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First observation of multiple paternity in loggerhead sea turtles, *Caretta caretta*, nesting on Dalyan Beach, Turkey

Fikret Sari [a,*], Aylin Koseler [b], Yakup Kaska [a]

[a] Pamukkale University, Faculty of Science and Arts, Department of Biology, Denizli, Turkey
[b] Pamukkale University, Faculty of Medicine, Department of Biophysics, Denizli, Turkey

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ABSTRACT

Sea turtles are promiscuous breeders with both males and females mating multiply. Due to this mating system, multiple paternity (MP) occurs in sea turtle clutches, and the frequency of MP varies greatly within and among species. In this study, the paternity of a population of loggerhead sea turtles (*Caretta caretta*) nesting on Dalyan Beach in Turkey was investigated using two highly polymorphic microsatellite markers (CcP2F11 and CcP7C06). Tissue samples collected from randomly selected hatchlings (a total of 522 hatchlings) from two to three successive clutches (a total of 25 clutches) of 10 nesting females were used for paternity analysis with an average sampling effort of 28.2% of offspring per clutch. Evidence of MP in seven out of 10 females (70%) was found, and it was detected that four out of these seven females mated with at least two males, whereas the remaining three females with at least three males. By analysing the successive clutches of females, it was detected that both the number and genotype of contributing sires was same in all clutches of a given female, possibly due to the lack of successful inter-nesting mating in this population. The high frequency of MP implies the possible high genetic diversity within this population. This study indicates that the density of individuals may be the reason of the high frequency of MP in this relatively small population because mating takes place mainly in a narrow area in Dalyan. The possible mating behaviours of the sea turtles and their population structures were discussed in light of the high frequency of MP within this population reported for the first time.

1. Introduction

If a species lives in marine habitats, it is very difficult to observe individuals while mating, and it is usually impossible to determine which mating individuals are actually successful (Karl, 2008). Molecular techniques provide an important tool for determining which individuals are contributing to species persistence in the lack of directly observing reproductive success, and molecular studies reveal aspects of mating system of an organism that otherwise would be impossible to detect (Karl, 2008). Specification of mating system of a species is an important part of understanding its natural history (Bjorndal et al., 1983; Wright, 1931). Mating systems are especially important within small populations because they can affect the genetic effective population size and the evolution of that species (Arden and Kapuscinski, 2002; Charlesworth, 2009; Frankham, 1995; Vucetich et al., 1997). Small population size and a skewed ratio of males to females that are available to mate at any nesting season, also known as operational sex ratio (OSR), may decrease genetic variation and the ability of that population to adapt to new environmental pressure (Kvarnemo and Ahnesjö, 1996; Montgomery et al., 2000). It is very important to estimate population size, population structure, and reproductive behaviour accurately in order to improve current conservation projects and make effective management decisions on endangered species. In populations whose mating system is polyandrous, multiple paternity (MP) affects the effective population size (Sugg and Chesser, 1994) and the genetic variability within a population (Baer and Schmid-Hempel, 1999). For decades, MP studies have been carried out in sea turtle species (i.e., Figgener et al., 2016; FitzSimmons, 1998; Harry and Briscoe, 1988; Jensen et al., 2006; Theissinger et al., 2009) as well as other species, and they provide valuable information regarding mating patterns and enable to understand population structure (Jensen et al., 2006). The frequency of multiple mating is critical for understanding of the evolution of the mating systems and for the conservation of endangered populations (Kichler et al., 1999; Moore and Ball, 2002).

Sea turtles are promiscuous breeders, with both males and females mating with multiple mates (FitzSimmons, 1998; Hamann et al., 2003; Miller, 1997), and males engage in intense and aggressive courtship behaviour to gain access to females (Lee and Hays, 2004). It is well known that MP is evident in all sea turtle species: loggerhead (Lasala et al., 2013; Zbinden et al., 2007), green (Alfaro-Núñez et al., 2015; FitzSimmons, 1998), leatherback (Figgener et al., 2016; Stewart and

* Corresponding author.
E-mail addresses: fikretsari1@gmail.com, fsari@pau.edu.tr (F. Sari).

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Dutton, 2011), olive ridley (Duran et al., 2015; Jensen et al., 2006), Kemp's ridley (Kichler et al., 1999), flatback (Theissinger et al., 2009), and hawksbill (Joseph and Shaw, 2011; Phillips et al., 2013). Frequency of the MP shows inter- and intra-specific variability (Jensen et al., 2006), and the percentage of clutches with MP can vary from 0 to >90% across these species. The factors causing these differences in MP among sea turtle species and between solitary and *arribada* rookeries include sperm storage (FitzSimmons, 1998; Pearse and Avise, 2001), sex ratio (Bollmer et al., 1999), inter-nesting interval (Kalb and Owens, 1994), abundance and density of individuals (Ireland et al., 2003; Jirotkul, 1999). Not only short term sperm storage but also sperm competition have been proven to be important aspects of sea turtle mating system (FitzSimmons, 1998; Phillips et al., 2013). Increased offspring viability and genetic diversity, fertilisation assurance and procurement of compatible gametes have been suggested to be some of the benefits of this behaviour (FitzSimmons, 1998; Jennions and Petrie, 2000; Uller and Olsson, 2008).

The loggerhead sea turtles (*Caretta caretta*) are long lived, and generally reach sexual maturity between 20 and 35 years of age (Conant et al., 2009; Snover, 2002); however, they typically reach sexual maturity earlier in the Mediterranean due to slower growth presumably as an adaptation to particular conditions (Casale et al., 2011). As a result, adult loggerheads in the Mediterranean have significantly smaller body size than the other populations around the world (Dodd, 1988; Margaritoulis et al., 2003). After sexual maturation, males are able to mate annually, whereas females mate every 2–3 years (Conant et al., 2009; Hays et al., 2010; Wibbels et al., 1990). Both females and males migrate asynchronously from foraging areas to breeding areas several weeks to months prior to the nesting season (Limpus, 1985). Males arrive a few weeks in advance of the females, but some males appear to be nonmigratory and may reside in breeding areas throughout the year (Henwood, 1987). Courtship and mating of loggerheads start in late March, and take place nearshore of adjacent nesting beaches. Females can store sperm and mate with more than one male in a breeding season (Moore and Ball, 2002; Pearse and Avise, 2001; Sakaoka et al., 2013), and may lay more than one clutch in a season with intervals of approximately two weeks (Dodd, 1988).

The loggerhead turtles have a circum-global distribution in temperate and subtropical regions including the whole of the Mediterranean (Bolten, 2003; Dodd, 1988). On the 2015 IUCN Red List, the loggerhead turtle is globally categorized as vulnerable (Casale and Tucker, 2015), while the loggerhead turtle in the Mediterranean is categorized as least concern (Casale, 2015). This categorization of the Mediterranean loggerheads demonstrates that long-term monitoring and conservation projects have recently started to give the results. The loggerhead is the most abundant sea turtle in the Mediterranean, having evolved local populations (Margaritoulis et al., 2003). Loggerhead turtles nesting in the Mediterranean have been shown to have diverged genetically from those in the Atlantic (Bowen et al., 1993; Laurent et al., 1993, 1998), although high numbers of loggerheads from the Atlantic enter the Mediterranean (Carreras et al., 2006; Laurent et al., 1998). The Mediterranean loggerheads are confined almost exclusively to the eastern basin, and the main nesting concentrations are found in Greece, Turkey, and Cyprus (Margaritoulis et al., 2003). Genetic analyses from several nesting areas in Turkey show differentiation among rookeries (Schroth et al., 1996). Loggerhead populations in the Mediterranean are fairly small in comparison with the world population; however, a significant part of the Mediterranean nesting population uses Turkey's beaches, and this demonstrates the importance of protecting sea turtles in this country.

Most of the behavioural knowledge of loggerhead mating systems comes from nesting beach-based studies. Although adult female sea turtles are more accessible nearshore or on the nesting beaches to direct observation (Bowen et al., 2004; Dodd, 1988), adult males are less accessible to direct observation as they generally remain in open waters. In this respect, MP studies have great importance, and they provide insights into the mating system and the population size and/or density of loggerheads. While several MP studies were conducted previously in different nesting loggerhead turtle populations (Bollmer et al., 1999; Harry and Briscoe, 1988; Lasala et al., 2013; Moore and Ball, 2002; Tedeschi et al., 2015; Zbinden et al., 2007), there was no MP study carried out in the Mediterranean loggerhead population nesting on not only Dalyan Beach but also any other Turkish beach. Dalyan Beach is one of the most important reproductive sites of the loggerhead turtles in terms of annual nest number (Canbolat, 2004), nesting density (Canbolat, 2004), hatching success and high proportion of male hatchlings produced (Sarı and Kaska, 2015), protection status (Türkozan and Yılmaz, 2008), and regular sea turtle surveys (Sarı and Kaska, 2015) not only for Turkey but for the Mediterranean region. This beach is therefore presumably the most appropriate place for conducting the studies of MP and population size on loggerheads. In this study, the paternity of the loggerhead sea turtle population nesting on Dalyan Beach (Turkey)—for the first time at a sea turtle nesting beach in Turkey—was investigated using microsatellite analysis. It was aimed to quantify the frequency of MP, to use this frequency of MP for estimating population size of the nesting females of Dalyan Beach, and to demonstrate the skew in male contributions within clutch and among the successive clutches of a female. In light of the findings, the questions of whether the mating takes place prior to nesting season, or inter-nesting mating that contributes to the hatchling genotypes occur, and whether correlations exist between the number of sires per clutch and female size, nesting date, and hatching success were also discussed.

2. Materials and methods

2.1. Study site

The Mediterranean coasts of Turkey are important nesting grounds for both loggerhead and green sea turtles (*Chelonia mydas*) (Türkozan and Kaska, 2010). Based on nest numbers, Turkey holds the second most important loggerhead sea turtle stocks in the Mediterranean (Margaritoulis et al., 2003). Female loggerhead sea turtles nest on the beaches of Turkey from late April until mid-August, and these nests hatch from late June until early October.

Dalyan Beach has been a part of Köyceğiz-Dalyan Specially Protected Area since 1988, and is one of the best protected nesting beaches in Turkey. The beach is used by both loggerhead sea turtles and the Nile soft-shelled turtles (*Trionyx triunguis*), and the reproduction of these two endangered species on the same beach increases the importance of the beach (Türkozan and Yılmaz, 2008). Public access is not allowed on Dalyan Beach from 20:00 to 08:00 h during the nesting and hatching season.

Dalyan Beach has a length of 4.5 km, and is located in the south-west of Turkey (Fig. 1). The beach is a crescent-shaped fine-sand dune. Behind the western two thirds of the beach is an extensive wetland with a labyrinth of reedy channels, and the wetland complex at the western edge of the beach opens to the sea through a channel. There is a small lake, named Alagöl, in the wetland, and mating of the loggerheads take place in this lake as well as the nearshore of the sea and labyrinth of reedy channels. Majority of the mating activities occurs in Alagöl and the labyrinth of reedy channels (Kaska, personal communication). Behind the eastern third is a small lake, named Iztuzu Lake, which is separated from the Dalyan estuary by a mountain ridge. There are several sand dunes at the lake side of the beach.

2.2. Sample collection

This study was approved by the Pamukkale University Animal Ethics Committee (No: PAUHDEK-2014/016), and samples from nesting female turtles and their offspring were obtained during the 2014 nesting season from mid-May to mid-September on the beach.