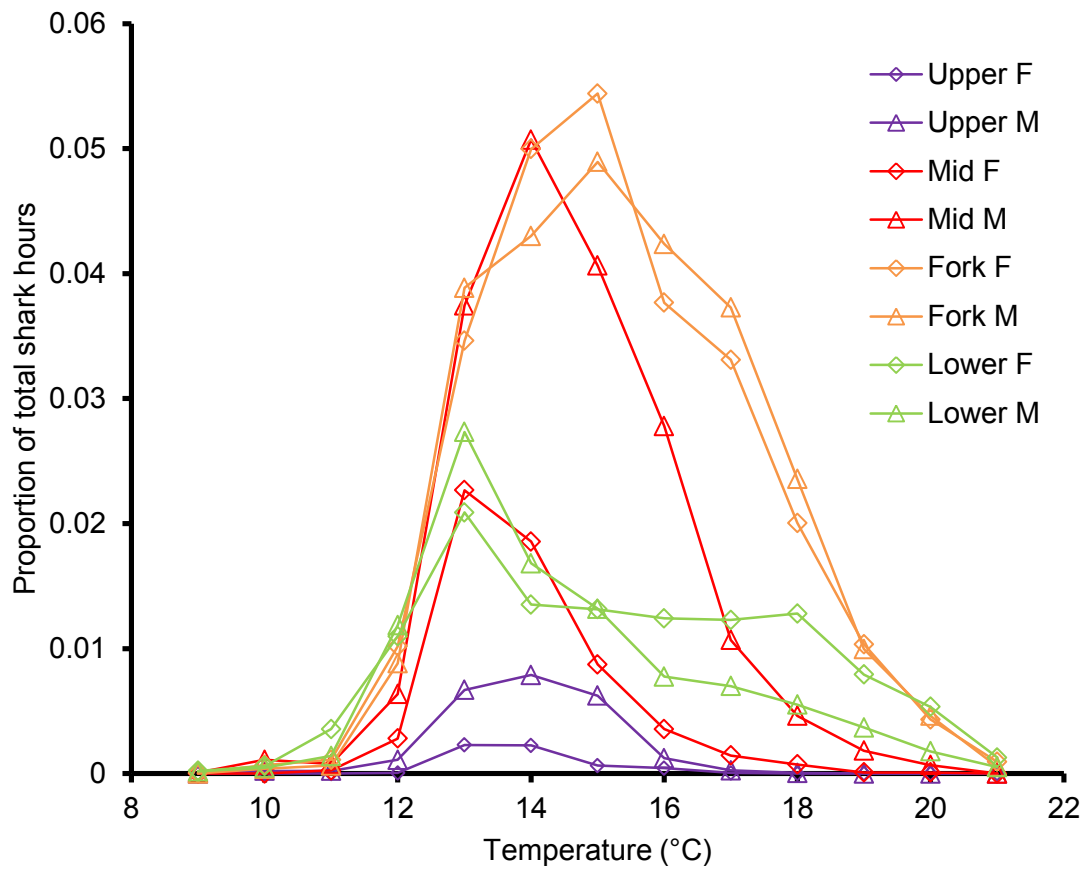
**Fig. 22** Water temperature when female and male Leopard Sharks were detected in **a** the Lower region and **b** ESNERR. An asterisk next to the area name indicates a significant difference in means.

When comparing water temperature at the time of detection for all individuals, Leopard Sharks were detected the greatest amount of time in the Fork region, at a wide range of water temperatures measured at the L01 mooring (Fig. 23). Sharks were present in Elkhorn Slough when water temperatures were between 9°C and 21°C at the L01 site in the Lower region. At lower temperatures ( $\leq 12^\circ\text{C}$ ), Leopard Sharks were relatively equal in their distribution in different regions of Elkhorn Slough. At mid temperatures, however, the proportion of time spent in the Mid region was greater than the proportion of time detected in the Lower region. When water temperature was 14°C at the L01 mooring, Leopard Sharks were detected twice as often in the Mid region than the Lower region. As expected, detections in the Fork region were greatest regardless of water temperature. Overall, Leopard Sharks were most abundant in Elkhorn Slough at temperatures between 13°C and 16°C.



**Fig. 23** Proportion of total hours Leopard Sharks were detected in the Lower, Fork, Mid, and Upper regions of Elkhorn Slough compared with water temperature at the time of detection from the MBARI L01 mooring and proportion of total hours these temperatures were recorded at the L01 mooring. Leopard Sharks were detected the greatest amount of time in the Lower, Fork, and Mid regions of Elkhorn Slough, at water temperatures ranging from 13°C to 16°C. The most frequently recorded water temperature at the L01 mooring was 13°C.

When these data were subdivided by sex, the proportion of time sharks spent in each region relative to water temperature only varied in the Mid region (Fig. 24). Male Leopard Sharks were detected a greater proportion of time than females in the Mid region at temperatures between 13°C and 16°C. This finding supports results of the cluster analysis by region as Group D comprised of five males and a single female where members were found to be present 33% of the time in the study area, whereas sharks from other groupings were present less than 13% of time detected in the Mid region. This finding may indicate that male and female Leopard Sharks were present in Elkhorn Slough when water temperatures range from 9-21°C, but males were more abundant in the Mid region of Elkhorn Slough when temperatures were between 13 and 16°C at the L01 mooring. Results of Chi-squared test of independence between sex and temperature was significant for each region (Lower: Chi-squared,  $\chi^2=49$ ,  $n=2,951$ ,  $df=3$ ,  $p<0.01$ ; Fork: Chi-squared,  $\chi^2=31$ ,  $n=8,256$ ,  $df=3$ ,  $p<0.01$ ; Mid: Chi-squared,  $\chi^2=228$ ,  $n=4,959$ ,  $df=3$ ,  $p<0.01$ ; Upper: Chi-squared,  $\chi^2=17.0$ ,  $n=652$ ,  $df=3$ ,  $p<0.01$ ). These results indicate that these factors are not independent of one another: the number of times male and female Leopard Sharks were detected in each region was dependent on the water temperature from the L01 mooring.



**Fig. 24** Proportion of hours Leopard Sharks were detected in the Lower, Fork, Mid, and Upper regions of Elkhorn Slough compared with water temperature at the time of detection from the MBARI L01 mooring, by sex.

The Marascuillo procedure revealed significant differences for all four regions through pairwise comparisons of the Chi-squared tests of independence. In the Lower region, males were detected more frequently at all temperatures with the exception of 16°C; both sexes had fewer detections at 16°C. The number of males and females detected at 16°C was significantly different than at water temperatures of 13°C to 15°C (Table 8). For the Fork region, females were more abundant at 14°C and 15°C than males, though frequency of detection was greater for both sexes at these temperatures. The number of detections from tagged male and female Leopard Sharks was significantly different at 14°C and 15°C than at 13°C and 16°C. Male Leopard Sharks were detected more frequently at the Mid region than females at all temperatures. Females were most abundant when water temperature was 13°C and decreased with increasing temperature. All pairwise comparisons for data from the Mid region were significant. Patterns of post hoc results were not as clear in the upper region of Elkhorn Slough. Male Leopard Sharks were detected nearly three times as frequently as female Leopard Sharks at 13°C and 14°C. Neither sex was frequently detected in the Upper region of Elkhorn Slough at water temperatures of 16°C. Pairwise comparisons indicated a difference among 13°C-14°C and 15°C, though no differences were detected with 16°C.

**Table 8** Results from post hoc pairwise analysis of Chi-squared test of independence, using the Marascuillo procedure, of number of detections by sex at temperatures ranging from 13°C to 16°C. The absolute value of the difference in the sample is reported along with the critical value, reported in parentheses. The pairwise comparison was deemed to be significant when the absolute value was greater than the critical value of the differences in sample proportions at a confidence interval of 0.95. An asterisk next to the test values indicates a significant difference in the pairwise comparison for that region.

Pair	Lower	Fork	Mid	Upper
13°C-14°C	0.012 (0.066)	0.066 (0.045)*	0.11 (0.047)*	0.033 (0.11)
13°C-15°C	0.066 (0.070)	0.055 (0.044)*	0.20 (0.048)*	0.16 (0.11)*
13°C-16°C	0.18 (0.075)*	0.001 (0.046)	0.26 (0.049)*	0.002 (0.21)
14°C-15°C	0.054 (0.076)	0.011 (0.041)	0.091 (0.044)*	0.13 (0.098)*
14°C-16°C	0.17 (0.081)*	0.067 (0.044)*	0.15 (0.045)*	0.035 (0.21)
15°C-16°C	0.11 (0.084)*	0.056 (0.042)*	0.063 (0.045)*	0.16 (0.21)



The water temperature and dissolved oxygen at the L01 mooring were compared from the last detection of Leopard Sharks departing Elkhorn Slough and their first detection returning to Elkhorn Slough. When Leopard Sharks vacated the study site, mean water temperature was  $14.1^{\circ}\text{C} \pm 0.17$  (SE) and  $14.3^{\circ}\text{C} \pm 0.17$  (SE) when Leopard Sharks returned to Elkhorn Slough. Similarly, mean dissolved oxygen concentration when sharks were departing was  $5.8 \text{ mg/L} \pm 0.16$  (SE) and  $6.2 \text{ mg/L} \pm 0.15$  (SE) when sharks returned to the study site. There was no significant relationship between temperature or dissolved oxygen concentration and when sharks departed or returned to Elkhorn Slough (temperature: Mann-Whitney U test,  $W=8.6 \times 10^3$ ,  $n=258$ ,  $df=1$ ,  $p=0.63$ ; dissolved oxygen: Mann-Whitney U test,  $W=9.3 \times 10^3$ ,  $n=254$ ,  $df=1$ ,  $p=0.12$ ). Water temperature at which male Leopard Sharks migrated in and out of Elkhorn Slough [mean  $14.6^{\circ}\text{C} \pm 0.02$  (SE)] was greater than temperatures during migrations for females [mean  $14.0^{\circ}\text{C} \pm 0.14$  (SE)]. When water temperatures at the time of migration were compared by sex, there was a significant difference (Mann-Whitney U test,  $W=6.6 \times 10^3$ ,  $n=258$ ,  $df=1$ ,  $p=0.02$ ). The same pattern was not observed when comparing dissolved oxygen concentration at the time of migration and sex (Mann-Whitney U test,  $W=8.8 \times 10^3$ ,  $n=254$ ,  $df=1$ ,  $p=0.20$ ). Mean dissolved oxygen concentration at the time of migration for females was  $6.1 \text{ mg/L} \pm 0.15$  (SE) and  $5.9 \text{ mg/L} (\pm 0.16 \text{ SE})$  for male Leopard Sharks.

## **Discussion**

Leopard Sharks exhibited high residency in Elkhorn Slough. A variety of studies have reported that Leopard Sharks are summer residents in nearshore bays and estuaries (Barry 1983; Talent 1985; Smith and Abramson 1990; Yoklavich et al. 1991; Carlisle and Starr 2009). Catch rates of Leopard Sharks from my study would suggest otherwise, however, as relative abundance was lowest during the summer (June to August). Acoustic data, however, showed congruence with results from previous studies (Talent 1985; Yoklavich et al. 1991; Carlisle and Starr 2009). Monthly amounts that tagged Leopard Sharks were detected on receivers in Elkhorn Slough increased, indicating sharks were present in the study site and relatively abundant. The combination of relative abundance and acoustic monitoring data provided a more robust snapshot of residency than using either alone.

Although Leopard Sharks are known to sexually segregate, rates of residency in Elkhorn Slough did not differ by sex. Mating likely occurs shortly after pupping in the spring, taking place between May and July most likely (Carlisle 2006). Ebert and Ebert (2005) suggested that sexual segregation of Leopard Sharks in Humboldt Bay occurred during and after the birthing season. In southern California, female Leopard Shark presence in the study site from San Clemente, CA to the Mexico border, was concurrent with the summer and winter solstice (Nosal et al. 2014). Additionally, the distribution of female Spottail Sharks in Australia differed based on season, whereas the same pattern

was not observed for males (Knip et al. 2012a). Residency in a habitat may not differ by sex, but specific areas, such as nurseries, may have differential residency.

ESNERR was determined to be an area of importance for both sexes. Previous literature stated its use as a nursery ground (Ackerman 1971; Barry 1983; Talent 1985; Yoklavich et al. 1991; Carlisle and Starr 2009) and females were abundant in ESNERR year round, though their use was mainly in the spring and summer (Carlisle and Starr 2009). Male and female Leopard Sharks were detected a similar amount of hours in Elkhorn Slough during the season from June through October. The mean daily number of hours detected for females was great throughout the season of residency but males also were found to be abundant and nearly equal amount of hours detected in December. These results indicate sexual segregation of Leopard Sharks in Elkhorn Slough is seasonal, with the greatest numbers beginning in June and ending in October/November, though the segregation lasts longer than the breeding season (May through July). Both sexes were detected throughout the season of residency in Elkhorn Slough, though use differed by region.

All sharks spent the majority of their time in the Fork region, but analyzing these movements by time of day revealed that males were continually detected in the Fork region throughout the day, and females were more abundant during early and late morning. Males were more abundant in the Mid region of Elkhorn Slough and females in the Lower region of Elkhorn Slough. Both sexes were relatively absent from the Upper region of the slough. Previous researchers have suggested that sexual segregation in elasmobranchs was related to a depth gradient (Wetherbee et al. 1997; Hight and Lowe

2007; Knip et al. 2012a). Based on the detections of Leopard Sharks within Elkhorn Slough, the opposite pattern was observed in this study. Males and females were detected in the Fork region (5-8 m) and ESNERR, where habitats can have depths less than 1 m at low tide. Males were distributed more frequently in the Mid region (2-5 m depth at low tide) compared with females that were frequently distributed in the Lower region (5-9 m depth at low tide). These analyses provided large scale patterns of use in Elkhorn Slough, though differences in use by habitat type were also observed.

Relative abundance estimates of Leopard Sharks in tidal creeks were less than other habitats and significantly less than channel habitats. Though they were less, this provides evidence that tidal creeks are still used by Leopard Sharks, a finding that is different than research conducted in the early 2000s, which did not report individuals moving in or out of tidal creeks during active tracking (Carlisle 2006). This may indicate that Leopard Sharks are re-inhabiting tidal creeks, either through adaptation or another change in species assemblage, as these areas were previously used as nurseries as few as 30 years ago (Barry 1983). Nursery use has likely shifted exclusively to tidal flat habitats of ESNERR (Carlisle 2006), as evidenced by the greater abundance of females in tidal flat habitats, though presence of both sexes was recorded throughout the season. The season of residency was long as one male shark (215M) was absent less than 26 days during the study period. This may indicate that some individuals are year-round residents.

Year-round resident behavior of Leopard Sharks during this season may be a result of the low amount of freshwater inputs via precipitation observed during the study

period. The “first flush” of freshwater did not appear until February. Normally, it is common between October and December, coincident with the end of the seasonal residency of Leopard Sharks in nearshore bays and estuaries. Total precipitation from 14 March 2013 to 9 March 2014 was 7.6 in, compared to Carlisle’s (2006) study period in which 17.8 in fell in 2003 and Elkhorn Slough received 16.2 in of precipitation in 2004 (MLML 2014).

Of the 13 sharks tagged as part of this study, 85% were detected during a second season (2014), which is in agreement with the idea that there is little movement between bays and estuaries along the coast (Talent 1985; Smith and Abramson 1992; Smith 2001). Carlisle (2006) detected only 3 of 11 (27%) tagged sharks the following year in Elkhorn Slough. During the course of their study, Smith and Abramson (1992) externally tagged 948 sharks in San Francisco Bay and only one shark was reported to have been caught in Elkhorn Slough. Despite literature evidence of little connectivity, at the time of publishing, three sharks tagged as part of this study were detected on other acoustic arrays located at Hopkins Marine Station (Pacific Grove, CA; 44M), San Rafael-Richmond Bridge in (214F), and Año Nuevo Island (Pescadero, CA; 41F). Shark 41F was detected in Elkhorn Slough in October 2013, Año Nuevo in December 2013, and returned to Elkhorn Slough in March 2014. Shark 44 also returned to Elkhorn Slough after being detected at Hopkins Marine Station. Movements of Leopard Sharks to nearshore locations may be related to environmental factors.

Environmental factors influencing Leopard Shark presence in the study site were not entirely clear. Nitrate levels are increased in Elkhorn Slough due to runoff from local

agricultural land (Hughes et al. 2011), however, large fluctuations were not observed in the L01 mooring data. During my study period, salinity levels were 17 parts per thousand (ppt) to 35 ppt, with values frequently in the range of 30 ppt to 34 ppt. Similar values were recorded at MBARI's L01 mooring in the lower slough in 2003 and 2004 during Carlisle's (2006) study, though salinity was variable in ESNERR and frequently was lower than 30 ppt. Dowd et al. (2010) tested Leopard Sharks in salinity treatments of 20.7 ppt, 27.6 ppt, and 33.3 ppt. They found in short-term experiments at lower salinities sharks swam consistently but in the long-term, low salinity required activity levels to be reduced to conserve energy. Nitrate concentration and salinity did not have as great an influence on Leopard Shark abundance in Elkhorn Slough as other environmental factors.

Low dissolved oxygen levels did not result in Leopard Sharks emigrating from Elkhorn Slough. This lack of response to low dissolved oxygen levels in Elkhorn Slough is likely a behavioral adaptation rather than a physiologically limiting factor. Female relative abundance in Elkhorn Slough was negatively correlated with dissolved oxygen concentration. Within ESNERR, both sexes were correlated with dissolved oxygen concentration. Though dissolved oxygen concentrations can be a factor limiting the amount of time an organism spends in a particular area, low dissolved oxygen levels were coincident with higher presence of Leopard Sharks in ESNERR. Much of ESNERR consists of habitat only available at high tides but there are some deeper pools and channels that sharks could utilize while waiting until adjacent habitat becomes available again. As observed by Hopkins and Cech (2003), it is likely that short-term low oxygen levels influenced shark abundance and habitat use more than long-term seasonal

fluctuations. In Tomales Bay, temperature and salinity were the most important environmental factors in the distribution and abundance of Leopard Sharks (Hopkins and Cech 2003). Levels of dissolved oxygen also are affected by water temperatures.

Water temperatures were correlated with Leopard Shark occurrence in Elkhorn Slough. Sharks were present at temperatures from 9-21°C, but abundance in the study site was greatest between 13 and 16°C. Talent (1985) observed Leopard Sharks were more common at temperatures above 14°C and larger sharks were more abundant at temperatures less than 11°C in Elkhorn Slough. Tagged Leopard Sharks in this study were most abundant in the Lower region, near Talent's sampling site, at temperatures greater than 12°C. In Catalina Island, Leopard Sharks were abundant throughout the day in the warmest sections of the study area (Hight and Lowe 2007) at temperatures above those recorded in this study. Leopard Sharks were most abundant in the Fork region at water temperatures of 16°C, though the abundance by sex was only different for the Lower (females more abundant) and Mid regions (males more abundant). Water temperature within Elkhorn Slough can vary by location based on depth, proximity to Monterey Bay, and residence time.

Comparing L01 and South Marsh environmental data, water temperatures in the South Marsh were nearly one degree Celsius greater in warmer months until the temperature at both stations decreased, and the South Marsh station was cooler than the main channel. Whereas the South Marsh station was not adjacent to station 4.5, it likely reflected much of the environmental conditions in areas within ESNERR, as habitats in ESNERR are generally shallow and can get relatively warm in the summer and cool in

the winter. These warm and shallow habitats are frequented by female Leopard Sharks (Hight and Lowe 2007; Nosal et al. 2014). In my study, the amount of time tagged sharks spent in each of the regions was contingent on sex and water temperature ranging between 13 and 16°C. Mean monthly temperature during this study was similar to the study period of Carlisle (2006), in which he observed Leopard Sharks arriving in Elkhorn Slough when water temperatures were above 10°C and reported that sharks vacated when temperatures fell below that level. In a laboratory study, the amount of oxygen consumed by Leopard Sharks increased as water temperature increased due to low metabolic temperature sensitivity of Leopard Sharks compared to other species such as Bat Rays (Miklos et al. 2003). The lack of greater metabolic rates at greater temperatures (20°C) allows Leopard Sharks to feed throughout the day (Miklos et al. 2003). This relates to diel patterns observed in this study, as Leopard Sharks were detected in the study side throughout the day with a slight decrease during the afternoon.

There are several potential causes for sexual segregation in elasmobranchs. One potential cause of sexual segregation could be female avoidance of mating attempts from aggressive males. This does not seem likely, however, as observations of mating between wild Leopard Sharks indicate that mating attempts are not aggressive, though pectoral biting during copulation has been observed (Smith 2005). Smith (2005) described an aggregation of Leopard Sharks circling and potentially exhibiting mating displays though sex identification was not possible from her vantage point. This circling and display behavior would not likely be as energetically demanding as aggressive mating observed in other species (Sims 2005). In Elkhorn Slough, large aggregations of



Leopard Sharks are difficult to observe due to turbid water. In Southern California, observations have occurred in large expanses of warm shallow water (e.g., Hight and Lowe 2007; Nosal et al. 2013; Nosal et al. 2014). That type of water clarity does not usually exist in temperate estuaries, making observations of large aggregations of Leopard Sharks difficult, though mating likely occurs somewhere in the estuary. No large wounds or damages were observed on the bodies of female Leopard Sharks caught in gillnets throughout Elkhorn Slough. Caught females frequently had red irritated areas or lines across their dorsal and ventral surfaces though the bodies of male Leopard Sharks had similar markings, making it unlikely that markings were a result of mating.

Other species of elasmobranchs segregate to reduce predation risk of offspring. ESNERR is nursery habitat for Leopard Sharks in Elkhorn Slough and likely the location of pupping in the spring. Despite this, males and females occurred in ESNERR throughout the spring and summer seasons. The number of daily hours of detection of female sharks in ESNERR was greater than males; however, both males and females were detected the greatest amount of time in the Fork region. The specific location of the nursery area within ESNERR was not identified in this study and assuming a more specific nursery exists, it is likely that the spatial scale of this habitat use is smaller than what was analyzed as part of this study. Conducting surveys with smaller mesh gillnets or beach seines throughout the season in different areas of ESNERR would be valuable in identifying spatially where neonate and juvenile Leopard Sharks occur to elucidate nursery use.

In this study, sex related differences in movement patterns in Elkhorn Slough could be the result of differences in foraging. Production of eggs and parental investment of female Leopard Sharks are energetically costly. Additionally, somatic growth and overall body condition of females is important for increased fitness of offspring. Male and female sharks may have different caloric requirements necessary for these basic functions. Though diets between males and females may not be entirely different, females may spend more time foraging for diverse prey types, whereas males are distributed in Elkhorn Slough where prey abundance is greatest. Diet studies of Leopard Sharks have occurred in Elkhorn Slough though differences in the diet were attributed to differences in size classes: sex related differences were not analyzed (Ackerman 1967; Kao 2000). Preliminary results of an ongoing capstone project for an undergraduate student at California State University Monterey Bay that is analyzing diets of Leopard Sharks captured in Elkhorn Slough have shown that fat innkeeper worms (*Urechis caupo*) occur exclusively in the diets of male Leopard Sharks (B. Machuca, California State University Monterey Bay, pers. comm).

Results from my study suggest that female Leopard Sharks may require a greater diversity of habitat types than male Leopard Sharks based on the hypothesis that their distribution is related to differences in foraging. Female sharks were abundant in the Fork region of Elkhorn Slough the greatest amount of time compared to other regions, but there was also a great percentage of time detected in the Lower region. Though there was not a significant difference in the length of departures from Elkhorn Slough, it is possible that females are leaving Elkhorn Slough for shorter periods of time than I

analyzed (<24 hr) or am able to detect. Detections in the Lower region of Elkhorn Slough may indicate females are transiting to areas adjacent to Elkhorn Slough instead of being present in the Lower region during that time. Carlisle and Starr (2009) found Leopard Sharks did not make trips to other coastal habitats until they had left Elkhorn Slough for the season. This would be difficult to determine with the current data, but a receiver on or near the Highway 1 bridge over the entrance to Elkhorn Slough may be able to provide valuable insight. The results of this study emphasize the need to include a diversity of habitat types within protected areas as they may provide areas for different biological functions to subsets of the population (Knip et al. 2012a).

Habitat degradation from anthropogenic and environmental sources are viable threats to estuarine habitats. Leopard Sharks were less abundant in tidal creeks than channel habitats. Erosion of tidal creek habitat in Elkhorn Slough forms more channel-like habitats (Lindquist 1998). Leopard Sharks used tidal creek habitats for nurseries in the 1970s (Barry 1983) but these habitats have stopped functioning in that capacity (Carlisle 2006). If tidal creek and channel habitats are similar, Leopard Shark abundance in channel habitats may be greater due to the area available. Leopard Sharks in channel habitats can allow transit along the length of Elkhorn Slough while foraging, which is not be possible with space restricted tidal creeks. Changes such as erosion likely changed the species assemblage (Carlisle 2006), potentially altering the abundance of Leopard Shark prey items.

The composition of an ecosystem's food web can be influenced by changes in abundance of other organisms or nutrients. These changes can be a cause for concern as

they can alter communities significantly (Hughes et al. 2013). Long-term effects can include changes in prey abundance and water quality, which would likely alter abundance and distribution of Leopard Sharks and other elasmobranchs (Carlisle and Starr 2009). Preservation of a diversity of estuarine habitat types will likely promote ecosystem health and diversity.

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