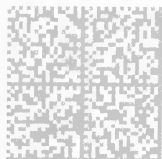


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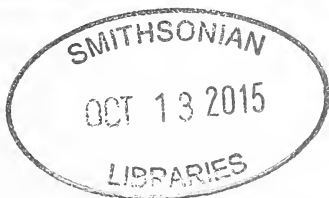
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## Effects of Ocean Recreational Users on Coastal Bottlenose Dolphins (*Tursiops truncatus*) in the Santa Monica Bay, California

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**Abstract.**—Coastal bottlenose dolphins (*Tursiops truncatus*) have been observed in proximity to swimmers, kayakers, stand-up paddle boarders and surfers along near-shore corridors in the Santa Monica Bay, California. From 1997 to 2012, a total of 220 coastal boat-based focal follows of dolphin schools were conducted in this area to determine a) the type and proximity of encounters between ocean recreational users and coastal dolphins, and b) the effects of these encounters on bottlenose dolphins' behavior. The majority of encounters involved dolphins and surfers (77.93%,  $n=145$  encounters), and overall, neutral reactions were observed in response to encounters (61.93%,  $n=176$  behavioral responses). Interactions between bottlenose dolphins and recreational users were recorded only once, and changes in dolphin behavior were observed more frequently when recreational users were at distances of less than three meters from a school. Although the current impact of human activities on coastal bottlenose dolphin behavior does not appear to be significant in the Santa Monica Bay, there is a need to: 1) adopt a precautionary approach in view of the increasing presence of ocean recreational users along this coastline, and 2) regularly monitor these encounters to determine potential changes in the type and proximity of encounters, as well as changes in dolphin behavioral responses.

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Bottlenose dolphins (*Tursiops truncatus*, hereafter bottlenose dolphins) are known to inhabit both pelagic waters and coastal regions, including bays and tidal creeks (Leatherwood et al. 1983). In the Pacific Ocean, a coastal and an offshore population of this species are currently recognized, showing morphological, osteological, and molecular differentiations (LeDuc and Curry 1998; Rossbach and Herzing 1999). Studies have suggested that coastal bottlenose dolphins are highly mobile within the inshore waters of the Santa Monica Bay, but also spend a large amount of time foraging and feeding in the bay (Bearzi 2005). Further, this species utilizes the region as a regular transit corridor between foraging hotspots along the California coast (Defran and Weller 1999; Bearzi 2005). An estimated 50 million tourists visit the Santa Monica Bay beaches each year<sup>1</sup>, many to partake in recreational activities including swimming, surfing, kayaking, and stand up paddle boarding. Swimmers, surfers, kayakers, and stand up paddle boarders are collectively defined as Ocean Recreational Users; hereafter ORUs. The year-round presence of both ORUs and bottlenose dolphins in the coastal waters of this region increases the likelihood of encounters between them.

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<sup>1</sup> Kreimann, S. H., Silverstrom, K. 2013. Beach and Marina Management Fact Sheet. County of Los Angeles Department of Beaches and Harbors. County of Los Angeles Department of Beaches & Harbors.

ORU presence has been proven to have adverse effects on dolphins in other areas worldwide. The occurrence of any vessel type, motorized or non-motorized, caused disturbances to dolphin behavior in Scotland (Pirootta et al. 2015). In New Zealand, Constantine (2001) observed sensitization and increased levels of avoidance with prolonged exposure to swimmers. Constantine (2002) also observed a decrease in bottlenose dolphin resting behavior when swimmers approached them in the wild. In Hawai'i, increased swimmer and kayak traffic led to decreased resting behaviors in spinner dolphins (*Stenella longirostris*; Samuels et al. 2000; Danil et al. 2005; Timmel et al. 2008; Ostman-Lind 2009). Spinner dolphins in Hawai'i also exhibited increased aerial behavior within their resting areas in correlation with the high number of swimmers in the area (Courbis and Timmel 2009). Indo-pacific dolphins (*Tursiops aduncus*) in Zanzibar displayed more frequent erratic (non-directional) behaviors in response to the increased presence of swimmers and boats (Stensland and Berggren 2007). Similarly, a study in West Cracoft Island, British Columbia found that when kayakers were present, killer whales (*Orcinus orca*) displayed avoidance behaviors, potentially resulting in changes to time spent feeding (Williams et al. 2011). Variations in behavioral states and decreased resting and feeding behaviors may cause a change in energetic demand, leading to changes in the lifetime fitness of the animal (Pirootta et al 2015; Williams 2011).

Based on the negative effects of these encounters between ORUs and cetaceans documented in other areas worldwide, the National Marine Fisheries Service (NMFS) has expressed concern that humans swimming with wild dolphins in the U.S. may qualify as harassment, leading to the disruption to their natural behavior (Spradlin et al. 1999). In an attempt to curb this disruption, the NMFS has advised vessels and swimmers to avoid approaching the animals at distance of less than 50 meters. Both ORUs and bottlenose dolphins have been frequenting the Santa Monica Bay since the 1930s and the tourism presence along this shoreline has increased, especially in recent times. The impact of ORU activities on bottlenose dolphins, however, has not yet been investigated in this area. This preliminary study describes the potential behavioral effects on coastal bottlenose dolphins of encounters with ORUs in this region, and provides suggestions for management and conservation measures aimed to mitigate the impacts on these animals.

## Materials and Methods

### *Study area*

The Santa Monica Bay study area (approximately 460km<sup>2</sup>, Fig. 1) is a shallow shelf bounded by the Palos Verdes Peninsula to the south (33°45'N, 118°24'W), Point Dume to the north (33°59'N, 118°48'W) and the edge of the continental shelf to the west. The bay contains two shallow water submarine canyons (Dume and Redondo) and the deeper Santa Monica Canyon. The Santa Monica Canyon begins at a depth of about 100m at the edge of the continental shelf. The bay has a mean depth of approximately 55m and a maximum depth of 450m. A shallow shelf between the Santa Monica and Redondo Canyons extends as a plateau from the 50m contour. Mild temperatures, short rainy winters and long, dry summers characterize the study area. Normal water surface temperatures range from 11 to 22°C.

### *Data collection and analysis*

This study utilizes data collected in the years 1997–2012 as a part of a long-term year-round marine mammal research project. The data presented in this paper were analyzed retrospectively and some of the reported information was opportunistic in nature.



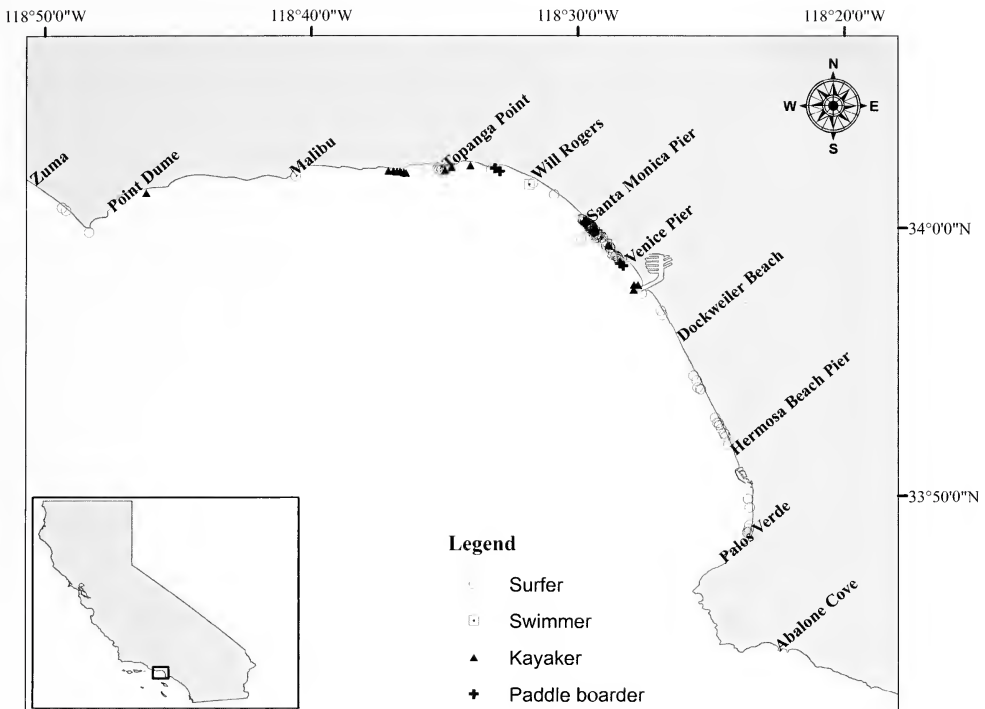


Fig. 1. Study area and locations of encounters between bottlenose dolphins and ORUs during surveys conducted in 1997-2012.

Coastal surveys (distance <1 km from shore) were conducted from February 1997 to September 2012 (excluding July 2002–August 2005, 2008 and 2010; Table 1), generally in the morning and early afternoon and in good weather conditions (Beaufort scale 2 or less, sea state 0 and visibility >300 m). Coastal surveys were conducted from 7m (1997–2000) and 10m powerboats (2001–2002, 2006–2007), and two 17m sailboats (2005–2006, 2009–2012), at an average speed of  $18\text{ km h}^{-1}$ . Boat speed was reduced in the presence of dolphins, and sudden speed or directional changes were avoided. Trained research assistants approximated the dolphins' position ( $\pm 30$  m from the boat) and speed with respect to the boat's position using GPS. Focal follows were conducted on all dolphin groups, each attempted for a minimum of 30 minutes and lasting up to 250 minutes. Prior to potential encounters between ORUs and coastal dolphins and throughout observation, the research vessel attempted to maintain a distance of 50m from ORUs and the dolphin focal group, paralleling the school and allowing the undisturbed recording of encounters (for full methodology: Bearzi 2003). Any boat disturbances, such as bowriding, were recorded (for definitions of boat disturbance: Bearzi 2003). The survey area was divided at the Marina del Rey harbor, and coastal surveys were conducted either to the north or south of the harbor depending on favorable weather conditions.

Data for coastal and offshore bottlenose dolphins were divided exclusively based on their distance from shore: all bottlenose dolphins observed during coastal surveys up to 1 km from shore were considered coastal; all bottlenose dolphins observed during surveys at >1km from shore were considered offshore. For this study, only data on coastal bottlenose dolphins were analyzed. Behavioral data collected opportunistically from July

Table 1. Summary of research effort for coastal surveys in Santa Monica Bay conducted from 1997 to 2012. No data were collected: July 2002–August 2005, 2008, and 2010. BD=bottlenose dolphins.

	1997	1998	1999	2000	2001	2002	2005	2006	2007	2009	2011	2012	Totals
N of surveys	16	55	39	33	27	9	3	14	8	3	6	7	220
N of sightings	7	58	32	16	18	6	6	21	16	5	13	11	209
Survey hours	123	214	155	119	121	60	16	23	41	11	29	25	937
Number of 5 min samples	68	722	345	212	273	38	68	147	135	19	182	72	2,281
Hours of BD observation	6	60	29	18	23	3	6	12	11	2	15	6	190

to December 1996 (58 hours of field observations) provided a framework of information to design the behavioral sampling procedures systematically adopted from January 1997 onward (Bearzi 2003). Data were collected with laptop computers and occasionally with tape recorders. Throughout all focal follows, the number of animals, behaviors of the dolphin group, and aggregation/associations with other marine mammal species were recorded at 5-minute intervals (Bearzi 2005). Behavioral data collected without ORUs present and before focal groups encountered ORUs were used as controls for the behavioral data in which ORUs were present. When more than one ORU was present in the study area, each ORU was recorded as one ORU. The number of dolphins was later verified through photo-identification and video analyses.

When coastal bottlenose dolphins were observed within 50m of ORUs, behavioral data continued to be recorded at 5-minute intervals to determine changes in school size, behavioral state, group formation, and surfacing mode as a result of their encounters with ORUs. Observed responses to potential disturbances to the bottlenose dolphins (i.e. the research vessel) and approximate distances between dolphin focal groups and ORUs were recorded. Data analyses were performed using R and Microsoft Excel 2011. A general linear model (GLM) was conducted in R and used to analyze which factors were most likely to be correlated with behavioral changes. All other data analyses on sighting length, number of dolphins involved in encounters, distances between dolphins and ORUs, rates of dolphins' behavioral changes were performed in Microsoft Excel 2011. Species distribution data were plotted with ArcGIS 10.2.1.

### Definitions

For the purposes of this study, the following definitions were used:

*Dolphin school*: dolphins in continuous association with each other and within visual range of the survey team (Weller 1991);

*Focal group*: any group of animals observed in association, moving in the same direction and usually engaged in the same activity (Shane 1990). Groups of animals not belonging to the observed focal group and spotted at distance were recorded, but their number was excluded from group size calculation;

*Behavioral state*: a broad category of activities, such as feeding behavior, which integrates several individual behavior patterns into a recognizable pattern (Weaver 1987; for additional definitions see Bearzi 2005);

*Encounter*: any instance in which at least one bottlenose dolphin was observed within 50 meters of any type and number of ORU;

*Association (A)*: an encounter between one or more dolphin and one or more of the four ORUs at a distance of 10-20 meters;

*Close Association (CA)*: an encounter between one or more dolphins and any ORU at a distance of 3 meters up to 10 meters;

*Potential Interaction (PI)*: an encounter between one or more dolphins and any ORU at a distance equal to or less than 3 meters;

*Interaction (I)*: observed physical contact between an ORU and one or more dolphins. Changes in behavioral states of the dolphin were defined as follows:

*Avoidance* – when one or more dolphins altered behavior to prevent a closer encounter with an ORU;

*Change in direction* – when one or more dolphins maintained the same speed but altered direction of approach to ORUs;

*Dive* - when one or more dolphins altered their behavior to display a dive longer than 15 seconds in the presence of ORUs;

*Aerial reaction* – when one or more dolphins displayed an aerial behavior (e.g., bow, leap) in the presence of ORUs;

*Vocal reaction* – when one or more dolphins displayed an audible response such as chuffing in the presence of ORUs;

*Stationary reaction* – when one or more dolphins displayed a motionless behavior on the surface for more than five seconds (e.g., floating, rafting) in the presence of ORUs;

*Percussive reaction* – when one or more dolphins hit the water with any portion of the body (e.g., breach, tail slap) in the presence of ORUs;

*Neutral reaction* – when one or more dolphins showed none of the above behavioral changes in the presence of ORUs.

## Results

Data were collected during 220 coastal surveys along the Santa Monica Bay coastline in the years 1997-2012, with an average of three surveys per month (Table 1). A total of 937 hours were spent searching for coastal bottlenose dolphin resulting in 209 sightings, 82.78% of which were conducted in good weather conditions (Beaufort scale 2 or less). A significantly higher number of surveys were carried out in the northern study area ( $t=3.24$ ,  $DF=26$ ,  $P<0.005$ ). Sightings lasted an average of 55.84 minutes ( $SD=37.74$ ,  $SE=2.61$ , range 5-250 minutes,  $n=209$ ).

During the study period, 145 encounters were recorded between 72 bottlenose dolphin schools and ORUs throughout the survey area (34.45%,  $n=209$  sightings; Fig. 1, Table 2). An average of nine dolphins were involved in each encounter ( $SD=4.66$ ,  $SE=0.03$ , range 2-19 individuals,  $n=145$  encounters). Few encounters lasted more than five minutes (4.55%,  $n=176$  encounters). It was common for a single bottlenose dolphin focal group to experience two or more encounters with an ORU during observation (40.28%,  $n=72$  schools; Table 2). Multiple ORUs were encountered by 16.67% of focal groups ( $n=72$ ), and surfers were the most common ORU encountered by focal groups (77.93%,  $n=145$  encounters; Table 2). Encounters occurred most commonly between ORUs and bottlenose dolphins within 3 to 10 meters (close associations; 40%,  $n=145$  encounters;  $\chi^2_3=1.41$ ,  $n=22$ ,  $p=0.02$ ; Fig. 2, Fig. 3). Physical contact (interaction) between an ORU and bottlenose dolphin occurred on only one occasion.

Bottlenose dolphins responded neutrally to 61.93% of encounters with ORUs ( $n=176$  behavioral responses, Fig. 4). Without ORUs present, behavioral changes occurred in 48.35% of 5-minute behavioral samples. When ORUs were present, however, behavioral changes occurred in 31.43% of 5-minute samples. This difference in the rates of change from one behavior to another was statistically significant ( $F_{1,20}=4.79$ ,  $p<0.05$ ),

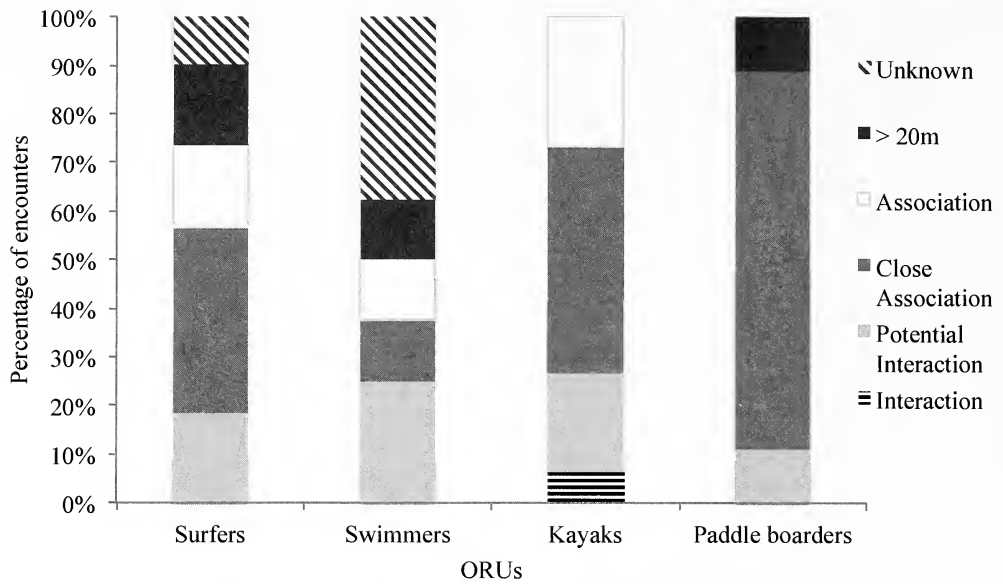


Fig. 2. Distances between different ORUs and bottlenose dolphin/s for each encounter.

suggesting that the presence of ORUs alters the rate of behavioral change in bottlenose dolphins. The most common behavioral changes observed by a focal group were either a change of surface mode (11.72%,  $n=176$  behavioral responses) or “other” reactions, which included activities such as “chin up” (3.84%), “tail up” (0.57%), mating (0.57%), circling (0.57%), splitting into subgroups (0.57%), or feeding (0.57%) (collectively: 6.25%,  $n=176$ ). The least common response to an encounter with an ORU was one or more dolphins displaying percussive or aerial behaviors (Fig. 4). Aerial reactions occurred solely as a result of encounters with surfers (2.14%,  $n=140$  responses; Fig. 4).

Focal groups responded to the presence of the research vessel by bowriding during 4.17% of the 5-minute samples in which an encounter occurred. If the dolphin group was bowriding in the 5-minute behavioral sample prior to the encounter, 75% of encounters resulted in a behavioral change. Focal groups did not avoid or approach the vessel in any 5-minute interval in which an encounter occurred. However, throughout focal follows of groups that encountered ORUs, 4.17% approached the vessel and 2.78% avoided the vessel ( $n=72$  schools). None of the focal groups that approached or avoided the vessel exhibited a behavioral reaction to an encounter with an ORU.

Table 2. Number of schools and encounters per ORU type and number of schools that experienced multiple ORU encounters.

	Surfers	Swimmers	Kayakers	Paddle boarders	Total
N of schools	48	7	11	6	72
Percentage of total schools	66.67%	9.72%	15.28%	8.33%	100%
N of encounters	113	8	15	9	145
Percentage of total encounters	77.93%	5.52%	10.34%	6.21%	100%
Schools with >1 encounter	24	1	2	2	29
Percentage of total schools	50.00%	14.29%	18.18%	33.33%	40.28%
Schools with >2 encounters	15	0	1	1	17
Percentage of total schools	31.25%	0%	9.09%	16.67%	23.61%



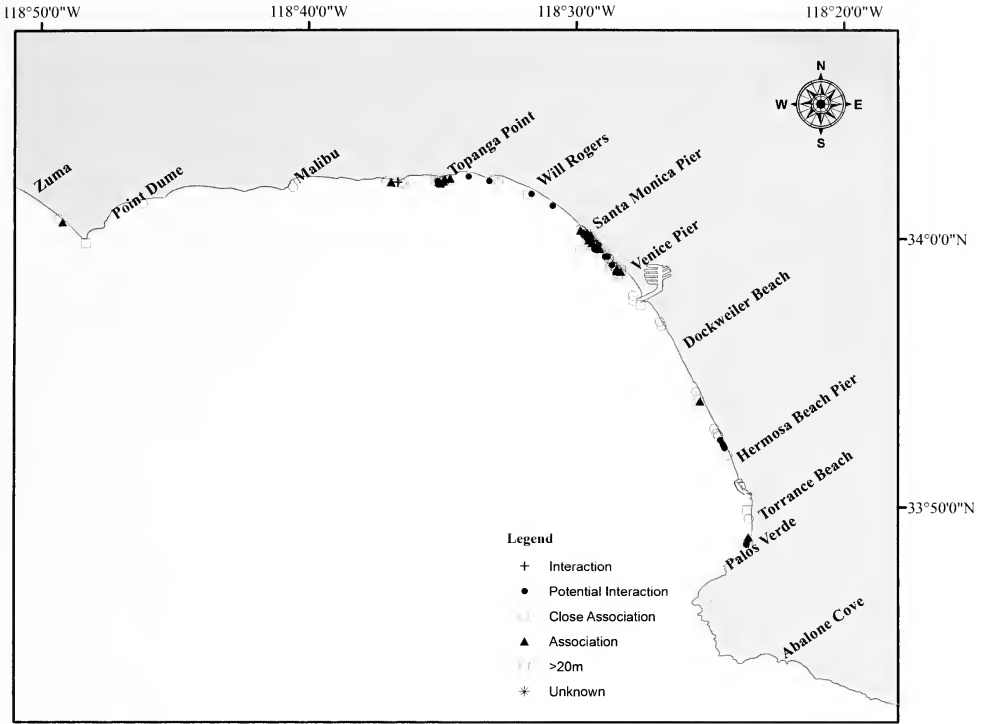


Fig. 3. Distance between ORUs and bottlenose dolphin/s at location of each encounter.

The results of a general linear model indicated that the group form of the focal dolphins during the 5-minute behavioral sample prior to the encounter might be a factor in determining whether a behavioral change would occur as a result of an encounter. Prior to encountering an ORU, dolphin groups that were at mixed distances ( $p < 0.05$ ,

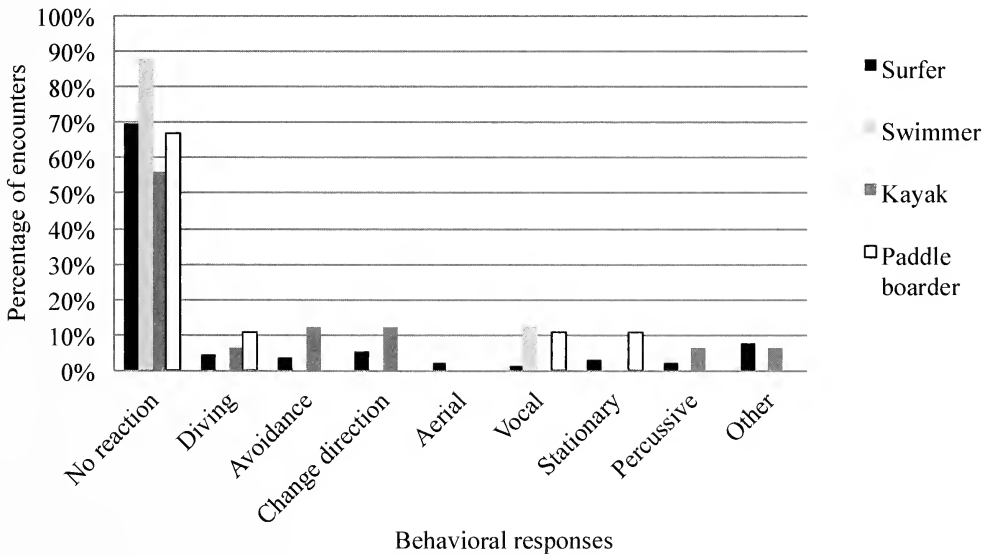


Fig. 4. Reactions (or lack of) to an encounter with one or more surfer, kayaker, stand-up paddle boarder, and/or swimmer during a 5-minute behavioral interval.

Table 3. Number of encounters and schools in which an ORU approached a dolphin or a dolphin approached an ORU.

ORU approach	Surfers	Swimmers	Kayakers	Paddle boarders	Total
Encounters	13	1	4	2	20
% of encounters	11.50%	12.50%	26.67%	22.22%	13.79%
Schools	7	1	1	2	11
% of schools	14.58%	14.29%	9.09%	22.22%	15.28%
Dolphin approach					
Encounters	5	0	1	0	6
% of encounters	4.42%	0%	6.67%	0%	4.14%
Schools	4	0	1	0	5
% of schools	8.33%	0%	9.09%	0%	6.94%

SE=0.153), widely dispersed with more than 50 meters between individuals ( $p < 0.05$ , SE=0.171), or in a tight form with less than one adult body length between individuals ( $p < 0.05$ , SE=0.462), were more likely to exhibit a behavioral change as a result of that encounter. Only one focal group involved in an encounter was described as being widely dispersed in the 5-minute behavioral interval prior to the encounter. No other behavioral data for this 5-minute interval were correlated with a behavioral change as a result of an encounter.

Bottlenose dolphins were approached by one or more ORU in 13.79% of all encounters, and dolphin focal groups approached ORUs in 6.94% of recorded encounters ( $n=145$  encounters; Table 3). When ORUs approached dolphins, behavioral changes occurred in 50% of encounters ( $n=20$ ), compared with 75% when dolphins approached an ORU ( $n=4$ ). When dolphins approached ORUs, all behavioral changes were changes in direction.

The distance between dolphins and an ORU during an encounter was an important factor in determining whether a behavioral change would occur as a result of the encounter (Fig. 5). This factor was more important than the type of ORU involved in the encounter (Fig. 5). Encounters classified as potential interactions were significantly more likely to lead to behavioral changes than encounters at distances greater than 3 meters ( $p < 0.001$ , SE=0.126). The type and number of ORUs present and whether a human or dolphin approached during the encounter were not significant when added to the model. Because the addition of these variables increased the AIC score of the GLM (177.76 to 187.15), they were excluded from the final version.

### Discussion

Surfers were the most common ORU encountered by dolphins in the study area. This result is likely due to the fact that Southern California has been a top US surf destination since the 1930's (Irwin 1973) and the sport continues to grow in popularity. On the contrary, swimmers were only occasionally involved in encounters with dolphins along this coastline. This may be explained by the presence of these ORUs close to the beach while coastal bottlenose dolphins tend to move slightly more offshore. In other areas where dolphins are found in extremely shallow waters, encounters with swimmers appear to be more likely, making these animals prone to being subjected to swim-with-the-dolphins programs and food-provisioned encounters. For instance, in Florida (Samuels and Bejder 2004; Cunningham-Smith et al. 2006), Tonga (Kessler et al. 2013), and New Zealand (Neumann and Orams 2006), dolphins are frequently exposed to swim-with

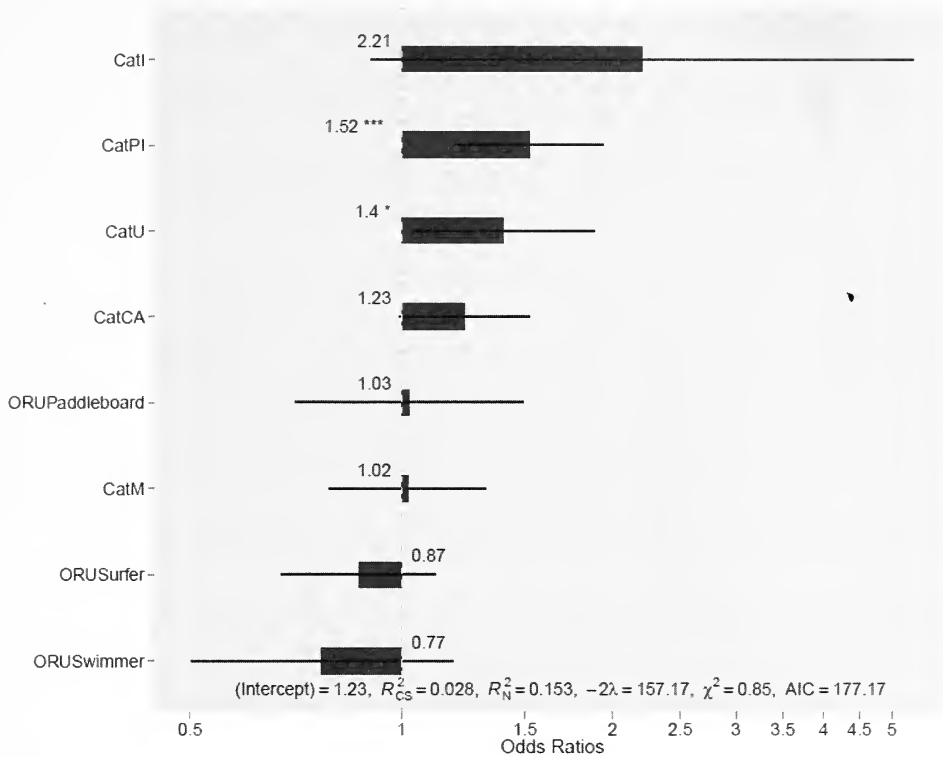


Fig. 5. Visualized results of a GLM depicting the effect of ORU type and distance from the focal group on dolphin behavioral responses. Cat I: Interactions, Cat PI: Potential Interactions, Cat U: Unknown Cat CA: Close Associations, ORU P: Paddle boarders, Cat M: More than 20 meters, ORU S: Surfers, ORU W: Swimmers.

dolphins programs and food-provisioned encounters with humans. In these situations, swimmers actively pursued dolphins. Based on this study, bottlenose dolphins in the Santa Monica Bay were neither discouraged (by frequent ORU encounters) nor encouraged (through food-provisioning) from interacting with ORUs. Because encounters in this region occurred by chance, there were likely fewer total encounters between dolphins and humans compared to those in incentivized or active pursuit settings like Australia and Brazil (Samuels et al. 2000).

Encounters between stand-up paddle boarders and bottlenose dolphins were recorded least often, and were observed mainly in the last few years of research. Stand-up paddle boarding was introduced in California in 2002, and by 2009 it was the fastest growing paddle-sport in North America<sup>2</sup>. This study shows that multiple encounters between ORUs and dolphins were common, but few encounters lasted more than five minutes. This could be attributed to several factors such as oceanographic conditions, specific behavioral patterns displayed by dolphins in this area (e.g., large amounts of time spent foraging; Bearzi 2005), and U.S. regulations such as the Marine Mammal Protection Act. As a comparison, in areas where swim-with-the-dolphin programs are allowed, this type of encounter typically was 35-60 minutes in duration (Constantine and Baker 1996; Samuels et al. 2003).

<sup>2</sup> Addison, Corran. 2010. The History of Stand Up Paddling. Editorial. *SUP World Mag* 2010.

Our results indicated that dolphins changed their behavior more often when no ORUs were present. The research vessel appeared to have a negligible effect on dolphin behavior. This suggests that the presence of ORUs may be altering dolphin behavior by preventing behavioral changes rather than increasing the amount of change. However, far more data were collected when no ORUs were present. The opportunistic nature of the study may have affected the number of ORU encounters observed. More targeted data collection on dolphin behavior in the presence of ORUs is needed to further elucidate this phenomenon.

In the Santa Monica Bay, only 20% of the dolphins approached by ORUs changed their direction of travel, compared to 40% in a New Zealand swim-with program (Constantine and Baker 1996). In several cases, dolphins that were highly habituated to ORUs and actively sought out human interaction displayed high rates of aggression toward ORUs (Samuels and Bedjer 2004, Scheer 2010) or have sustained an anthropogenic injury (Samuels and Bedjer 1998). On one occasion, aggressive behavior by a dolphin resulted in a human death (Santos 1997). Our study did not reveal any instances of bottlenose dolphin aggression toward ORUs or vice versa, but as dolphins become increasingly habituated to ORU presence, aggression may become a concern.

As expected, our preliminary results indicated that the proximity of ORUs to dolphins during encounters was the best predictor of whether a behavioral reaction would be elicited from the dolphin. If one or more dolphin and an ORU came within three meters of one another during an encounter, a behavioral change was likely to occur. Increased dolphin behavioral changes as a result of close encounters with ORUs are consistent with Bedjer et al. (1999) findings, which determined that the distance between an ORU and dolphins during an encounter was the most reliable predictor of a change in dolphin behavior. Williams (2011) also found that orcas (*Orcinus orca*) were more likely to exhibit avoidance behaviors when approached by kayaks at close range. Kayakers may be of particular interest for looking at these types of interactions, as they can elicit the same response from a dolphin school as a powerboat (Lusseau 2003), and have been found to associate with dolphins more often than motorized vessels in the same area (Nichols et al. 2001).

### Conclusions

This preliminary study shows that coastal bottlenose dolphins in the Santa Monica Bay are not subjected to prolonged encounters with ORUs, and these dolphins appear to be generally “habituated”<sup>3</sup> to ORU presence. The apparent reduction in behavioral changes in response to ORUs, as well as the high occurrence of “no reactions,” are in accordance with Filby et al. (2014) findings that habituated dolphins display reduced avoidance behaviors.

Coastal bottlenose dolphins are now well recognized as a sentinel species<sup>4</sup> and key indicators of coastal habitat health (Simberloff 1998; Wells et al. 2004; Bossart 2011; Reif 2011). Although the current impact of ORU activities on bottlenose dolphin behavior does not appear to be significant in Santa Monica Bay, there is a need to adopt a precautionary approach in view of: a) the increasing presence of ORUs along this

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<sup>3</sup>Thorpe (1963) defines habituation as “the relative persistent waning of a response as a result of repeated stimulation, which is not followed by any kind of reinforcement.”

<sup>4</sup>Barometers for current or potential negative impacts on individual-and-population-level animal health (Bossart 2011)



coastline, and b) studies in other regions showing the adverse effects of human recreational activities on coastal bottlenose dolphins.

Dolphin responses to increased human presence can have lasting population effects. For instance, habituation due to increased human presence may have intensified the probability of boat strike mortality in Hector's dolphins (Stone and Yoshinaga 2000). In New Zealand, the Hector's dolphin population decreased due to a rise in dolphin ecotourism (Bejder et al. 2006), and dolphins abandoned previously favored habitat (Bedjer 1997) as a result of encounters with humans. Martinez et al. (2011) suggested that encounters that seem positive (i.e. dolphins approaching swimmers) can still cause a reduction in crucial behavior such as feeding. Additionally, it has been demonstrated that dolphin presence can cause a significant increase in ORUs, thereby increasing the disturbance (Östman-Lind 2009). Kayakers in Hawaii changed their behavior when dolphins were present in an attempt to get closer to the dolphin school (Timmel et al. 2008). Considering the growing popularity of recreational activities along the Santa Monica Bay coastline, there could be a risk of a similar response in this area. Efforts should be directed to ensure that ORUs are aware of marine mammal observation guidelines, such as the requirement to maintain a minimum distance of 50 meters during an encounter with a dolphin.

Educational programs conducted in marine protected areas to inform the public of the importance of marine mammals have been shown to aid in the enforcement of the parameters of the Marine Mammal Protection Act and decrease disturbances to marine mammals (Gunvalson 2011). Similar educational programs designed to explain marine mammal observation guidelines to ORUs along the Santa Monica Bay coastline could further minimize the effects of ORU presence on bottlenose dolphins.

In conclusion, this preliminary investigation suggests the need of regular monitoring of coastal bottlenose dolphins and encounters with ORUs to determine potential changes in these animals' behavior. Also, it suggests the necessity of implementing public education program and management measures to ensure that dolphins remain undisturbed by the growing number and diversity of anthropogenic presence in the bay.

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## Salt Marsh Reduces Fecal Indicator Bacteria Input to Coastal Waters in Southern California

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*Abstract.*—We investigated fecal indicator bacteria (FIB) concentrations in water and sediment from Carpinteria Salt Marsh, a medium-sized (93 ha), mostly natural southern California coastal wetland. High FIB concentrations, exceeding recreational water quality standards, were found at inlet sites after winter storm events and during a summer dry weather sampling event. Runoff entering the wetland had the highest concentrations of FIB after large rain events and after rain events following extended periods without rain. The watersheds with the greatest agricultural and urban development draining into the wetland generally contributed the highest loads of FIB, while the largest and least developed watershed contributed the lowest FIB concentrations. Surface water exiting the wetland at the ocean contained relatively low concentrations of FIB and only exceeded recreational water quality standards after the largest rain event of the year. Bacterial concentrations in sediment were only elevated after rain events, suggesting wetland sediment was not a reservoir for bacteria. Our results provide evidence that moderate-sized tidal wetlands at the base of moderately urbanized watersheds can attenuate FIB, improving coastal water quality.

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Runoff from coastal watersheds carries bacteria to the ocean, causing human health risks. Fecal indicator bacteria (FIB), including *Enterococcus* (ENT) and *Escherichia coli* (EC), are natural components of human and other mammal, reptile and bird intestinal fauna used to indicate the likely presence of human pathogens that cause unhealthy conditions for people recreating in coastal water (Balarajan et al. 1991; Haile et al. 1999). Sources of FIB include faulty or overflowing sewage systems, homeless populations and domestic and wild animals (including birds) (Mallin et al. 2001; Crowther et al. 2002). FIB are generally present in high concentrations in sewage and in urban and agricultural runoff during wet weather conditions (Wyer et al. 1994, 1996, 1998; Kay et al. 2005). They may be concentrated on fine (<6 $\mu$ m) particles (Brown et al 2013), and can come from streambed sediments (Wilkinson et al. 1995; Solo-Gabriele et al. 2000), intertidal sediments (Obiri-Danso and Jones 2000; Ferguson et al. 2005) and watershed stores that are flushed by rainfall (Sanders et al. 2005).

For example, stormwater runoff from the Santa Ana River in California was identified as a significant source of near-shore pollution, carrying sediment, FIB, fecal indicator viruses, and human pathogenic viruses (Jeng et al. 2005). FIB concentrations are used as a guide to determine when Southern California beaches should be closed to recreational

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activities. In one of the only epidemiological studies of coastal ocean bathing water, Haile et al. (1999) determined that thresholds of 10,000 cfu of total coliform (TC), 104 cfu of *E. coli* (EC) and 400 cfu of enterococcus (ENT) per 100 ml of sample had potentially harmful human health effects at southern California beaches. These values are incorporated in California Department of Health Services regulations, which furthermore state that if the TC/EC ratio is  $<10$ , then TC must be  $\leq 1000$  cfu/100ml.

Coastal tidal wetlands could mitigate the risk of bacterial contamination. It is well known that freshwater wetlands perform water treatment functions (Kay and McDonald 1980; Breen et al. 1994; Kadlec and Knight 1996; Davies and Bavor 2000). Constructed freshwater wetlands may remove over 85% of FIB (Kadlec and Knight 1996; Davies and Bavor 2000). While tidal wetlands may perform similar functions, few studies have addressed this topic. Dorsey et al. (2010) found bacterial loads were significantly reduced in a southern California coastal wetland during daylight hours. A tidal wetland behind a flood defense wall reduced flux and concentration of fecal indicator bacteria (FIB) in coastal waters by 97% (Kay et al. 2005). An analysis of 32 years of coliform data for Newport Bay wetland and tidal embayment in southern California revealed a gradient of reduced bacterial concentration between inland sites and the ocean (Pednekar et al. 2005). The highly urbanized Talbert Salt Marsh watershed had a gradient of high to low FIB during dry weather run off, with highest concentrations in the upstream watershed and lowest at adjacent coastal waters (Reeves et al. 2004).

Coastal wetlands are a potential source of FIB since animals, such as birds, attracted by the wetland produce FIB-laden feces. Bacteria either from within wetland or outside sources may settle in slow-moving wetland waters where they can accumulate in sediment and possibly re-grow (Solo-Gabriele et al. 2000; Desmarais et al. 2002; Ferguson et al. 2005). Bacteria harbored in the sediment may be tidally flushed out to coastal bathing waters (Sanders et al. 2005). High concentrations of FIB were observed in California coastal wetlands when sediments were resuspended during strong ebb flows (Dorsey et al., 2010; Dorsey et al., 2013). At Talbert Salt Marsh, a small (10 ha), restored southern California tidal wetland, outflow from the wetland increased bacterial concentration in coastal waters (Grant et al. 2001). The reduced size of this wetland, less than  $1/100^{\text{th}}$  its original 1200 ha, and restored condition likely affected its ability to attenuate bacterial populations. This study and others pointed to bird populations as a potentially important bacteria source (Abulreesh et al. 2004). However, a modeling study of the same wetland by Sanders et al. (2005) indicated that bird feces were a minor contributor to surface water contamination, although they suggested that feces contributed to sediment FIB loads and tidal flushing deposited bacteria in coastal waters.

Water entering the ocean from coastal wetlands is most likely to cause poor ocean water quality after large rain events, when runoff flowing into the wetland has high volume and FIB concentrations, or when water has been stored for long periods in the wetland without tidal flushing (Reeves et al. 2004; Gersberg et al. 1995). For example, immediately following the breaching of San Elijo Lagoon in San Diego County, water quality close to the wetland mouth at an adjacent marine bathing beach was unhealthy (Gersberg et al. 1995); the authors predicted healthy bathing conditions would return to coastal waters within two weeks after breaching and one week after any large rain events. Jeong et al. (2008) found that as the volume of runoff entering Talbert Salt Marsh declined, the wetland was better able to attenuate FIB loads and coastal water quality improved.

Table 1. Land uses, areas and elevations of subwatersheds that drain into Carpinteria Salt Marsh. Table adapted from Page and Court (unpublished data).

Subwatershed	Drainage area	Maximum elevation	Greenhouse		Orchard	
	km <sup>2</sup>	m	ha	%	ha	%
Western Creek	3.41	1175	36.7	10.8	91	26.7
Franklin Creek	11.60	533	63.3	5.4	68.8	5.9
Santa Monica Creek	15.61	1192	6.1	0.4	8.1	0.5

The capacity of a wetland to remove FIB is affected by a variety of physical and ecological factors including: wetland size, sediment size, tidal flow, bird and other animal populations, vegetation type, size and abundance and tidal creek length and shape. Larger, more pristine wetlands, with longer tidal creeks for runoff to travel through and longer residence time of bacteria and sediment-attached bacteria, likely are better at reducing FIB loads to the coastal ocean. Carpinteria Salt Marsh (CSM) was selected as the study location because it is a moderate-sized, mostly natural southern California wetland. To determine if CSM acted to attenuate or exacerbate FIB loads to coastal waters, we evaluated FIB concentrations at all the inlet sites where watershed runoff entered the wetland and at the wetland-ocean interface where watershed runoff flowed to the ocean after passing through the wetland. Our purpose was to investigate whether this wetland protected coastal water quality.

## Materials and Methods

### Study Area

CSM is a 93 ha (230 acre) wetland of pickleweed habitat [*Sarcocornia pacifica* (= *Salicornia virginica*)]. Located at 34°24'N and 119°31'30"W in Santa Barbara County, California, it is influenced by a Mediterranean climate with heavy, intermittent rainfall in the winter and dry, usually rainless summer months. Nearly 90% of average annual rainfall occurs between November and April, carrying materials stored during the summer from the watershed into the wetland<sup>1</sup>. The bird population, estimated by monthly two-hour bird counts at high and low tides in 2003, is estimated to be between 150 (June) and 1000 (October) including all bird species (shorebirds, water fowl etc.)<sup>2</sup>

The watershed of CSM is composed of three subwatersheds that are drained by Franklin and Santa Monica creeks and a western coastal plain area (Table 1; Fig. 1). Land use cover within sub-watersheds was delineated by Page and Court<sup>3</sup> using a Geographic Information System (GIS) and a USGS 30 m digital elevation model (DEM). By combining the GIS with a 1999 aerial photograph of the study area, they divided land use within each sub-watershed into five categories, 1) greenhouse agriculture, 2) open-field agriculture, 3) orchard, 4) urban/residential and 5) undeveloped (Table 1).

Franklin and Santa Monica Creeks originate in the Los Padres National Forest, a mountainous area whose foothill communities are composed of chaparral vegetation,

<sup>1</sup>Ferren, W.R., Page, H.M. and Saley, P. 1997. Carpinteria Salt Marsh: Management Plan for a Southern California Estuary, Environmental Report No. 5, Museum of Systematics and Ecology, Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara.

<sup>2</sup>Brooks, A.J. 2003. Unpublished data. Marine Science Institute University of California, Santa Barbara, CA 93106-6150

<sup>3</sup>Page, H.M. and Court, D. 1997. Unpublished data. Marine Science Institute University of California, Santa Barbara, CA 93106-6150

Table 1. Extended.

Open field		Total agriculture		Urban		Undeveloped		Total
ha	%	ha	%	ha	%	ha	%	ha
26.3	7.7	154	45.1	50.9	14.9	136.4	40	341
45.6	3.9	177.4	15.3	270.7	23.3	714.9	61.5	1163
5.5	0.4	19.7	1.3	32.5	2.1	1509	96.7	1561

with several kilometers of downstream coastal plain that are covered by a mixture of urban and agricultural development, including greenhouses and fields for commercial flower production and lemon and avocado orchards. The Franklin Creek sub-watershed (1107 ha) is the furthest east and has the lowest elevation, lying partially in the foothills but primarily within the coastal plain, where a large portion of the land is developed with multi-use agriculture, residential areas and light commercial facilities<sup>4</sup>. The Franklin Creek watershed is the most developed of the three subwatersheds, with 271 ha of urban development and 177 ha of agricultural land. Most of Franklin Creek (75%) is concrete lined with a concrete bottom (Robinson et al. 2002). Franklin Creek provides water to a restored section of CSM. Both Franklin and Santa Monica creeks have been dredged by County Flood Control, creating wide, deep, straight channels through the wetlands. The Santa Monica Creek sub-watershed (1561 ha) is the largest and least-developed sub-watershed, with over 90% composed of undeveloped land in the foothills and southern slopes of the Santa Ynez Mountains. The portion of Santa Monica Creek flowing from the northern edge of the city of Carpinteria into the salt marsh is channelized and concrete lined with a concrete bottom.

The Western creeks drain a much smaller area (340 ha) that lies entirely within the coastal plain. The Western subwatershed is nearly 50% agricultural and 15% urbanized (Robinson et al. 2002). The creek water is entirely from coastal plain runoff, flowing through a riparian corridor before entering the western side of CSM at three locations. Two of these creeks (Creeks B1 and B2) flow together, but upon intersecting with the railroad track located just outside the wetland border, Creek B1 diverges and flows easterly until it enters the salt marsh at a separate location. The most northwestern creek (Creek A) primarily drains greenhouse runoff. Degradation of the salt marsh due to anthropogenic pollutants entering from urban and agricultural runoff has been documented since the 1970s<sup>5</sup> (Page et al. 1995; Hwang et al. 2006).

### *Field Sampling*

To investigate the change in FIB concentrations as water moves through CSM, we sampled water and sediment at the main water inlet and outlet sites to the wetland. Inlet sites included one site each from Franklin Creek and Santa Monica Creek and three sites from the Western subwatershed (Fig. 1). The mouth was sampled approximately 100 m upstream from the wetland/ocean interface.

<sup>4</sup>Ferren, W.R. 1985. Carpinteria Salt Marsh: Environment, History, and Botanical Resources of a Southern California Estuary, Santa Barbara, CA: Herbarium, Dept. of Biological Sciences, University of California, Santa Barbara.

<sup>5</sup>MacDonald, K. 1976. The natural resources of Carpinteria Marsh. Their status and future. Report to the California Department of Fish and Game. Coastal Wetland Series #13.





Fig. 1. Carpinteria Salt Marsh with inlet sites where creeks drain into the marsh and the mouth site identified. (Image from Google Earth.)

Tidal cycle may affect ENT loads, with highest populations occurring during spring ebb tides (Boehm and Weisberg 2005), so collection of all samples was initiated within an hour after the tide had changed from in-coming to out-going. Samples were always collected during daylight but at different times of day to accommodate the tide. Thus, different samples may have been exposed to UV radiation for different lengths of time on different sampling dates. This would have had little influence on differences among sites for a sampling date since all samples were collected within a few hours of each other, but could potentially lead to differences between dates. However, UV exposure did not appear to have an overriding influence on results since high FIB concentrations exceeding health standards occurred in samples taken in the afternoon after extended UV exposure. There were also differences in cloud cover, stream flow, and other environmental variables that could have led to variability in FIB concentrations.

One surface water sample was collected at each site except on February 26, 2004 and March 3, 2004 when five water samples were collected, and July 8, 2004 when three water samples were taken in Franklin Creek (site F). No water sample was collected from Franklin Creek in December. Samples were placed in sterile 50 ml Falcon tubes and maintained on ice in a dark container immediately after collection until they were processed within 6-8 hours. Water column salinity was measured at each site using a YSI 85 meter.

Samples were collected three times during dry weather between Nov 30, 2003 and Dec 10, 2003 and during dry weather on July 8, 2004. Samples also were taken seven times during the winter rainy season in 2003/2004. Samples were taken immediately following significant rain events of 0.5" or greater on Feb 3 (0.85"), Feb 19 (0.5"), Feb 26 (2.8") and Mar 3 (0.5") in 2004; on Dec 16, 2003, one day following a small 0.12" rain event that occurred after a month without rain; and on Jan 16 and 17, 2004 during the wet season but not following a significant rain event. Precipitation measurements were taken from the Carpinteria Fire Station (34°23'53" N, 119°31'06" W) (Santa Barbara County Flood Control District 2004; <http://www.countyofsb.org/pwd/water/hydro.htm>).



Table 2. Salinity during water and sediment sampling. Dashes indicate no salinity reading was taken. **Bold text** indicates 5 sediment samples taken, normal text indicates 3 sediment samples were taken, and sites with grey text boxes were not sampled for sediment. Asterisk indicates that lab tests failed on Dec 4 for Western Creek B2 although salinity was measured.

Station	Dry weather				Wet weather						
	Nov 30	Dec 4	Dec 10	Jul 8	Dec 16	Jan 16	Jan 17	Feb 3	Feb 19	Feb 26	Mar 3
Mouth	<b>36</b>	35	36	-	36	35	35	34	34	6	32
Franklin Creek	<b>35</b>	36	33	-	35	-	-	35	32	2	2
Santa Monica Creek	-	32	32	-	37	-	-	19	4	2	2
Western Creek B2	-	25*	23	-	7	-	-	4	8	3	2
Western Creek B1	<b>29</b>	25	11	-	5	-	6	5	3	2	5
Western Creek A	<b>30</b>	15	21	-	14	11	-	10	5	3	7

Sediment samples of at least 5 g of material were scraped from the top 1-3 cm of the tidal creek substrate closest to the water's edge during an outgoing low tide. In an unpublished experiment, we found no difference in sediment bacterial concentrations at different lateral locations on the tidal creek bank. The samples from each location were stored in individual plastic bags. In general, three sediment samples one meter apart were collected at each site, although this varied somewhat with five sediment samples taken at most sites on Nov 30, 2003 and no samples on Feb 26 and Mar 3, 2004 (see Table 2).

### Sample Analysis

Each sediment sample was homogenized and a 5 g sample was suspended in 35 ml of phosphate buffer solution (0.3mM  $\text{KH}_2\text{PO}_4$ , 2mM  $\text{MgCl}_2$ ) based on Standard Method 9221 A-3 (Greenberg et al. 1992). Samples were shaken by hand for one minute and then centrifuged at 4°C for five minutes at 1000 rpm (Evanson and Ambrose 2006). Three sediment samples and one water sample were processed per site. Using standard procedures for Idexx Colilert®-18 and Enterolert® 97-well Quanti-trays, ten ml of each sample of sediment supernatant and water were added to 90ml of dilution water and analyzed for TC, EC and ENT. The highest value of FIB that could be measured was 2500 MPN/100 ml since sample dilutions were not made; bacteria levels exceeding this maximum detection limit were not quantified.

### Results

The Santa Monica Creek subwatershed, which was the largest (15.6 km<sup>2</sup>) but least developed (97% undeveloped) catchment draining into Carpinteria Salt Marsh (Table 1), generally had the cleanest water and sediment (Fig. 2 and 3). Santa Monica Creek water EC levels were below health standards at all times and TC was relatively low. The subwatersheds with high amounts of urbanization and agricultural development (Table 1), Franklin Creek and the Western subwatersheds, had runoff with higher FIB concentrations. Franklin Creek subwatershed was the most developed and had the highest FIB in runoff entering the wetland. High levels of TC and ENT were present in Franklin Creek water during wet and dry weather, with ENT exceeding health standards during each sampling event except on December 10 and 16, 2003. The Western subwatershed also had high FIB levels with water exceeding ENT health standards on at least one occasion at each site during both wet and dry weather.

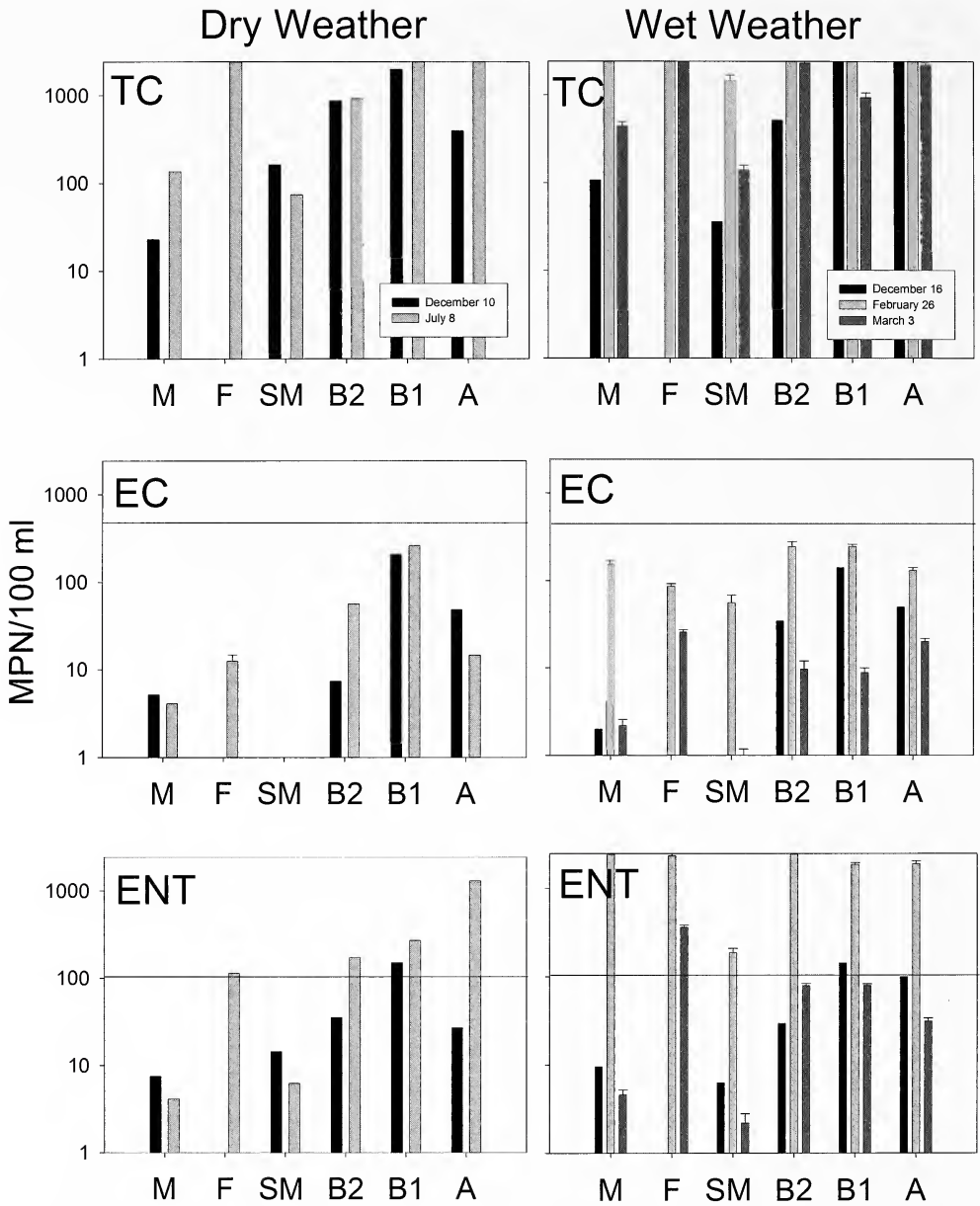


Fig. 2. Concentrations of total coliform (TC), *E. coli* (EC) and *Enterococcus* (ENT) bacteria in Carpinteria Salt Marsh water samples. Five inlet sites [Western Creek A (A), Western Creek B (B1 and B2), Franklin Creek (F), Santa Monica Creek (SM)] and one mouth (M) site were sampled during two dry weather (Dec 10 and Jul 8) and three wet weather (Dec 16, Feb 26 and March 3) sampling events. No water sample was collected from Franklin Creek in Dec. Error bars indicate MPN confidence interval based on SE of five method replicates for Feb 26 and Mar 3, three for Jul 8. Horizontal lines indicate the single-sample water quality standards for EC and ENT; the single-sample standard for TC (10,000 cfu) is above the maximum detection limit (2,500 MPN/100 ml) for the samples.

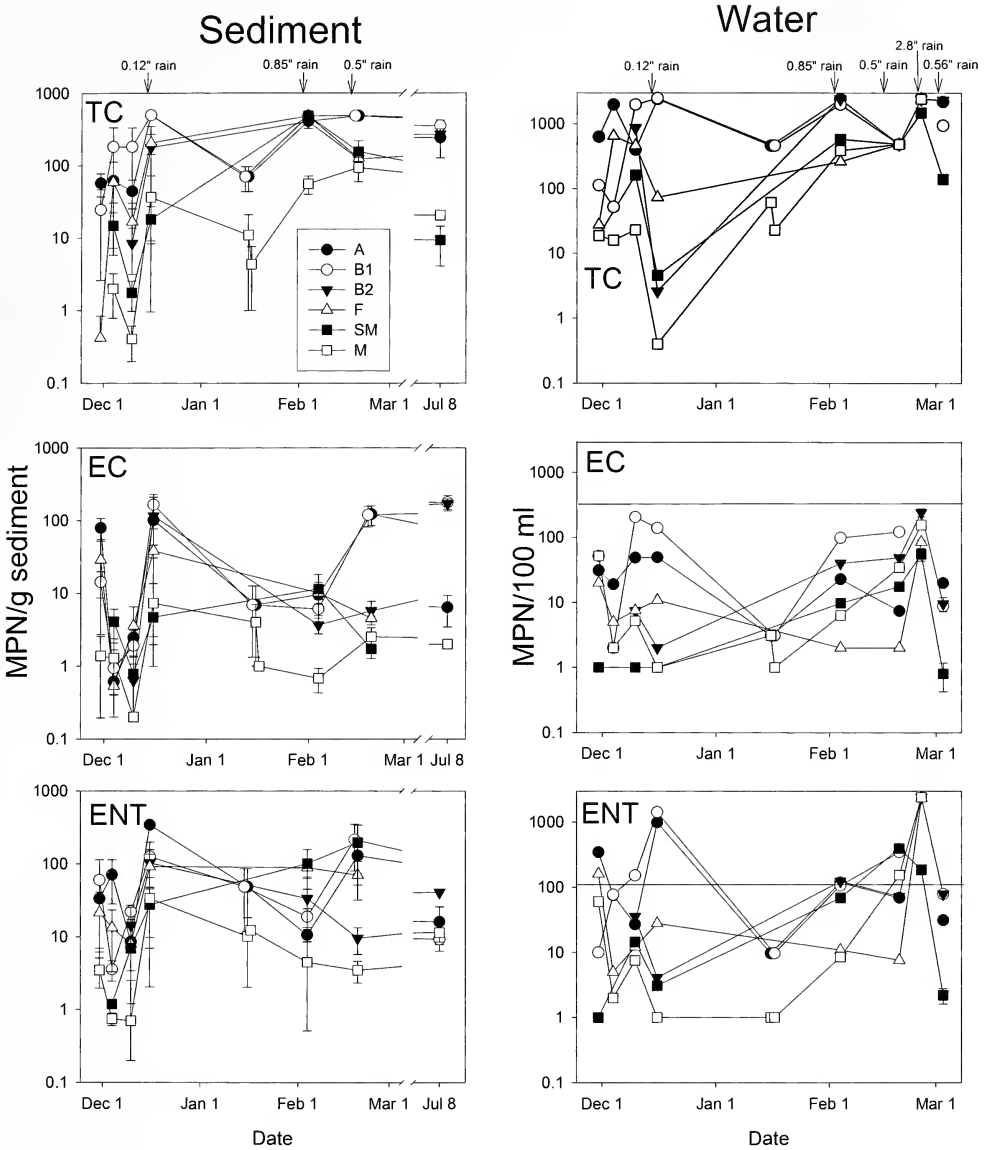


Fig. 3. Concentrations of total coliform (TC), *E. coli* (EC) and *Enterococcus* (ENT) bacteria in sediment samples taken between November 30, 2003 and July 8, 2004 and in water samples taken between November 30, 2003 and March 3, 2004. Five inlet sites [Creek A (A), Creek B (B1 and B2), Franklin Creek (F), Santa Monica Creek (SM)] and one mouth (M) site were sampled. For sediment samples, error bars are based on SE of three replicate samples; where error bars do not show, the error bar is smaller than the symbol except for B2 for July 8, when only one replicate analysis was successful. Arrows indicate rainfall events, with amount of rain noted. Horizontal lines indicate the single-sample water quality standards for EC and ENT; the single-sample standard for TC (10,000 cfu) is above the maximum detection limit for the samples (2,500 MPN/100 ml). Standards have not been established for sediment.

The largest winter rain event [7.1 cm (2.8") on February 26, 2004] produced the highest ENT and TC values at all sites (Fig. 2). The ENT health standard was exceeded at all sites. This was the only occasion when site B2, which generally had the lowest bacteria concentrations in the Western subwatershed, had similar or higher FIB levels than A and

B1. Only after this largest rain event did Santa Monica Creek water entering the wetland exceed ENT standards. It also was the only occasion when water draining from the wetland mouth into the ocean exceeded health standards and had concentrations of TC over 2500 MPN/100 ml (Fig. 2).

Generally, FIB levels were low during dry weather and EC levels were low, not exceeding health standards, during both wet and dry weather (Fig. 3). On December 10, 2003, both creek flow rates and FIB levels were low. Health standards were not exceeded and TC was low compared to wet weather values. However, on July 8, 2004 after several months without rain, FIB values were relatively high. ENT water quality standards were exceeded at all Western and Franklin Creek sites and TC values were over 2500 MPN/100 ml for Creeks A and B1 and Franklin Creek (Fig. 2). During this time creek flow rates were also high, indicating an upstream water source other than rainwater, likely from agricultural irrigation of greenhouses and/or field crops.

FIB loads in sediment were affected by rain events, although the pattern of increased FIB concentrations following storms was not as pronounced as in water samples, possibly due to generally low bacteria concentrations, particularly for EC and ENT (Fig. 3). At site B1 sediment mean values of EC varied between 1 and 166 MPN/g and ENT values varied between 4 and 215 MPN/g, while in water mean EC and ENT values at the same sites were as high as 1396 and 1848 MPN/g, respectively. Elevated TC was detected in both water and sediment after rain events (Fig. 3). While EC and ENT in sediment were generally low, relatively high values of ENT occurred at Western creek sites after the Dec 15 rain event, when values in water were also high. (Sediment data were not available following the largest winter rain event on February 26, 2004). Santa Monica Creek sediment also had a relatively high ENT value during dry weather (Fig. 3).

At the wetland mouth, where water entered the ocean, FIB concentrations were usually low, not exceeding recreational water quality standards (Fig. 2). FIB concentrations were only elevated at the mouth following the largest winter rain event (on February 26, 2004), when TC and ENT were over 2500 MPN/100 ml, vastly exceeding ENT health standards.

Salinity varied widely by sampling location and time (Table 2). The mouth site had near-seawater salinity during all sampling times except February 26, 2004, which was after the largest rain event. Franklin and Santa Monica Creeks also were usually close to seawater salinity, but salinities were reduced after rainfall. The Western Creek sites had lower salinities, even during dry weather, indicating their influence by persistent freshwater inflow not related to storms.

## Discussion

### *Watershed Input to Wetland*

FIB concentrations entering CSM were related to the amount of watershed urbanization rather than watershed size. The largest, least-developed watershed draining into the marsh had water with low FIB, while the smaller, more highly developed watersheds produced much higher FIB concentrations. Watershed land use has been correlated with FIB concentrations in coastal waters around the United Kingdom (Crowther et al. 2002; Kay et al. 2005). Urbanization was the primary predictor of EC concentrations in popular bathing beaches around Clacton, UK as well as for EC and ENT concentrations in surface waters of the 1583 km<sup>2</sup> Ribble drainage basin (Kay et al. 2005).

In CSM, high FIB values occurred after rain events, as has been found in other southern California wetlands. For example, TC in Santa Monica Bay and the Santa Ana river wetlands peaked on the same day as the rain event and decreased within one day



(Haile et al. 1999; Evanson and Ambrose 2006) and ENT and EC in the Santa Ana river wetlands peaked on the day of the storm or within several days (Evanson and Ambrose 2006). Overall the highest FIB values occurred following the largest rain event of the year on February 26, 2004 (2.8") and after the December 15, 2003 rain event that followed over a month without precipitation, the longest dry period preceding a rain event during this study. The 7.1 cm (2.8") rain event likely was large enough to saturate soil and produce field runoff as well as high volumes of impervious surface runoff. Although the December 15 rain event was small 0.3 cm (0.12"), it likely flushed bacteria that had accumulated over a long duration (relative to the dry period duration preceding other storms sampled).

Western Creek flow rates in July, while not quantified, appeared similar to those that occurred the day after rain events rather than the typical dry weather flow, likely due to agricultural and greenhouse irrigation runoff from facilities as close as a kilometer upstream of the wetland (Page et al. 1995). Some July dry weather values of TC, EC and ENT in water were similar to or higher than bacterial concentrations from creek water sampled directly following rain events.

### *Bacteria Removal*

While surface waters entering CSM often had high FIB concentrations (during both wet and dry weather), they generally exited the wetland with low FIB values. Although the number of samples taken during this study was relatively low, sediment and water samples were collected simultaneously at five inlets sites and the wetland mouth, providing a synoptic view of FIB inputs and output over a season that included both wet and dry weather sampling. The lower FIB concentrations at the wetland mouth compared to water entering the wetland suggest that bacteria populations decreased as a result of flowing through the wetland.

Bacteria removal from CSM waters likely was the result of processes such as predation, destruction by ultraviolet light, and sedimentation (i.e. adsorbing to particles that then settle to the bottom) (Alkan et al. 1995; Noble et al., 2004; Dorsey et al., 2010; Dorsey et al., 2013). While an estimated 65-85% of the total fecal coliform, EC and ENT remain free-floating in the water column and do not settle (Jeng et al. 2005; Schillinger and Gannon 1985), the low flow rate within CSM tidal channels allows for sedimentation and increased exposure of FIB to harmful solar radiation, thereby reducing FIB concentrations in a similar manner to a reservoir system or a constructed wetland (Kay and McDonald 1980; Kay et al. 1999). As with freshwater wetlands, UV was probably important for FIB destruction since sunshine is abundant year-round in southern California and CSM tidal creeks are shallow, allowing high UV exposure. FIB concentrations also may have been reduced due to dilution by tidal water, although this factor was minimized by sampling during an outgoing tide.

FIB loads at site B2 in the Western subwatershed were generally lower than at sites A and B1, possibly because this water travelled approximately 100 yards along the wetland fringe before entering the wetland. This area beside the railroad track, while not wetland habitat, was a dirt ditch lined with plants. The extra amount of both travel time and exposure likely contributed to FIB removal.

Within-wetland sources, such as bacterial growth in the sediment or feces from bird populations, did not appear to significantly contribute to surface water FIB loads. Storm flow re-suspension during winter could have contributed to increased bacterial populations in the water (Steets and Holden 2003), but sediment FIB were generally

low and unlikely a large contributor during our study. Low values of bacteria in the sediment indicated FIB were not stored there nor did they re-grow to high concentrations in wetland sediment. Grant et al. (2001) suggested that Talbert Marsh, a small (10 ha) southern California wetland with a similar bird population (1180 individuals) to CSM (a population maximum of 171-2200 individuals<sup>6</sup>), exacerbated FIB concentrations in coastal runoff, pointing to bird populations as an important within-wetland FIB source. A subsequent model of FIB loads to Talbert Marsh, which included urban runoff, erosion of contaminated sediments, bird feces, and combinations of these factors, indicated that direct runoff of bird feces was not likely to be a major source in this small wetland (Sanders et al. 2005). The low FIB concentrations at the mouth (wetland-ocean interface) of CSM suggest that birds in CSM were not significantly increasing FIB loads entering the ocean, despite the frequent concentration of birds near the wetland mouth (personal observations).

Besides removal of bacteria, lower FIB concentrations could be due to dilution by seawater. Salinity varied widely during sampling (range 2-37). Some dilution undoubtedly occurred at times because salinity was over 30 at many stations during at least some sampling times, particularly during dry weather and at the mouth. We minimized dilution effects by sampling on the falling tide. Nonetheless, reductions in FIB concentrations would have been due to a combination of dilution and removal processes.

The capacity of a wetland to remove contaminants is related to the volume of water flowing through the wetland and wetland size. Not surprisingly CSM, at 93 ha at the base of a 3,000 ha watershed, of which 350 ha was urbanized, was better able to attenuate FIB than Talbert Marsh, a small 10 ha marsh located at the bottom of a highly developed 3,400 ha watershed. Jeong et al. (2008) indicated that Talbert Salt Marsh was able to remove FIB more efficiently as the volume of storm water runoff entering the marsh decreased. They concluded that a wetland may have a maximum capacity to attenuate contaminants; when loads exceed this value the wetland becomes a net source of contaminants to coastal waters.

Our work suggests that a moderate-sized wetland was able to attenuate FIB during most rain events. Reducing the size of a wetland, such as occurred at Talbert Salt Marsh (historically 1200 ha, now 10 ha), reduces its capacity to remove contaminants. Expansion of existing wetland area through restoration may partially restore its capacity for attenuating FIB, although this possibility remains to be tested.

### Conclusions

This work provides evidence that a 93 ha southern California wetland is an adequate size to allow for natural removal of FIB when the contributing watershed(s) have low to moderate levels of development. With relatively little loss of original wetland habitat and only moderate levels of development in its watershed, CSM is able to provide a valuable ecosystem service of improving the quality of water before it reaches the coastal ocean. Coastal water quality appeared to only be compromised by runoff during a large storm event when high volumes of bacteria-laden water overwhelmed the wetland's ability to reduce loads through sedimentation, die off, and/or dilution.

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## Asian Fish Tapeworm (*Bothriocephalus acheilognathi*) Infecting a Wild Population of Convict Cichlid (*Archocentrus nigrofasciatus*) in Southwestern California

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**Abstract.**—In September 2007 and May 2014, the Asian fish tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Bothriocephalidea), was found in populations of the non-native convict cichlid (*Archocentrus nigrofasciatus*) and mosquitofish (*Gambusia affinis*) collected from the discharge channel of a water treatment plant in Los Angeles County. Prevalence and mean intensity of infection of 450 convict cichlids and 70 mosquitofish were 55.3%/9.3 and 11%/1.4, respectively. Overall prevalence and mean intensity of infection in the convict cichlid was higher in 2007 (92%/12.3) than in 2014 (37%/5.4). In 2007, parameters of infection were size-dependent. The highest prevalence/mean intensity of infection was revealed in small fish (100%/15.5) and the lowest in large fish (66.7%/1.5). No statistically significant differences in infection parameters were found in convict cichlids of different size classes in 2014. This paper provides the first documented record of the Asian fish tapeworm infecting a wild population of the convict cichlid in the U.S.

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Introduction of exotic fish into novel aquatic ecosystems is sometimes accompanied by the unintentional transmission of additional species dangerous to populations of endemic fish, commercial fish and aquaculture (Bauer et al. 1973, Hoffman and Shubert 1984, Scholz 1999, Salgado-Maldonado and Pineda-Lopez 2003). One such invader, the Asian fish tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Bothriocephalidea), was imported from East Asia to Europe and the Americas during the 1960s and 1970s with herbivorous cyprinids, predominantly grass carp (*Ctenopharyngodon idella*), to control growth of aquatic vegetation in freshwater ecosystems (Hoffman 1999, Williams and Jones 1994, Choudhury and Cole 2012). The Asian fish tapeworm (hereafter, Asian tapeworm) has a simple life cycle that requires only two hosts: a definitive host, a fish in which larval stages develop into adult worm producing eggs, and an intermediate host, a cyclopoid copepod, which is a transmitter of the early larval stage (Liao and Shin 1956). The entire life cycle is temperature-dependent, and under optimal temperature, 25° C, can be completed in eighteen days (Bauer et al. 1973).

Due to low specificity for both intermediate and definitive hosts, and by colonizing other cyprinid as well as poeciliid hosts, the Asian tapeworm easily became established within native fish populations in new regions and continents, eventually resulting in its

current global distribution (Hoffman 1999, Font 2003, Choudhury and Cole 2012). Presently, it has been reported in 104 fish species in 14 families and seven orders from almost every continent except Antarctica (Salgado-Maldonado and Pineda-Lopez 2003). It is pathogenic to wild fish and aquaculture stock and may cause disease and even mortality events (Bauer et al. 1973, Scott and Grizzle 1979, Hoffman 1980, Granath and Esch 1983c, Hoole and Nissan 1994, Heckmann 2000, Hansen et al. 2006, Han et al. 2010, Britton 2011). In the U.S., after the initial discovery of the Asian tapeworm in Florida in 1975 (Hoffman 1980), it has been reported from 13 additional states (Arizona, California, Colorado, Hawai'i, Kansas, Michigan, Nevada, New Hampshire, New Mexico, North Carolina, Texas, Utah and Wisconsin), both in the wild or in fish hatcheries (Hoffman and Schubert 1984, Heckmann and Deacon 1987, Riggs and Esch 1987, Heckmann et al. 1993, Brouder and Hoffnagle 1997, Kuperman et al. 2002, Bean et al. 2007, Pullen et al. 2009, Archdeacon et al. 2010, Choudhury and Cole 2012). In California, the Asian tapeworm was first discovered in 1987 in grass carp, collected from irrigation reservoirs in Riverside and Imperial counties and in golden shiners (*Notemigonus crysoleucas*) collected from a fish farm in San Diego County (Chen 1987).

Surveys conducted in 1999-2001 revealed seven additional fish species (six cyprinid, one poecillid) in southern California infected by the Asian tapeworm (Kuperman et al. 2002). Of the six infected cyprinids, the arroyo chub (*Gila orcutti*) and Mojave tui chub (*Siphateles bicolor mohavensis*) are native, while the other four, common carp (*Cyprinus carpio*), golden shiner, goldfish (*Carassius auratus*) and fathead minnow (*Pimephales promelas*), are introduced. The single infected poecillid is the introduced mosquitofish. In June 2007, a population of convict cichlids (*Archocentrus nigrofasciatus*) was reported from the perennial discharge channel of a water treatment plant in Los Angeles County (Hovey and Swift 2012). The convict cichlid is native to Central America and is a tropical thermophilic species with a minimum temperature tolerance of 20 C (Conkel 1993, Bussing 1998). The first U.S. records of the convict cichlid were in Nevada where the fish were discovered in two natural warm springs (Deacon et al. 1964, Hubbs and Deacon 1965). In Mexico, introduced convict cichlids (as, *Cichlasoma nigrofasciatus*) were found to be infected by the Asian tapeworm (Salgado-Maldonado and Pineda-Lopez 2003), but no information on fish infection by this parasite was known for the U.S. The goal of the present study was to investigate whether the recently discovered population of the convict cichlid in California was infected by the Asian tapeworm.

#### Materials and Methods

Fish were collected for parasitological examination from a discharge channel with elevated water temperature 26° C [ $\pm 1.5^\circ$  C]. The source of the thermally elevated water was the treated discharge from the Rio Vista Water Treatment Plant that feeds directly into the Santa Clara River, Los Angeles County (34.423806, -118.540511; WGS84). The willow riparian scrub vegetation supported by the perennial discharge channel is restricted to the southern bank of the much wider, dry sandy river bed of the Santa Clara River. The outflow travels approximately 800 m before flowing subsurface. It is believed that the established convict cichlid population at this location originated from released aquarium fish (Hovey and Swift 2012). Other fish species that occurred at the study site were the native arroyo chub, and the non-native mosquitofish, prickly sculpin (*Cottus asper*), black bullhead (*Ameiurus melas*), goldfish, and common carp (var. koi) (Hovey, unpub. field notes). Of them, only mosquitofish were available for parasitological examination.

Table 1. Prevalence and mean intensity of infection of convict cichlids (*Archocentrus nigrofasciatus*) and mosquitofish (*Gambusia affinis*) by the Asian fish tapeworm (*Boithrioccephalus acheilognathii*) in 2007 and 2014.

Size class	Sample size (N)	Fish total length (TL) range, mm	Prevalence (%)	Intensity	
				Mean $\pm$ SD	Range
Convict cichlids					
September 2007					
Entire sample	150	25 - 130	92.0	12.3 $\pm$ 12.8	1 - 101
Class 1, small fish	100	25 - 59	100 <sup>A*</sup>	15.5 $\pm$ 13.7 <sup>B*</sup>	1 - 101
Class 2, medium fish	35	61 - 86	80.0 <sup>C*</sup>	3.9 $\pm$ 4.5 <sup>D*</sup>	1 - 22
Class 3, large fish	15	88 - 130	66.6 <sup>E*</sup>	1.5 $\pm$ 0.7 <sup>F**</sup>	1 - 3
May 2014					
Entire sample	300	39 - 112	37.0	5.4 $\pm$ 5.2	1 - 24
Class 1, small fish	74	39 - 59	32.4 <sup>G</sup>	4.8 $\pm$ 4.5 <sup>H</sup>	1 - 19
Class 2, medium fish	155	60 - 80	41.9 <sup>G</sup>	5.7 $\pm$ 5.4 <sup>H</sup>	1 - 24
Class 3, large fish	71	88 - 112	25.4 <sup>G</sup>	3.9 $\pm$ 3.5 <sup>H</sup>	1 - 14
Mosquitofish					
May 2014					
Entire sample	70	43 - 65	15.7	1.4 $\pm$ 0.7	1 - 3

A-H: Within the category, mean values sharing the same letter are not significantly different ( $P \leq 0.05$ )

\*  $p$ -value  $< 0.001$

\*\*  $p$ -value  $> 0.05$

A total of 450 convict cichlids and 70 mosquitofish were used for this study. On 11 September 2007, only three months after the discovery of convict cichlids in the channel, 150 convict cichlids were collected to be examined for the presence of the Asian tapeworm. A second fish collection took place on 1 May 2014 and included 300 convict cichlids and 70 mosquitofish. Fish were captured by seine net and placed into 5-gallon buckets containing channel water. Within three hours of being captured, the fish were removed from the water, transferred into plastic bags and placed into a freezer. The fish were then transported while still frozen, and stored at San Diego State University in a freezer until the commencement of parasitological examinations. After being thawed, total length (TL) of each individual was measured to the nearest mm. The TL of convict cichlids collected in 2007 ranged from 25 mm to 130 mm and in 2014 ranged from 39 mm to 112 mm (Table 1). To calculate infection parameters, convict cichlids were separated into three size classes: class 1 (small), class 2 (medium) and class 3 (large) (Tables 1, 2). We arbitrarily selected the range for each of the three size classes based on the clustering of sizes. The body cavities were opened and digestive tracks removed. After a longitudinal incision of the intestine, tapeworms were carefully teased from the intestinal wall, rinsed in 0.85% saline and placed into Petri dishes with the same solution. Tapeworm identification was made using the reference keys by Bykhovskaya-Pavlovskaya et al. (1964) and Hoffman (1999). Tapeworms from each fish were enumerated to determine the prevalence, the proportion of the hosts infected, and mean intensity of infection, the mean number of parasites in the infected hosts (Bush et al. 1997). The number of fish sampled, prevalence and mean intensity are provided in Table 1. A total number of tapeworms found in fish collected in 2007 and 2014, number of tapeworms in each size class of fish and the percentage of immature and mature tapeworms are presented in Table 2. Images of immature and mature tapeworms were obtained by light microscopy (LM) and scanning electron microscopy (SEM). For LM, 10 tapeworms and several

Table 2. Number and percentage of immature and mature Asian fish tapeworms (*Bothriocephalus acheilognathi*) recovered from convict cichlids (*Archocentrus nigrofasciatus*) in 2007 and 2014.

Size class of convict cichlids	Number of tapeworms	Stage of tapeworm development, %	
		Immature	Mature
September 2007			
Entire sample	1710	43.2	56.8
Class 1, small fish	1558	45.3	54.7
Class 2, medium fish	137	24.1	75.9
Class 3, large fish	15	13.3	86.7
May 2014			
Entire sample	597	83.9	16.1
Class 1, small fish	133	86.5	13.5
Class 2, medium fish	393	86.1	13.9
Class 3, large fish	71	64.8	35.2

pieces of intestinal wall with tapeworms attached were examined with a Nikon Eclipse E200 microscope (Melville, NY) and photographed under magnification x40. For SEM, eight mature tapeworms fixed in 70% alcohol were rinsed in phosphate buffer saline, post-fixed in 1% osmium tetroxide, dehydrated in ascending concentrations of ethanol from 50% to 100%, critical-point dried, sputter-coated with platinum, and examined using a FEI Quanta 450 scanning electron microscope (Hillboro, OR). A series of 10 preserved Asian fish tapeworms collected from the convict cichlids was deposited into the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska (HWML 64742).

Prevalence of infection in convict cichlids was tabulated by fish size (small, medium, large) for 2007 and 2014 separately. The resulting 2x3 contingency table was analyzed using a Pearson's chi-squared test. Mean intensity in fish from three size classes in 2007 and 2014 were estimated using the two-sample independent Mann-Whitney U test.

## Results

The Asian tapeworm was the only intestinal parasite found in the 450 convict cichlids collected from the discharge channel of a water treatment plant in September 2007 and May 2014. The prevalence and mean intensity of fish infections were higher in the 2007 sample than in the 2014 sample (Table 1). In the 2007 sample, parameters of infection were different among fish from the three size classes (Table 1). The highest prevalence and mean intensity of infection was found in small fish while the lowest were found in large fish (Table 1). Intensity of infection in fish from different size classes varied widely (Table 1). The highest parasite loads in small, medium and large fish were 101, 22, and 3, respectively. Both mature and immature Asian tapeworms were recovered from fish. Mature Asian tapeworms had a heart-shaped scolex with deep long bothria, a flattened attachment disc (Fig. 1A), and a perfectly segmented strobila composed of wide proglottids containing rosette-shaped ovaries filled with eggs (Fig. 1B, C). Immature tapeworms were represented by individuals at various developmental stages, ranging from worms having a small scolex and non-segmented body, to worms with a well-shaped scolex but still poorly segmented strobila and an underdeveloped reproductive system (Figs. 1D, E). In 2007, almost 60% of tapeworms recovered from the convict cichlids were represented by mature worms (Table 2). The highest percent of mature tapeworms was found in large (class 3) fish, and small (class 1) fish contained an almost equal



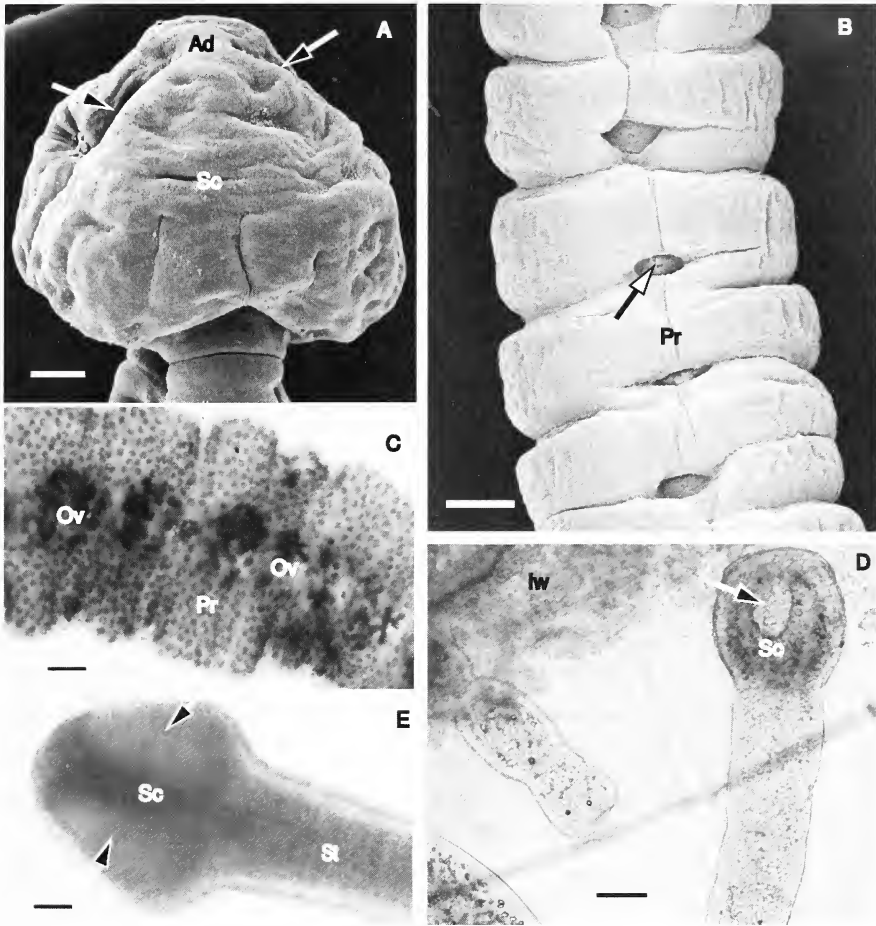


Fig. 1. Representative scanning electron microscope micrographs (A, B) and light microscope micrographs (C-E) of the Asian fish tapeworm *Bothriocephalus acheilognathi*. A) Mature worm - heart-shaped scolex with long bothria and flattened attachment disc; B) Mature worm - segmented strobila with mature proglottids and uterine pores; C) Mature worm - segmented strobila with mature proglottids and rosette-shaped ovaries; D) Immature worm - small scolex with short bothria, pre-proglottid formation of strobila; E) Immature worm - well developed scolex and early stage proglottid formation of strobilla. Ad - adhesive disk; lw - intestinal wall; Ov - ovary; Pr - proglottid; Sc - scolex; St - strobila. Black-head arrows indicate bothria, white-head arrow indicates uterine pore. Scale bars: 20 $\mu$ m.

number of mature and immature tapeworms (Table 2). In the 2014 sample, overall prevalence and mean intensity of infection in convict cichlids were 2.5 times and 3 times lower, respectively, than the 2007 sample (Table 1). Contrary to the 2007 results, no significant difference was found in the infection parameters of fish from the three size classes (Table 1). The highest load of Asian tapeworms at 24 individuals was found in a medium (class 2) fish. In contrast to 2007 results, about 84% of Asian tapeworms recovered from convict cichlids were immature (Table 1). The highest percent of mature Asian tapeworms was found in large (class 3) fish (Table 2). Of the 70 mosquitofish, also collected in May 2014, only eleven were infected by Asian tapeworms, with the lowest infection level being one tapeworm (Table 1). Of the fifteen Asian tapeworms found, 87% were immature (Table 2).

## Discussion

The present paper documents the first record of the Asian tapeworm in a wild population of the convict cichlid in the U.S. Finding the Asian tapeworm in years 2007 and 2014 indicates the presence of a persistent reservoir of infection in the channel conveying the thermally elevated discharge from the Rio Vista Water Treatment Plant in Los Angeles County. As only convict cichlids and mosquitofish were available for parasitological examination, we have no information on the infection in five other species of fish inhabiting this channel. We cannot exclude that three fish species, arroyo chub, goldfish and common carp, all well-known for their susceptibility to Asian tapeworm (Kuperman et al. 2002), could contribute to the persistence of the parasite at this site.

The artificially elevated water temperature of  $26^{\circ}\text{C} [\pm 1.5^{\circ}\text{C}]$  was optimal for the growth and development of the Asian tapeworm. Stimulating effect of high temperature on parasite transmission, infectivity, development and infrapopulation structure has been previously reported (Bauer et al. 1973, Sankurathri and Holmes 1976, Granath and Esch 1983a, b, c, Dobson and Carper 1992, Khan 2012). In our study, the overall infection rate in the autumn sample (2007) was higher than in the spring sample (2014). These results appear to be largely in agreement with the most common pattern of the seasonal dynamics of populations of the Asian tapeworm, in which elevation of water temperature was considered a critical factor controlling infectivity, development and infrapopulation structure (Bauer et al. 1973, Granath and Esch 1983a, b, c).

However, the water temperature at our collection site remained nearly constant throughout the year. Our sampling effort, separated by seven years, is long enough for significant changes to have occurred in the ecosystem we examined. Based on our limited sampling effort we were unable to identify alternative abiotic factors that, acting singly or synergistically with biotic factors, might affect fish infection by the Asian tapeworm. The last ones may include fluctuations in the biomass of zooplankton including cyclopoid copepod community (the intermediate host of the Asian tapeworm), shortage in biomass of phytoplankton (the food web for copepods), copepod species diversity (not all copepods are an efficient intermediate host for the Asian tapeworm) and changes in the structure of the fish community inhabiting the collection site. Water quality may also contribute to the rate of fish infection. It is possible that the chemical composition of the discharged water from the water treatment plant may affect both fish and cyclopoid copepods known for their high sensitivity to water chemistry (Ferdous and Muktaadir 2009). It is also known that in the case of fish infected by the Asian tapeworm, the pattern of high prevalence of infection may be followed by low prevalence (Heckmann and Deacon 1987, Archdeacon et al. 2010), and we cannot exclude the possibility that our samplings do not fit this seasonal pattern because of the different seasons and years of sampling.

Different parameters of infection were recorded in convict cichlids in the autumn 2007 and spring 2014 samples; overall values varied among size classes. In the 2007 sample, both prevalence and mean intensity of infection were size-dependent. Prevalence of infection reached 100% in small (class 1) fish but only 66.7 % in large (class 3) fish (Table 1). There was an inverse relationship between the size class of fish and the number of worms they harbored (Table 1). The highest parasite load of 101 Asian tapeworms was carried by one of the smallest fish (TL 31 mm). Lower values of infection rate in larger fish may be associated with the elimination of heavily infected individuals, the expelling of a number of worms due to their competition for food source, or stronger immunity of large fish compared to the smaller fish. The stage of worm maturation was inverse to the

intensity of infection, and consequently to fish size (Table 2). For example, large fish carried a maximum of three Asian tapeworms, most of them mature, while in the heavily infected smaller fish, the percent of mature and immature worms were nearly equal at 45.2% and 54.7%, respectively. Based on the rate at which the Asian tapeworm developed at 26° C [ $\pm 1.5^\circ$  C], the predominance of mature tapeworms infecting fish in 2007 indicates that this infection was at least one month old (Bauer et al. 1973, Williams and Jones 1994). In the spring sample (2014) we documented comparatively low infection levels in convict cichlids, regardless of fish size (Table 1). The highest parasite load of 24 Asian tapeworms was found in a medium (class 2) fish (TL 73 mm). There was an inverse relationship between the size class of fish and the number of worms they harbored (Table 1). In contrast to the fall sample (2007), the percent of mature tapeworms for all three fish size classes was lower (Table 2). Approximately 86% of the tapeworms recovered from the small (class 1) and medium (class 2) fish were immature, predominantly in the early stages of development, while 64.8% of the tapeworms from the large (class 3) fish were immature (Table 2). The predominance of immature stages of the Asian tapeworms infecting convict cichlids in the spring season (2014) indicates that the intermediate host, a cyclopoid copepod carrying infective larval stage of the procercoids, had been recently consumed. Low infection parameters and the same pattern of worm development were recorded in the mosquitofish. The seasonal patterns of infection levels and development stages of the Asian tapeworm discussed above are in agreement with previous reports of mosquitofish infections (Kuperman et al. 2002). Although we advocate for the removal of introduced and deleterious species when possible, this thermally isolated population of an infected tropical fish species in an artificially elevated and nearly constant temperature environment, provides a unique opportunity to study alternative factors influencing the seasonal population dynamics and ecological relationships of the intermediate host (cyclopoid copepods), the Asian tapeworm, and the final host, infected fish.

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## Food Selection of Coexisting Western Gray Squirrels and Eastern Fox Squirrels in a Native California Botanic Garden in Claremont, California

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Southern California is home to one native and one introduced species of tree squirrel. The native Western Gray Squirrel (*Sciurus griseus*; here on gray squirrel), is a highly arboreal tree squirrel that can be found inhabiting mixed oak and pine forest habitats and tree dominated parks and gardens in suburban areas within California (King 2004; Muchlinski et al. 2009). Gray squirrels feed primarily on fungi, pine nuts, acorns, and bay fruit. They have also been documented to feed on *Eucalyptus* seeds, samaras, and berries (*Morus* and *Phoradendron* spp.) along with bird eggs and nestlings (Carraway and Verts 1994). Fungi are one of the gray squirrel's most highly utilized food items. By consuming fungi, gray squirrels assist in providing a healthy soil environment for the development and growth of oak-woodland communities (Maser et al. 1981).

The introduced Eastern Fox squirrel (*Sciurus niger*; here on fox squirrel) is an invasive generalist species (Tatina 2007) typically found in upland areas, open forests, or areas neighboring open spaces such as agricultural lands and pastures (Sexton 1990). The presence of the fox squirrel in California has been a concern of the general public, land managers, and researchers. The Los Angeles County Agricultural Commission considers the fox squirrel a pest species and potentially aggressive. In their native range, the fox squirrel has been important ecologically in the succession of grasslands to forests by caching their food within open grasslands (Stapanian and Smith 1986). Seeds cached and fed on by the fox squirrel come from persimmon, blue gum *Eucalyptus*, cottonwood, pines, and many others (Koprowski 1994). Fox squirrels incorporate animal foods in their diet such as insects, butterflies, ants, birds, and bird eggs (Koprowski 1994). It is reported that the fox squirrel takes advantage of fruits found within backyards such as avocados, oranges, and strawberries, an activity often disliked by human occupants (Becker and Kimball 1947; Salmon et al. 2005).

Very little is known regarding food preferences of the two species within Southern California and detailed information is limited. This study sought to gain information on what foods each species selects, and which food items overlap and differ between gray and fox squirrels. Knowledge of food preferences among species promotes making management decisions that sustain their populations. For example, improving habitat by adding particular plants or trees preferred by the gray squirrel can aid in the recovery of its population (Linders and Stinson 2006). Information on food selection may also reveal a high degree of overlap such that competition is possible in years of food shortage. Competition could lead to extirpation of the gray squirrel where food selection is limited. Muchlinski et al. (2009) established that fox squirrels replace gray squirrels at locations

within Southern California. Food availability could be a factor in the replacement of gray squirrels from habitats invaded by the fox squirrel.

Observations on food selection were conducted at Rancho Santa Ana Botanic Garden (RSABG) in Claremont, California. RSABG is a native California garden of approximately 35 hectares containing a heterogeneous mixture of trees, shrubs, and grasses. Food available within the garden is all natural with very little human influence (e.g. trash, birdfeeders). Tree species present include but are not limited to *Quercus*, *Juglans*, *Pinus*, *Umbellularia*, and *Sequoia*. The study was conducted March 2013 to February 2014. Three transect lines and surrounding trails within the garden were visited in the same order for each observation period. Observations occurred as follows for a total of 124 hrs: (1) every other week from 14:00 to 17:00 hrs (72 hrs, 6 hrs/month, 24 observational days), (2) during a monthly census of the squirrels (36 hrs, 3 hrs/month, 12 observational days), and (3) during general behavioral observations conducted as a separate study (16 hrs total, 2 observational days per species). Data were collected using binoculars (8x30mm) and recorded creating a list of food items consumed by each species per observation day. The number of individuals consuming the food item was not recorded; however, the total number of days a food item was selected by each species was documented (Table 1). Food items were recorded only if the squirrel was eating at the time of the encounter.

Twenty-nine food items were consumed during the year by gray and/or fox squirrels (Fig. 1). In instances when observations are separated by at most three months it is assumed the species utilized that food item during the time between observations. Eleven food items including *Pinus* spp. (female cone), *Sequoia* spp. (female cone), *Quercus* spp. (acorn, flower bud, leaf/insect, and catkin), *Juglans* spp. (walnut and catkin), *Fragaria* spp. (fruit), *Aesculus* spp. (fruit/husk) and bark/insects from various species were consumed by both gray and fox squirrels (Table 1). Abundantly available acorns were utilized by both species the entire year while less abundant pine cones were utilized the first half of the year (January-July). Walnuts off the branch or from cached stores were utilized by both species most of year. Remaining food items were consumed seasonally, prior to spoilage or drying out (personal observation), when alternative food items were unavailable.

Gray squirrels consumed 7 food items that fox squirrels did not (Table 1), including *Fremontodendron* spp. (flower bud, flower/nectar, and fruit), *Umbellularia californica* (flower bud, fruit), *Arctostaphylos* spp. (fruit) and fungi. Gray squirrels utilized fruits from the California Bay Laurel (*Umbellularia californica*) from July to February. Fungi were documented as a food item for the gray squirrel October through January.

Fox squirrels consumed 11 food items not consumed by the gray squirrel (Table 1). Food items eaten by fox squirrels included *Washingtonia* spp. (leaf), *Liquidambar* spp. (fruit), *Heteromeles* spp. (fruit), *Arctostaphylos* spp. (flower), *Rosa* spp. (flower bud), *Mahonia* spp. (fruit), *Comarostaphylis* spp. (fruit), *Cornus* spp. (fruit), *Berberis nevadensis* (fruit), *Pinus* spp. (male cone), and *Allium* spp. (bulb). Such foods fill the fox squirrel's diet when acorns or pine seeds were unavailable. Many food items were utilized for only one to two months. Fruits of the American Dogwood (*Cornus*) served as a food source for a majority of the year.

Both species preferred a variety of food items at RSABG, yet observations at several urban/suburban parks indicated gray squirrels were limited in food choices (Ortiz 2014). Gray squirrels at these parks ate acorns, female and male cones (*Pinus* spp.), black berries from an unknown ornamental tree, and fruit from the California Bay Laurel

Table 1. Number of days food items were selected by *Sciurus griseus* and *Sciurus niger* out of 38 total observational days at Rancho Santa Ana Botanic Garden in Claremont, California from March 2013 to February 2014.

Food item	<i>S. griseus</i> *	<i>S. niger</i> *
<i>Fragaria</i> spp. (Fruit)	4	4
<i>Washingtonia</i> spp. (Leaf)	0	2
Fungi	3	0
<i>Umbellularia californica</i> (Fruit)	10	0
<i>Umbellularia californica</i> (Flower Bud)	1	0
<i>Mahonia</i> spp. (Fruit)	0	1
<i>Liquidamba</i> spp. (Fruit)	0	1
<i>Heteromeles</i> spp. (Fruit)	0	1
<i>Cornus</i> spp. (Fruit)	0	4
<i>Comarostaphylis</i> spp. (Fruit)	0	1
<i>Berberis nevini</i> (Fruit)	0	1
<i>Arctostaphylos</i> spp. (Fruit)	5	0
<i>Arctostaphylos</i> spp. (Flower)	0	1
<i>Aesculus</i> spp. (Fruit/Husk)	2	1
<i>Pinus</i> spp. (Male Cone)	0	1
<i>Pinus</i> spp. (Female Cone)	3	2
<i>Fremontodendron</i> spp. (Fruit)	1	0
<i>Fremontodendron</i> spp. (Flower Bud)	1	0
<i>Fremontodendron</i> spp. (Flower/Nectar)	4	0
<i>Rosa</i> spp. (Flower Bud)	0	1
<i>Sequoia</i> spp. (Female Cone)	3	1
<i>Juglans</i> spp. (Walnut)	13	11
<i>Juglans</i> spp. (Catkin)	1	3
Various spp. (Bark/Insect)	3	5
<i>Allium</i> spp. (Bulb)	0	1
<i>Quercus</i> spp. (Flower Bud)	1	1
<i>Quercus</i> spp. (Leaf/Insect)	2	4
<i>Quercus</i> spp. (Catkin)	4	6
<i>Quercus</i> spp. (Acorn)	28	21

\* Total number of food items consumed by each species.

(*Umbellularia californica*). Gray squirrels expanded their diet in the city of Redlands to include oranges from neighboring orchards. Fox squirrels had a broader diet in the parks including fruits and buds of *Eucalyptus* spp., fruit of the *Plantanus* spp., bark and leaves from various tree species, samaras (*Ulmus* spp.), peaches, cones from *Casuarina*, legumes, seed pods (*Jacaranda* spp.) and *Areaceae* fruits. Fox squirrels also supplemented their diet with peanuts and dry dog food supplied by park visitors and food in trash cans (personal observation). In contrast, King (2004) found gray squirrels did not supplement their diet with food from trash cans during her study in a park with a high level of human activity.

Food choices of gray and fox squirrels within their native and non-native ranges have been documented through observation and stomach analyses (Ingles 1947; Cross 1969; Steinecker and Browning 1970; Byrne 1979). Many of these food studies show an overlap in diet between the species. Each species also consumed unique food items. Food preferences found at RSABG are in line with many published works (Cross 1969; Wolf and Roest 1971; Steinecker 1977; Byrne 1979; Carraway and Verts 1994; Crabtree 2008); however, this study was the first to document several native plants of California.



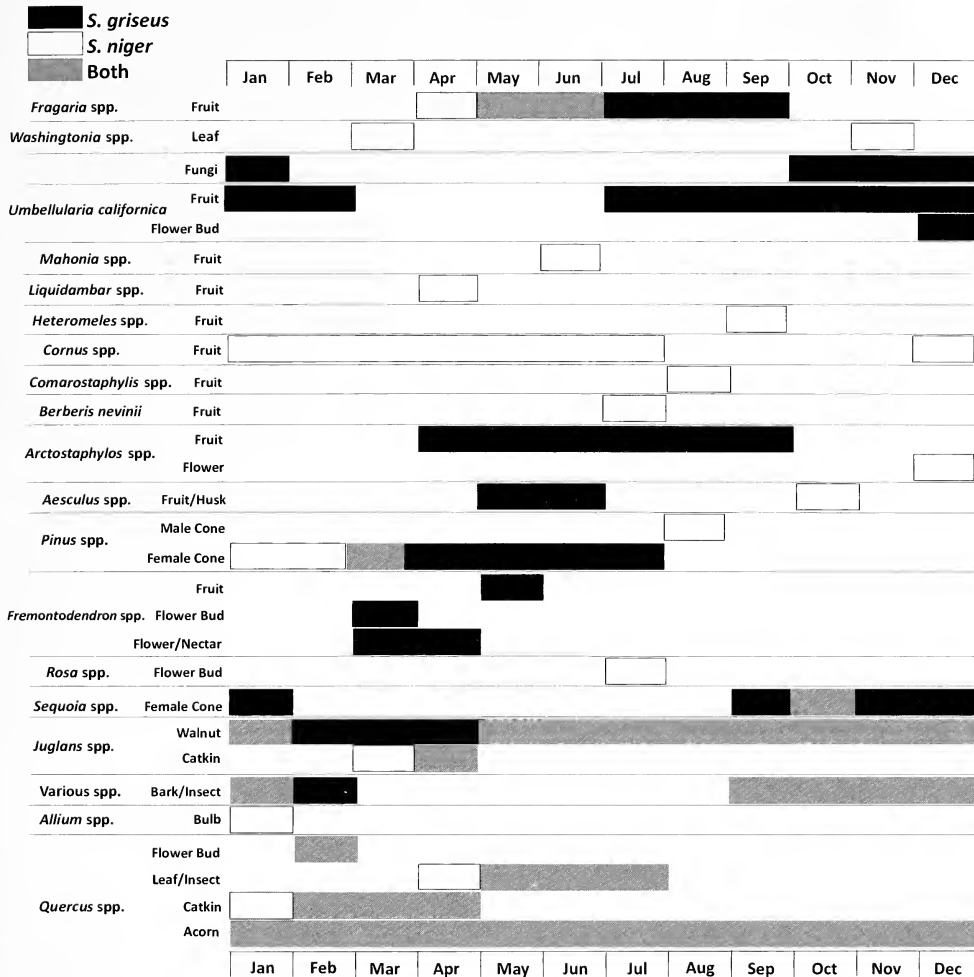


Fig. 1. Food items consumed by *Sciurus griseus* and *Sciurus niger* from March 2013 to February 2014 at Rancho Santa Ana Botanic Garden in Claremont, California. Black bars indicate food items consumed by *S. griseus*, white bars indicate food items consumed by *S. niger*, and gray bars indicate food items consumed by both species.

Fox squirrels consumed a wider variety of foods including fruits/seeds of RSABG natives and exotic species at local parks. A broader diet allows the fox squirrel a more stable, year-round food supply. Even with California’s on-going drought affecting the production of fruits, fox squirrels are able to supplement their diet with birdseed and hand-feeding from humans (King 2004). Food items previously documented include fruits of *Eucalyptus globulus* (Boulware 1941; King 2004), *Ulmus parvifolia* flowers (King 2004), samaras of *Acer macrophyllum* (King 2004; personal observation), plus other food items unique to the fox squirrel.

Gray squirrels continued to be restricted in food choices based on the habitat in which they were found. Although there were alternative trees with additional food items available, gray squirrels still fed almost exclusively on acorns and pine nuts. Gray squirrels move away from acorns and pine nuts when seasonally unavailable. They have been documented to eat bay fruit, pecans, almonds, cypress, mulberry, maple, and elm in

other locations (Ingles 1947). Yet none of these food items, with the exception of bay fruit, were emphasized in publications as part of gray squirrels' diet in Southern California. Gray squirrels in South Pasadena were found to have consumed seeds of *Eucalyptus* (Little 1934), which has only been observed once in Trabuco Canyon, California where oaks were drastically affected by a drought, producing little to no acorn crop (personal observation). Cross (1969) showed the importance of fungi in their diet, with specialty in subterranean fungi but also epigeous fungi and gill mushrooms (Steinecker 1977; Byrne 1979).

Although gray and fox squirrels overlap in many food choices including fungi (Carraway and Verts 1994; Koprowski 1994), fox squirrels were not observed consuming fungi during our study. The population of fox squirrels at RSABG may not need to utilize fungi since the garden contains a variety of food items such as fruits and catkins to consume instead. Utilization of fungi by the gray squirrel is reported to occur most during spring and summer (Carraway and Verts 1994), whereas fox squirrels utilize fungi during the summer and winter (Koprowski 1994). Timing of fungi consumption by the gray squirrel varies from year-long usage (Cross 1969) to primarily late summer (Byrne 1979). The benefit of fungi to their diet remains unknown.

Conserving the native Western Gray Squirrel will prove to be a complex issue. As of now, the best conservation method in urban/suburban habitats is to preserve isolated populations of gray squirrels that currently exist. Habitat improvements such as planting trees like the California Bay Laurel and conifers and shrubs like *Fremontodrendon* may sustain the isolated populations of gray squirrels for a longer period of time.

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## CONTENTS

Effects of Ocean Recreational Users on Coastal Bottlenose Dolphins ( <i>Tursiops truncatus</i> ) in the Santa Monica Bay, California. Amber D. Fandel, Maddalena Bearzi, and Taylor C. Cook .....	63
Salt Marsh Reduces Fecal Indicator Bacteria Input to Coastal Waters in Southern California. Monique R. Myers and Richard F. Ambrose .....	76
Asian Fish Tapeworm ( <i>Bothriocephalus acheilognathi</i> ) Infecting a Wild Population of Convict Cichlid ( <i>Archocentrus nigrofasciatus</i> ) in Southwestern California. Victoria E. Matey, Edward L. Ervin, and Tim E. Hovey.....	89
Food Selection of Coexisting Western Gray Squirrels and Eastern Fox Squirrels in a Native California Botanic Garden in Claremont, California. Janel L. Ortiz and Alan E. Muchlinski .....	98