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Seasonal reproduction of female round stingrays (*Urobatis halleri*): Steroid hormone profiles and assessing reproductive state

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ABSTRACT

This study characterizes the seasonal reproductive cycle of female round stingrays (*Urobatis halleri*) in an open coastal site at Seal Beach, CA and a protected estuary at the Seal Beach National Wildlife Refuge (SBNWR). Female round stingrays were sampled from August 2004 to July 2006, and assessed for reproductive parameters (GSI, maximum ova diameter, pregnancy status) and sex steroid (estradiol (E_2), progesterone (P_4) and testosterone (T)) concentrations. E_2 and P_4 increased at the time of ovulation (June and July) and remained elevated until parturition (October and November); recently partruded females were observed until November. Mature females were absent from Seal Beach in August and September, the same time period that abundance of mature females peaked in the SBNWR. This aggregation of predominantly mature females in the upper reaches of the SBNWR was seasonal, and was observed from April to September. To better understand the aggregation behavior, sex steroid hormones were assayed in SBNWR females. In July and August, E_2 and P_4 concentrations in females at the SBNWR were 1.5-fold and 2-fold higher, respectively, than concentrations in mature females at Seal Beach, and correlated with elevated water temperature in the estuary. Pregnancy was confirmed in aggregating females by increased levels of E_2 and P_4 and the presence of developing embryos. Our data suggest that coastal estuaries may play a crucial role in round stingray reproduction, perhaps by providing a thermal refuge for pregnant females during gestation.

1. Introduction

The majority of reproductive studies in elasmobranch species have been conducted on sharks, while batoids remain underrepresented in the literature. Because batoids such as round stingrays (*Urobatis halleri*), and other rays are a common occurrence in coastal marine communities and are likely to be affected by human activities, there is an increasing need to understand the reproductive biology and population dynamics of these elasmobranchs, ideally using less invasive methods.

Few batoid species have been studied, and most reproductive studies have focused on the Atlantic stingray (*Dasyatis sabina*). The genus *Urobatis* contains six species of short tail round stingrays found in the western north Atlantic and Eastern Pacific (Compagno et al., 2005). Of the six species, only the round stingray and yellow stingray (*Urobatis jamaicensis*) have been the subject of reproductive studies (Babel, 1967; Hamlett et al., 1999; Fahy et al., 2007; Mull et al., 2008), though relatively little is known about female reproductive function in these species.

Round stingrays are a common nearshore elasmobranch in Southern California (Babel, 1967), where large aggregations can occur in shallow, fine sediment beaches and embayments with low wave action (Allen et al., 2002; Babel, 1967; Hoisington and Lowe, 2005; Vaudo and Lowe, 2006; Lowe et al., 2007). Female round stingrays are viviparous, bearing from one-six pups each season, depending on the size of the mother (Babel, 1967). The predominant form of matrotrophy is aplacental yolk sac and histotroph (Wourms and Demski, 1993). Mating typically occurs from March to June with parturition reported to take place approximately three months later, a rapid gestation period for a viviparous elasmobranch (Babel, 1967; Nordell, 1994). Because both female and male round stingrays are believed to reach sexual maturity at approximately 30 months, and retain their reproductive capability throughout much of their life span, round stingrays have a high fecundity relative to other elasmobranchs (Babel, 1967). This is in contrast to other species of elasmobranchs that only reproduce once every two to three years and only give birth to a single pup (Wourms and Demski, 1993).

A common observation among sharks and batoids are aggregations of females during the reproductive period (Economakis and Lobel, 1998; Hight and Lowe, 2007). These aggregations may play some role in reproduction although no direct evidence has supported this hypothesis. Elasmobranch aggregations are often

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observed in shallow water that is warmer than surrounding areas, and pregnant females may exhibit thermotaxis as a way to increase the rate of embryo development and reduce gestation time (Economakis and Lobel, 1998; Wallman and Bennett, 2006), although this has never been directly tested.

With a high fecundity and ideal habitats extremely dense ray aggregations can result, such as observed in Seal Beach, CA. High densities of round stingrays have been observed at Seal Beach near a thermal outfall at the mouth of the San Gabriel River (Babel, 1967; Hoisington and Lowe, 2005; Vaudo and Lowe, 2006; Lowe et al., 2007) and appear be correlated with warmer water temperatures and low surf conditions (Hoisington and Lowe, 2005). Although water temperatures near the San Gabriel River outfall are higher than surrounding areas, they are highly variable, and can change up to 10 °C in a single tidal cycle (Babel, 1967; Hoisington and Lowe, 2005). While round stingrays are present at Seal Beach throughout the year, abundance varies with season and the residence time of individuals is approximately 2 weeks (Vaudo and Lowe, 2006). Interestingly, during six years of monthly sampling at Seal Beach no evidence of mating activity (e.g., fresh female mating scars) was ever observed (Hoisington and Lowe, 2005; Vaudo and Lowe, 2006; Lowe et al., 2007; Mull et al., 2008).

Prior to this study, the effects of photoperiod and water temperature on sex hormones during the reproductive cycle in round stingrays had not been investigated, and the possibility of a bimodal or dual breeding season had not been fully examined in large aggregations of round stingrays. Bimodal distribution of ovulation has been suggested for certain populations of round stingrays, and is hypothesized to result from a portion of "out of phase" females (Babel, 1967). Yellow stingrays (*U. jamaicensis*) exhibit a dual reproductive season with females ovulating twice each year, and pregnant females present year-round (Fahy, 2007). In addition to round stingrays, double reproductive seasons have also been suggested for thorny stingrays (*Dasyatis centroura*) (Capapé, 1993) during years with optimal conditions; however, these hypotheses regarding plasticity have yet to be fully examined. We hypothesized (1) that round stingrays in Seal Beach, CA, would exhibit a single reproductive event each year, (2) the timing of reproductive events would be correlated with changes in steroid hormone concentrations, and (3) changes in steroid hormone concentrations would be correlated with changes in temperature and photoperiod.

To address these hypotheses, we sampled round stingrays at Seal Beach, CA, monthly for 16 months to assess seasonal changes in reproductive physiology. Individuals were assessed for plasma steroid hormone estradiol (E_2), testosterone (T), and progesterone (P_4) concentrations, gonadal morphology and GSI. In addition, female round stingrays in the Seal Beach National Wildlife Refuge (SBNWR) were sampled for 16 months to examine the demographics, reproductive condition, and seasonality for comparison with the Seal Beach individuals. This allowed us to also examine the potential use of a coastal estuary as a breeding ground by round stingrays.

2. Materials and methods

2.1. Field collection

Round stingrays were collected monthly at Seal Beach, CA, from August 2004 to July 2006. Rays were captured using a large (3 m × 10 m) beach seine near the mouth of the San Gabriel River (33°44′ N, 118°06′ W) (Fig. 1). All rays captured were sexed and measured for disc width. In addition, a subset of adult female rays was brought back to California State University, Long Beach (CSULB). These rays were then re-measured, weighed, and euthanized via immersion in a seawater ice slurry, in accordance with approved CSULB IACUC protocol # 212. Blood samples were taken

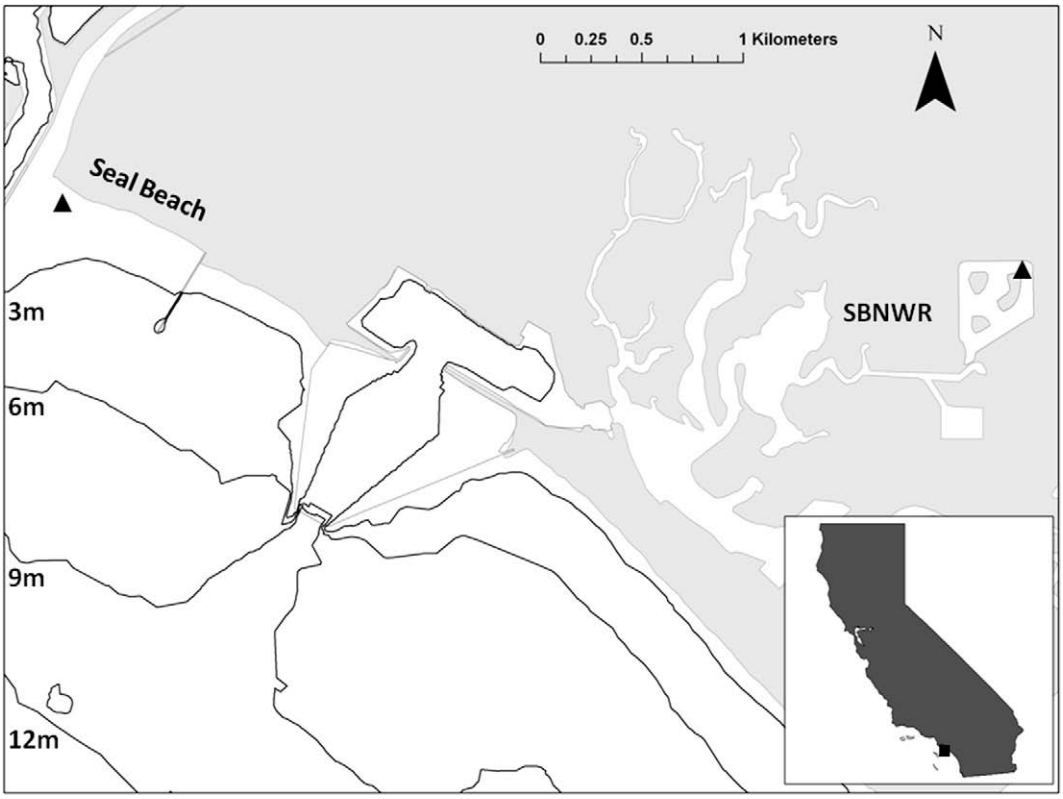

Fig. 1. Overview map of the sampling sites (▲) in Seal Beach, CA and the Seventh Street pond of the Seal Beach National Wildlife Refuge (SBNWR). ■ in inset shows the location of Seal Beach and the SBNWR in California. Gray indicates land areas.