GENETIC STRUCTURE AND HATCHLING BEHAVIOR OF SEA TURTLE POPULATIONS IN THE EASTERN MEDITERRANEAN

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ABSTRACT

GENETIC STRUCTURE AND HATCHLING BEHAVIOR OF SEA TURTLE POPULATIONS IN THE EASTERN MEDITERRANEAN

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As ancient species, living sea turtles history laid back to 110 milion years. There are seven sea turtle species occupying different niches among the Earth's oceans. There are two species of sea turtles breeding in the Mediterranean Sea; loggerhead turtle; *Caretta caretta*, green turtle; *Chelonia mydas*. The Mediterranean subpopulations of green and loggerhead turtle are in IUCN Red list and categorized as critically endangered and least concern respectively. The first step to protect these species is learning more of their biology and ecology. Although there are many studies conducted on ecology and conservation of sea turtles, application of new technologies to sea turtle monitoring and understanding sea turtle population genetics at finer spatial scales are still needed. At the base of this concern the aim this thesis was; understanding the sea turtle populations' genetic structure and testing a new monitoring method on sea turtle hatchlings emergence behavior at the eastern Mediterranean, that are explained under two chapters respectively.

In the first chapter, to understand the genetic structure of sea turtle populations in the Eastern Mediterranean, a nuclear DNA intron marker (R35: RNA Fingerprint Protein) and a mitochondrial DNA marker (COI) were used for sea turtle populations. Samples were collected from Antalya-Belek, Mersin-Erdemli, Hatay-Samandağ and Northern Cyprus from loggerhead, green turtle and softsheled Nile turtle as an out group. A non-invasive sampling method was used that based on sampling muscle and dermal tissues from dead hatchlings. The results were compared with the database samples to understand relationships between them. In the present

study, 115 haplotypes were revealed from 240 sequences with 50 and 54 private haplotypes for loggerhead and green turtle respectively. The highest amount of polymorphism and haplotype observed among Mersin (METU Erdemli Campus) loggerhead turtle population. According to gene flow (Nm) and genetic differentiation (Fst) estimations eastern Mediterranean green turtle populations grouped as Antalya and Hatay/Mersin/North Cyprus nesting colonies while loggerhead turtle grouped as Mersin/Antalya and North Cyprus. Furthermore, in total 30 hybrid individuals detected from Antalya and Mersin samples, as the result of interbreeding between female loggerhead and male green turtle. Additionally, multiple paternity observed on 9 loggerhead turtle nests among hybridization events with at least two males; one is loggerhead, one is green turtle.

In the second chapter, to understand the emergence patterns and behavior of hatchlings, IR cameras were installed on one green and four loggerhead turtle nests located in METU Erdemli Campus during 2014 and 2015 nesting season. Hatchling emergences were recorded continuously and analyzed temporal pattern, incubation duration and group emergence pattern accordingly. According to results; hatchlings emerged from the nests asynchronously in varying numbers of groups and different group sizes. 98.6% of hatchlings emerged during night with a peak activity between 21:00 and 00:00. Total emergence activity continued at least 60-65 days after the egg deposition and 1-22 days and after the first emergence.

The present study provides a better understanding of genetic structure of eastern Mediterranean sea turtle populations. According to the study each nesting beach should be considered as different management units in the eastern Mediterranean. Furthermore, even small beaches such as METU Erdemli Campus beach, may have significant contribution into the metapopulations in varied ways. On the other hand implementation of IR camera provided accurate and extensive information about hatchling behavior. IR camera is a promising complementary tool which will facilitate a better management policy along restricted areas such as METU Erdemli Campus.

Key words: *Chelonia mydas*, *Caretta caretta*, Population Genetic, Conservation, IR camera

ÖZ

DOĞU AKDENİZ DENİZ KAPLUMBAĞASI POPULASYONLARININ GENETİK YAPISI VE YAVRU DAVRANIŞI

Oğul, Fatıma Nur

Yüksek Lisans, Deniz Biyolojisi ve Balıkçılık Bölümü

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Çok eski bir tür olan ve günümüzde de yaşayan deniz kaplumbağalarının tarihi 110 milyon yıl önceye dayanmaktadır. Dünya okyanuslarında farklı nişlerle yayılım gösteren toplamda yedi deniz kaplumbağası türü vardır. Akdenizde ise üreme davranışında bulunan iki tür bulunmaktadır; İribaş deniz kaplumbağası (Caretta caretta) ve yeşil deniz kaplumbağası (Chelonia mydas). Yeşil ve iribaş deniz kaplumbağalarının Akdeniz alt populasyonları IUCN Kırmızı listesindedir ve sırasıyla kritik olarak soyu tükenmekte olan ve soyu tükenme tehlikesi altında olan şeklinde katagorize edilmiştir. Bu türleri korumak için atılan ilk adım, onların biyoloji ve ekolojileri hakında daha fazla bilgi sahibi olmaktır. Deniz kaplumbağalarının ekolojileri ve korunması hakkında yürütülen bir çok çalışma olmasına rağmen, daha kapsamlı izleme çalışmaları ve genetik yapılarının anlaşılması adına yeni teknoloji uygulamaları gerekmektedir. Bu bağlamda tezin amacı, deniz kaplumbağası populasyonlarının genetik yapısının anlaşılması ve Doğu Akdeniz'de deniz kaplumbağası yavru çıkışı üzerine yeni gözlemleme metodunun test edilmesi olarak iki bölüm altında sırasıyla incelenmiştir.

Birinci bölümde, Doğu Akdeniz'de bulunan deniz kaplumbağalarının genetik yapısını anlamak için genetik analizlerde çekirdek DNA kodlanmayan bölgede bulunan R35: RNA Parmakizi Proteini ve mitekondriyal DNA COI: sitokrom oksidaz altunite I gen bölgeleri kullanılmıştır. Örnekler, Antalya-Belek, Mersin-Erdemli, Hatay-Samandağ ve Kuzey Kıbrıs bölgelerinden iri baş ve yeşil deniz kaplumbağaları ve bir dış grup olarak yumuşak kabuklu nil kaplumbağasından toplanmıştır. Örneklem hiçbir canlıya zarar verilmeden, ölü yavruların kas ve deri dokuları üzerinden gerçekleştirildi. Aralarındaki ilişkiyi anlamak adına çalışmanın sonuçları genetik veritabanından elde edilen örneklerle karşılaştırıldı. Bu çalışmada, 240 sekanstan elde edilen

toplam 115 haplotipten; iribaş deniz kaplumbağası için 50 ve yeşil deniz kaplumbağası için ise 54 haplotip ortaya çıkarılmıştır. En yüksek derecede polimorfizm ve haplotip Mersin (ODTÜ Erdemli Kampüsü) iri baş deniz kaplumbağası üreme populasyonunda görülmüştür. Gen akışı (Nm) ve genetik farklılaşma (Fst) tahminlerine göre Doğu Akdeniz yeşil deniz kaplumbağası populasyonu Antalya ve Hatay/Mersin/Kuzey Kıbrıs yuvalama kolonileri olarak gruplanırken; iribaş deniz kaplumbağaları Mersin/Antalya ve Kuzey Kıbrıs olarak gruplandı. Ayrıca Antalya ve Mersin örneklerinden, dişi iri baş deniz kaplumbağası ve erkek yeşil deniz kaplumbağası türleri arası üreme sonucunda ortaya çıkan toplam 30 hibrid birey saptanmıştır. Buna ek olarak, hibrid bireylerin bulunduğu iribaş deniz kaplumbağası yuvalarından 9 tanesinde; biri iribaş deniz kaplumbağası, diğeri de yeşil deniz kaplumbağası olmak üzere en az iki erkekle ortaya çıkan çoklu babalık tespit edilmiştir.

İkinci bölümde ise; 2014 ve 2015 yıllarında ODTÜ Erdemli Kampüsünde, yavru çıkış desenlerini ve yavruların çıkış davranışlarını anlamak için bir yeşil deniz kamplumbağası ve dört iribaş deniz kaplumbağası yuvası gece görüşlü kameralar ile izlenmiştir. Yavru çıkışları devamlı olarak kayıt altına alınmış, grup çıkışı ve zamansal desenleri, kuluçka süreleri analiz edilmiştir. Çalışma sonucunda yavru çıkışlarının değişken grup sayıları ve büyüklüklerde belirli bir zamanlama olmaksızın gerçekleştiği görülmüştür. Toplam yavru çıkışının %98.6'sı gece gerçekleşmiş olup, en yüksek aktivite 21:00 ve 00:00 saaatleri arasında meydana gelmiştir.Toplam yavru çıkış aktivitesi, yuva kurulumundan sonra 60-65 gün, ilk yavru çıkışından sonra ise 1-22 gün devam etmiştir.

Bu çalışma, Doğu Akdeniz deniz kaplumbağası üreme populasyonlarının genetik yapısının daha iyi anlaşılmasını sağlamaktadır. Çalışmaya göre Doğu Akdeniz'de bulunan her bir yuvalama sahili farklı yönetim birimleri olarak göz önünde bulundurulmalıdır. Buna ek olarak, ODTÜ Erdemli Kampüsü sahili gibi küçük sahillerin bile, metapopulasyonlara çeşitli şekillerde önemli katkılarının olabileceği gözlenmiştir. Ayrıca gece görüşlü kamera uygulaması ile, yavru çıkış davranışı hakkında oldukça kapsamlı ve güvenilir bilgi elde edilmiştir. Gece görüşlü kamera uygulaması, özellikle ODTÜ Erdemli Kampüsü gibi sınırlandırılmış alanlarda deniz kaplumbağası izleme çalışmalarında daha verimli bir koruma politikası geliştirmek adına gelecek vaat eden bütünleyici bir araçtır.

Anahtar Kelimeler: *Chelonia mydas, Caretta caretta,* Populasyon Genetiği, Doğa Koruma, Gece Görüşlü Kamera

To wonder women

Sebahat, Satı and Arzu…

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INTRODUCTION

1.1. General information about sea turtles

1.1.1. Taxonomy and history

As an ancient group, sea turtles were common in the Cretaceous (130 million years ago) and their fossil records laid back to at least 200 million years (Marquez, 1990). The extant sea turtles originated between 60 and 110 million years ago and evolved in the period from the Eocene to the Paleocene (Kear and Lee, 2006; Figure 1). There are seven sea turtle species alive today, grouped into one superfamily (*Chelonioidea*) represented by two families: *Cheloniidae* and *Dermochelyidae*. *Dermochelyidae* has only one species: the leatherback (*Dermochelys coriacea, Vandelli, 1761*) and *Chelonidae* has six species: the green turtle (*Chelonia mydas, Linnaeus, 1758*), the loggerhead (*Caretta caretta, Linnaeus, 1758*), the olive ridley (*Lepidochelys olivacea, Eschscholtz, 1829*), the Kemp's ridley (*Lepidochelys kempii, Garman, 1880*), the hawksbill (*Eretmochelys imbricata, Linnaeus, 1766*), the flatback (*Natator depressus Garman, 1880*).

According to a study conducted among the Pacific, Indian and Atlantic oceans using complete mito-genome analyses of all sea turtle species, the flatback turtle is placed as the sister taxon to *Chelonia* rather than to the *Eretmochelys, Lepidochelys,* and *Caretta* (Duchene et al., 2012). The study suggested that the divergence between Pacific and Atlantic clades of loggerhead and olive ridley has occurred more recently than that of the hawksbill and green turtle. Additionally, it is revealed that the Atlantic and Pacific loggerhead samples were more divergent, while olive ridley Atlantic samples were clustered with the Pacific samples. The results indicated that the genetic isolation mechanisms are different for each sea turtle species and the biogeographic history of sea turtles have been shaped by different events (Duchene et al., 2012). Similar results were found according to mitochondrial DNA restriction site analyses of the loggerhead turtle: there is substantial phylogeographic structure among the Pacific, Indian, Atlantic oceans and the Mediterranean Sea with tendency of natal homing (Bowen et al., 1994). Bowen et al. (1994) also suggested that there is more recent interoceanic gene flow based on the analyses of loggerhead turtle populations. The reason is explained by the ability of temperature adaptation of loggerhead turtle enabling them to utilize the habitats around southern Africa mediated (Bowen et al., 1994). Results show that variation of ecology and geographic ranges of sea turtles can affect their population structure, globally. Moreover, Reece et al. (2005) reported that the nesting habitat

preferences have been correlated with the genetic structure patterns of the loggerhead, hawksbill and green turtle Atlantic and Mediterranean populations as well as reflected historical responses to climatic cycles. Reece et al. (2005) also reported that the loggerhead turtle distribution may be affected by the increasing temperature. However, further studies using nuclear DNA and detecting sex biased gene flow are needed for a more comprehensive understanding.

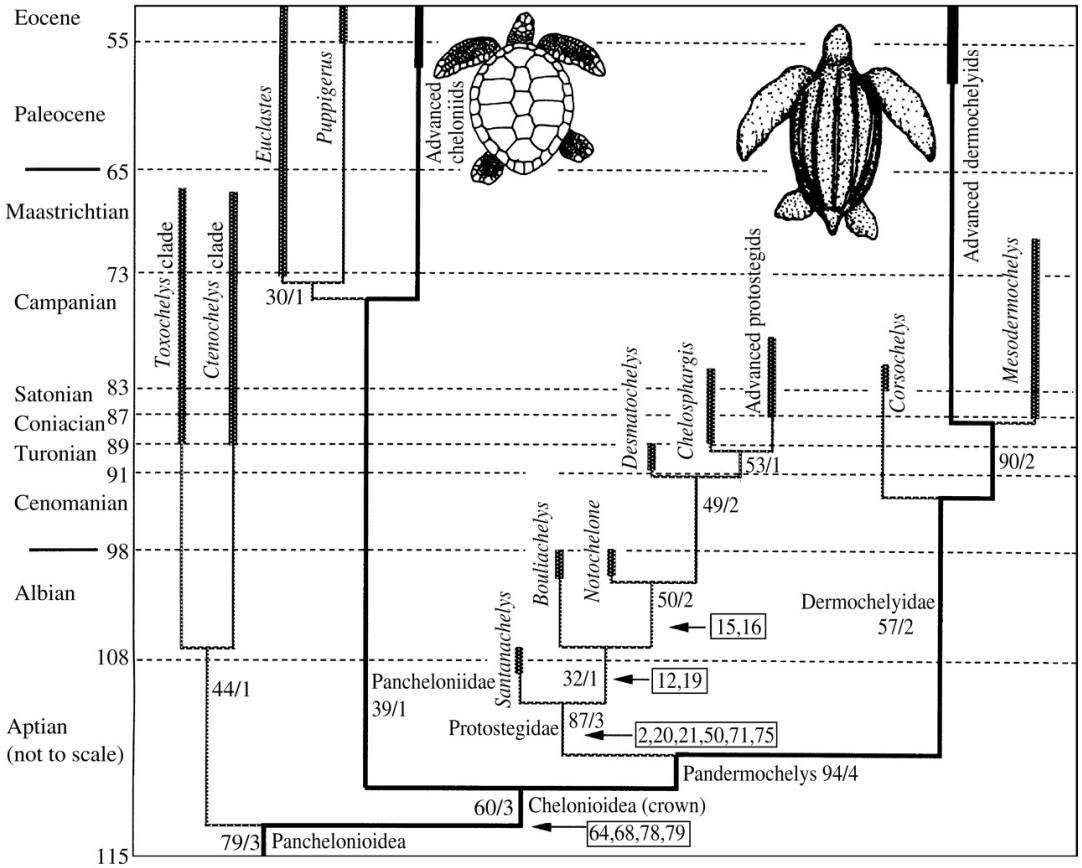


Figure 1.1.1.1 Phylogeny and stratigraphic record of sea turtles. Black lines denote living lineages, grey lines denote extinct lineages. Boxed numbers at selected nodes refer to synapomorphies (derived traits in share of common ancestor; see Kear and Lee, 2006 for more information) and other numbers refer to bootstrap/Bremer support. (Retrieved from Kear and Lee, 2006)

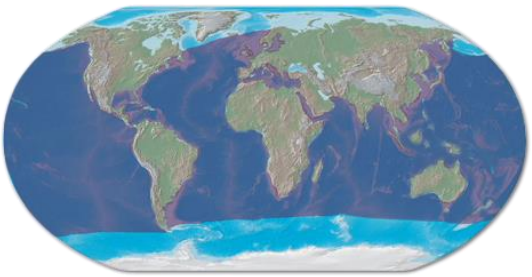
1.1.2. Distribution

Sea turtles are full migrant species distributed all around the world oceans excluding polar region depending on their habitat and temperature preferences (WWF, 2006). Leatherback, loggerhead and green turtles are more likely to be seen at offshore ocean while leatherback turtle has the widest distribution among sea turtle species since it is more cold-tolerant; and followed by loggerhead and green turtles for distribution range, respectively (SeaWorld Parks & Entertainment, 2017). Hawksbill and the olive ridley turtles are inclined to be along shallow coastal waters while Kemp's ridley and flatback are indigenous species to golf stream and Australia coasts, respectively.

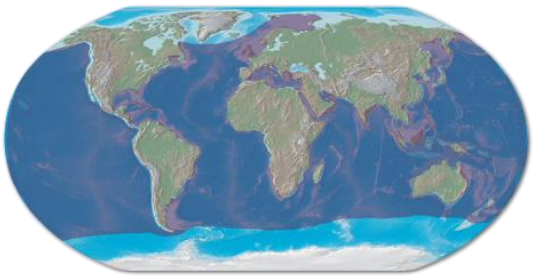
Previous studies on the distribution of the loggerhead and green turtle populations in the Mediterranean revealed important patterns. According to a satellite tracking study conducted in the Mediterranean, ten foraging grounds in total were identified for green turtles with two major areas in Libya (Stokes et al., 2015). They also revealed that Libya and Egypt coastline were occupied by migratory turtles before and after nesting seasons using the pelagic corridor from western Turkey and Cyprus to Egypt (Stokes et al., 2015). Another study conducted in the Mediterranean revealed that there is a clear overlap between loggerhead and green turtle migratory corridors and foraging areas (Snape et al., 2016); corroborating the previous study conducted by Broderick et al. (2007). Moreover, there are some evidence of migration into the Ionian/Adriatic Sea from Cyprus and Turkey loggerhead hatchlings and adult females revealed by modelling studies and stable isotope analyses (Casale and Mariani, 2014; Clusa et al., 2014). Casale and Mariani (2014) suggested that the Levantine basin should be considered as a key spot for the Mediterranean population of the both species by the evidence of dispersal patterns. Similar results observed on tracked loggerhead turtles (3 females and 1 male) released from Italy during autumn (Bentivegna, 2002). According to the study 2 females migrated to the eastern Mediterranean, while the male migrated to Greece.

Sea turtles are known for their fidelity to their nesting sites and natal homing behavior. However, some exceptional cases of non-fidelity for loggerhead turtles have also been previously reported (Margaritoulis, 1998; Ehrhart et al., 2014). Additionally, it has been recently reported that two tagged green turtles laid nests in different beaches with 70 and 100 km distance from each other in the same nesting season in the eastern Mediterranean (Sönmez et al., 2017). The reasons of this unexpected behavior of nesting sea turtles may be disorientation, disturbance at the nesting beaches or a change in their nesting beaches for more accessible and appropriate areas (Margaritoulis, 1998; Sönmez et al., 2017).

1.1.2.a



1.1.2.b



1.1.2.c

1.1.2.d

1.1.2.e

1.1.2.f

1.1.2.g

Figure 1.1.2.1. Global distribution of sea turtles.

a. Green, **b.** Loggerhead, **c.** Leatherback,

d. Hawksbill, **e.** Olive ridley, **f.** Kemp's ridley,

g. Flatback turtles.

(Taken from: https://seaworld.org/en/animal-info/animal-infobooks/sea-turtles/habitat-and-distribution)

1.1.3. Life Cycle

All of the seven sea turtle species occupy different niches yet share the same complex life-cycle with the periodic migration between breeding and foraging areas as well as extreme navigation skills (Bowen and Karl, 2007). Sea turtles spend most of their lives in the open waters and all of the sea turtle species excluding flatback turtle have juvenile oceanic phase in their life cycle (Bowen and Karl, 2007). The migration of of all species is explained as females and males migrate from foraging areas to breeding areas near-shore, the males return to the foraging areas after the breeding, while females continue to the nesting areas after mating (Miller, 1997; Musick and Limpus, 1997). It is known that female sea turtles tend to return their natal beaches and re-nest at the same beach, which is called "natal homing" (Carr, 1967; Bowen and Karl, 2007). Male sea turtles may reproduce every year (Limpus, 1993), while female sea turtles usually reproduce at 2 to 8 year intervals (Miller 1997). The females crawl to beaches during night and may lay several clutches per season with 50 to 200 eggs in one clutch depending on the species (WWF, 2006). Incubation period of the hatchlings is varying between 45 and 82 days depending on the species and sand temperature (Bustard and Greenham, 1968; Matsuzawa et al., 2002). At the end of the incubation, hatchlings emerge mostly during night and swim to the offshore currents (Bowen and Karl, 2007) and they drift several years in the ocean water pelagic zone until juvenile period, named as "lost years" due to limited knowledge (Carr,1986). After this oceanic phase, juveniles recruit actively to the demersal habitats in coastal waters and move seasonally between foraging areas till they approach to maturity (Musick and Limpus, 1997). Sea turtles grow slowly and it takes decades for them to reach maturity (WWF, 2006). Sexual maturity is varying between 15 and 50 years depending on species and geographic area (e.g. 13-14 years for the leatherback, 15-20 years for the loggerhead, 20-50 years for the green turtle) (Balazs, 1982; Bjorndal and Zug, 1995; Davenport, 1997). Once sea turtles reach their maturity, they start to migrate between foraging areas and nesting beaches seasonally (Musick and Limpus, 1997; Bowen and Karl, 2007). Although juvenile and mature foraging areas may overlap for some populations, those areas are usually distant from each other (Musick and Limpus, 1997).

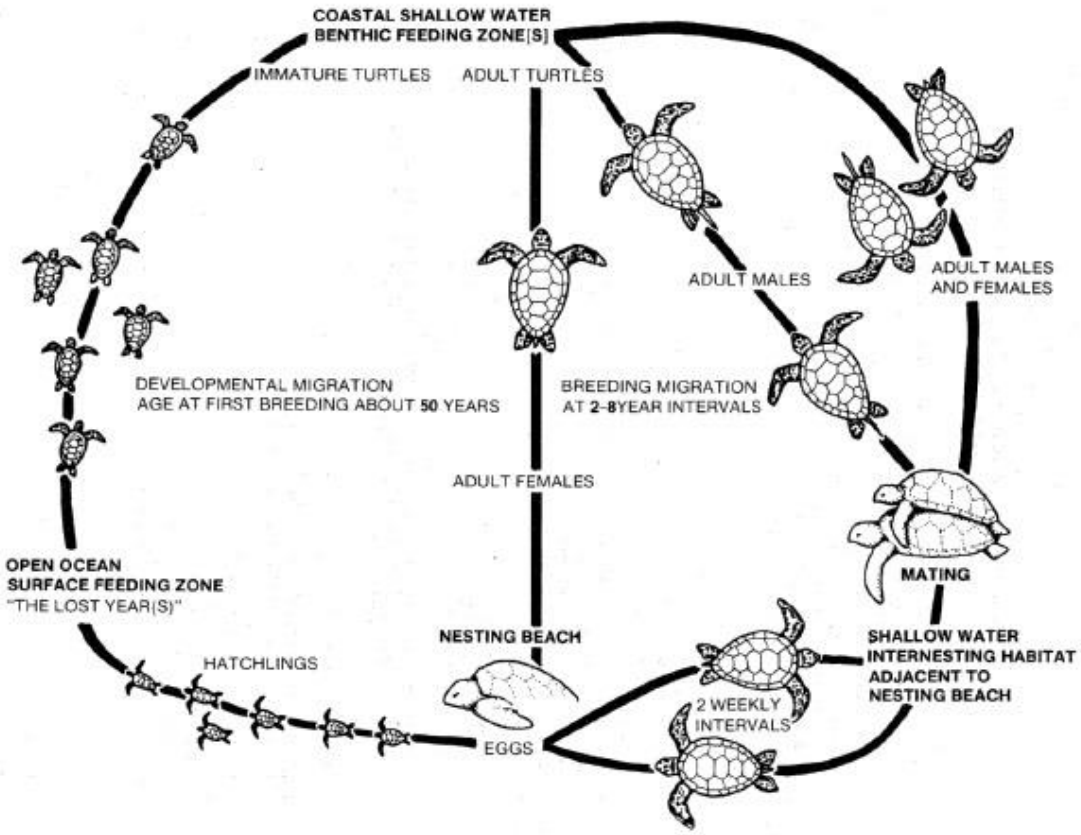


Figure 1.1.3.1. Green turtle life cycle diagram.

(Taken from: https://www.coraldigest.org/index.php/Turtles)

1.1.4. Global conservation status and threats

Mortality is very high at each life stage of sea turtles (Hamann et al., 2010). There are several negative pressures on sea turtle populations, such as climate change, coastal development, pollution, destruction of nesting and foraging habitats, incidental fisheries catch or conscious catch, egg collection, diseases and predation (Seminoff, 2004; Abreu-Grobois and Plotkin, 2008; Mortimer and Donnelly, 2008; Wallace et al., 2013; Casale and Tucker, 2015). Six of the sea turtle species are on the red list of International Union for Conservation of Nature (IUCN). According to the red list, global populations of the loggerhead, the leatherback, the olive ridley are categorized as vulnerable, the green turtle is categorized as endangered, and the hawksbill and Kemp's ridley are categorized as critically endangered. Those six sea turtle species' global populations have decreasing trend according to IUCN (Marine Turtle Specialist Group, 1996;

Red List Standards and Petitions Subcommittee, 1996; Seminoff, 2004; Abreu-Grobois and Plotkin, 2008; Mortimer and Donnelly, 2008; Wallace et al., 2013; Casale and Tucker, 2015). Furthermore, the Mediterranean sub-population of the loggerhead is categorized as least concern and the current population trend is increasing (Casale, 2015). Atlhough the green turtle is listed as critically endangered (Hilton-Taylor, 2004), there is a recent increasing trend detected in northern Cyprus green turtle nests (Stokes et al., 2014).

The studies and conservation efforts have mostly focused on the sea turtle nesting beach monitoring and conservation where female individuals and hatchlings can be readily observed (Bowen and Karl, 2007). However, there were also several studies focused on hatchling emergence, nesting selections, and anthropogenic effects on sea turtles. Adam et al. (2007) conducted a study in Greece to understand the loggerhead hatchling emergence patterns. The results showed that loggerhead turtles emerge mostly during night with moderate synchrony in Kyparissia Bay. Adam et al. (2007) suggested that asynchronous emergence caused by temperature difference within the clutch and decrease the predation risk both on land and in sea. Kaska et al. (2010) conducted a study in Dalaman in order to understand the factors affecting loggerhead turtles' nest site selection. According to the study, the loggerhead turtle chose undeveloped parts of the nesting beaches and avoid the disturbance of hotel complex and its facilities (Kaska et al., 2010). There is another study conducted in Akyatan to determine the nesting preferences for both species demonstrates that green turtles prefer vegetated areas while loggerhead turtles prefer non-vegetated areas (Türkozan et al., 2011). It was also suggested that the green turtle nests in vegetated areas have higher success. Although the existing studies on several nesting sites, more knowledge is needed to fully comprehend the pressures on sea turtle breeding sites.

The impacts on sea turtle populations can be grouped as anthropogenic and natural impacts. The fishery has one of the highest anthropogenic pressure on sea turtle populations' survival for several reason; bycatch or post release mortality (decompression), destruction of foraging areas by trawling, ghost fishing by drift nets (Rees et al., 2016). Snape et al. (2016) suggested that there is a high threat of bycatch among important neritic habitats in Libya and Tunisia. Additionally, it was suggested that the small scale fisheries might cause death along North Cyprus, Syria and Egypt near shores (Snape et al., 2016). Another study reported 67 dead or injured sea turtles and main causes were identified as fishing activity related injuries and drowning due to entanglement in trawling nets (Kaska et al., 2004). There are some strategies for fisheries to reduce the pressure: national legislation, international agreements, marine protected

areas, closures of fisheries (spatial or temporal), gear engineering solutions such as usage of turtle excluder devices for bottom trawling fisheries (Epperly, 2003), LED lights (Wang et al., 2010) and buoyless nets (Peckham et al., 2016).

Coastal development and pollution (solid debris, chemical, light, noise etc.) comprise the other main anthropogenic impacts on sea turtle populations (Hamann et al., 2010). According to study conducted in Brazil, 13.2% of the 38 juvenile green turtles died because of the anthropogenic debris (Bugoni et al., 2001). Another study conducted in Samandağ with green turtles shows that the amount of solid waste is increasing during summer and early autumn which is negatively correlated with the amount of hatchlings reaching to sea (Özdilek et al., 2006). A study revealed that hatchlings orientation was negatively effected in the presence of artificial lights (Lorne and Salmon, 2007). To mitigate the negative impact of light pollution, usage of low sodium pressure lamps and light shield across nesting beaches have been proposed (Salmon et al., 2000), More effective sea turtle conservation often requires development of ecotourism (Tisdell and Wilson, 2005), public education (Pretty and Smith, 2004) and volunteer programs (Campbell and Smith, 2006).

All species of sea turtles have temperature dependent sex determination like all the other reptile species, with the higher chance of female offspring produced at the higher temperatures (Yntema & Mrosovsky, 1980). Therefore climate change is one of the biggest concerns of researchers due to its effects on the sex ratio and survival success of sea turtles hatchlings (Girondot & Kaska, 2014; Santidrián Tomillo et al., 2015). According to a modelling study conducted using air temperature showed that loggerhead populations in America will become highly female biased with 1°C warming, and experience high level of hatchling mortality with 3°C warming (Hawkes et al., 2007). Özdilek et al. (2016) also revealed that the sex ratio is highly female biased in Samandağ. There are several strategies suggested such as shading or relocation of nests as well as sprinkling to reduce the impact of climate change on sea turtle nests (Hill et al., 2015). However, more knowledge about the risks and effectiveness of these measures are needed before any implementation (Rees et al., 2016). The climate change also affects the sea levels with consequent impacts on nesting habitat morphology (Katselidis et al., 2014), wave regimes and currents (impact on foraging areas) (Osorio et al., 2014), and sea turtle dispersal potential (Boyle et al., 2014). It is also reported that the timing of the loggerhead nesting has been getting earlier following the sea temperature (Weishampel et al., 2004) corroborating the previous studies showing similar shifts in migration and breeding phenology associated with global warming (Walther et al., 2002).

There are many different natural predators of sea turtles all around the world's nesting beaches depending on the region; such as dogs, coatis, vultures (Fowler, 1979), foxes, jackals (Kasparek et al., 2001), sea birds, ghost crabs (Hendrickson, 1958). The effect of natural predation may be substantial. Accordingly, predator cages (Yerli et al., 1997), predator removal and meso-predator release (Barton and Roth, 2008) have been proposed as conservation measures against predation. Furthermore, diseases also have impact on the sea turtle survival. Fibropapillomatosis (FP) is a disease caused by herpesvirus (Ackermann et al., 2012), widespread among sea turtle populations. FP was associated with disrupted ecosystems (indirect anthropogenic impact), however the mechanism is still unknown (Rees et al., 2016). Electrotherapy (Brunner et al. 2014) and phototherapy (Sellera et al., 2014) haven been suggested for treatment against FP.

Sea turtles distribute across wide geographic ranges along breeding and feeding areas occupied by adults and geographically distinct habitats for immature sea turtles at each ontogenetic life stage (Musick and Limpus, 1997). Sea turtles have complicated population structures formed by female nest site fidelity and natal homing behavior, male mediated gene flow and population overlaps during migration (Bowen and Karl, 2007). Understanding this complex population structure is essential to quantify threats and design conservation management as response to those threats (Bolker et al., 2007). Hamann et al. (2010) reported that it is important to determine relevant spatial scale that status assessments should cover for useful conservation efforts. Moreover, determining the status assessments at global and regional scales are important to design and implementing the conservation tools assisting national management policy. Furthermore, an essential problem in studies of sea turtles demography is determination of the demographic units (Chaloupka and Musick, 1997) along with their abundance, fecundity, sexual age maturity and sex ratios, and survival rates. Rees et al. (2016) suggest that it is important to understand the demography for population models and conservation planning. Therefore, integration of different methods such as genetic analyses and site based monitoring may facilitate accurate definitions of population segments at multiple biological and spatial scales to address different management and research challenges (Wallace et al., 2010).

1.2. Mediterranean Sea turtles

There are two sea turtle species breeding in the Mediterranean Sea: the loggerhead and green turtles. Additionally, 3 species were also recorded as vagrants on previous studies in the Mediterranean Sea: leatherback, Kemp's ridley and hawksbill turtles. The records of hawksbill and Kemp's ridley are very limited in the Mediterranean. Laurent and Lescure (1991) reported

seven records for the the hawksbill turtle in the Mediterranean. It is suggested that nesting beaches may occur along Red Sea in the Egypt and Sudan (Meylan and Donnelly, 1999). For the Kemp's ridley there are captured records in Malta, Spain, Italy (Brongersma and Carr, 1983; Tomas et al., 2003; Insacco and Spadola, 2010). There have been several records on the occurrence of leatherback sea turtle in the Mediterranean, in total 411 individuals in the Mediterranean with 152 of them from Italy (Casale et al., 2003). There are additional capture data from Syria and Israel (Rees et al., 2004; Levy et al., 2005). The leatherback turtle has also been observed along Turkish coasts of the Mediterranean with the first record in 1983 in Antalya (Baran and Kasparek, 1989). There has been several observations from İskenderun Bay, Mersin and İzmir (Oruç et al., 1996; Taşkavak and Farkas, 1998; Sönmez et al., 2008, Taşkavak et al., 2015). Furthermore there is softhsheled Nile turtle (*Trionyx triunguis*) identified as brackish water, African species but also distribute widely along the Mediterranean coasts; Turkey, Israel, Egypt, Syria, Lebanon (Kasparek, 2001).

The loggerhead and green turtles have nesting beaches in different countries along the Mediterranean coast. The most important loggerhead nesting beaches in the Mediterranean are found Greece (Margaritouilis et al. 2003), Turkey (Baran and Kasparek 1989), Libya (Laurent et al. 1997) and Cyprus (Broderick et al. 2002), respectively. The other nesting beaches with lower density are in Tunisia, Syria and Israel (Kasparek et al. 2001). For the green turtle the most important nesting beaches in the Mediterranean are found in Turkey (Baran and Kasparek, 1989; Yerli and Demirayak, 1996) and Cyprus (Broderick et al., 2002).

According to studies conducted in the Mediterranean Sea and Atlantic Ocean using microsatellite (Carreras et al., 2011) and mtDNA control region (Naro-Maciel et al., 2014), the gene flow was very low and genetic structuring was high between Atlantic and Mediterranean loggerhead and green turtle populations (Carreras et al., 2011; Naro-Maciel et al., 2014). According to Carreras et al. (2011) despite the Atlantic individuals and Mediterranean loggerhead populations sharing the same feeding grounds in western Mediterranean, there was no gene flow between two populations. Carreras et al. (2011) explained this as either the individuals coming from different populations were juvenile or the encounter probability is very low because of the frequency difference of their presence. This suggestion was also supported by mtDNA evidence of samples taken from bycatch juveniles in the Mediterranean nesting areas (Laurent et al., 1998).

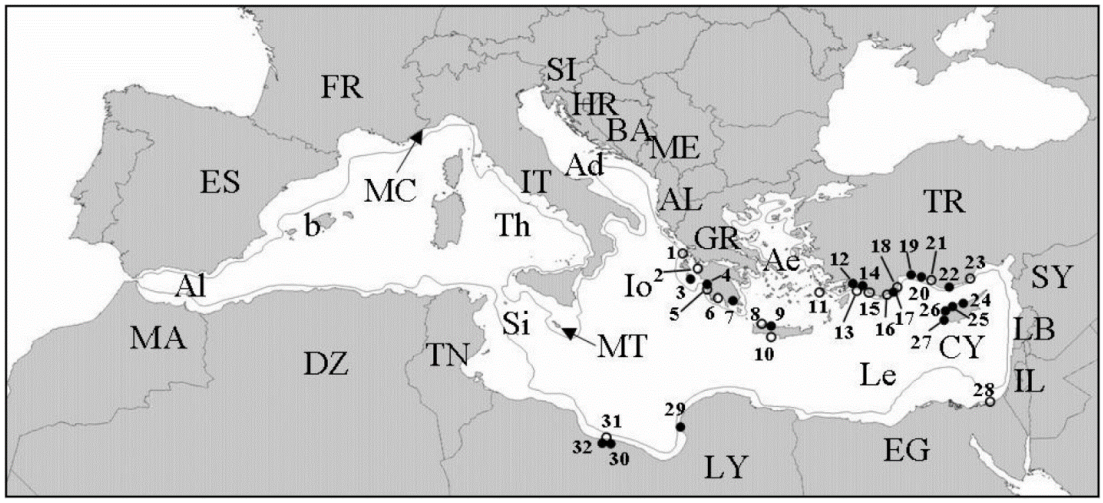


Figure 1.2.1. Major nesting beaches (≥50 nests/yr) of loggerhead turtle in the Mediterranean. Closed circles: >100 nests/yr, open circles: 50-100 nests/yr. (Retrieved from Casale and Margaritoulis, 2010). 1: Lefkas Isl, 2: Kotychi, 3: Zakynthos Isl., 4: Kyparissia Bay, 5: Beaches adjacent to Kyparissia town, 6: Koroni, 7: Lakonikos Bay, 8: Bay of Chania, 9: Rethymno, 10: Bay of Messara, 11: Kos Isl., 12: Dalyan, 13: Dalaman, 14: Fethiye, 15: Patara, 16: Kale, 17: Finike-Kumluca, 18: Çıralı, 19: Belek, 20: Kızılot, 21: Demirtaş, 22: Anamur, 23: Göksu Delta, 24: Alagadi, 25: Morphou Bay, 26: Chrysochou Bay, 27: Lara/Toxeftra, 28: Areash, 29: Al-Mteafla, 30: Al- Ghbeba, 31: Al-thalateen, 32: Al-Arbaeen.

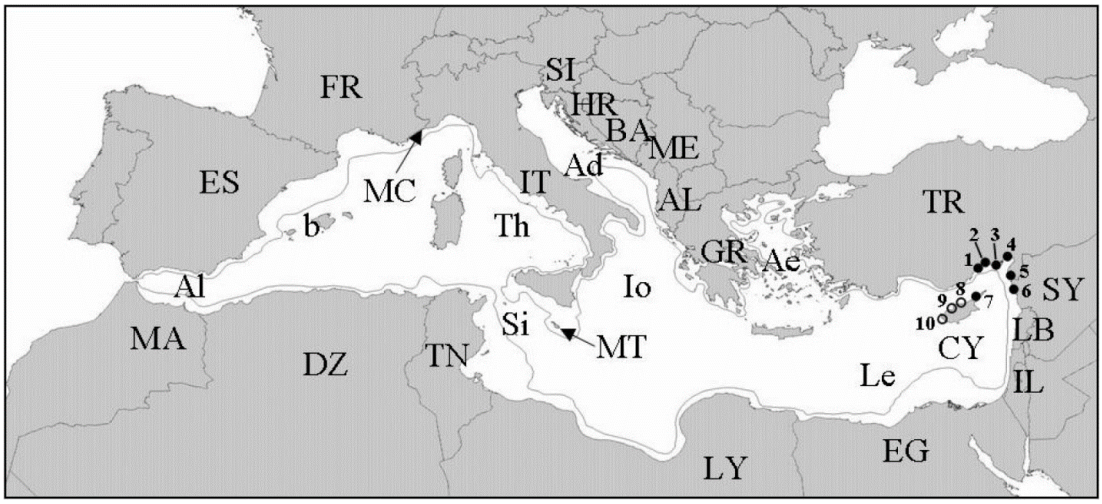


Figure 1.2.2. Major nesting beaches (>40 nests/yr) of green turtle in the Mediterranean Closed circles: >100 nests/yr, open circles: 40-100 nests/yr. (Retrieved from Casale and Margaritoulis, 2010). 1: Alata, 2: Kazanlı, 3: Akyatan, 4: Sugözü, 5: Samandağ, 6: Latakia, 7: North karpaz, 8: Alagadi, 9: Morphou Bay, 10: Lara/Toxeftra.

Previous studies demonstrated that there is also a genetic structuring within the Mediterranean populations. Carreras et al. (2007) suggested that according to nDNA data from Greece, Israel and Cyprus loggerhead turtle populations indicated that these countries form different breeding population units as well as Turkey which is relatively close to the Cyprus population. Another study showed that there was significantly high genetic variation in the loggerhead turtle populations in Turkey and Greece/Cyprus (Laurent et al., 1998). A study conducted in Turkey and North Cyprus nesting beaches on green turtles using both mtDNA d-loop and microsatellite markers suggested that every nesting beach should be considered as different management units in Mediterranean (Bagda et al., 2012).

In addition to the phlogeny and population studies, there are other studies about hybridization and multiple paternity among the Mediterranean populations. A study that conducted in Zakynthos revealed multiple paternity using microsatellite markers in 14 nests out of 15 with at least five males contributing to two nests (Zbinden et al., 2007). Another multiple paternity study was conducted in Dalyan and revealed multiple paternity among 7 out of 10 females (Sarı et al., 2017). The only hybridization and introgression event was recorded in Sicily within the Mediterranean Sea between loggerhead, hawksbill and green turtles (Garofalo et al., 2012).

1.2.1. Sea turtle conservation in Turkey

Sea turtles nest along 2577 km of the Mediterranean coast of Turkey, 606 km of which are suitable beaches (Baran and Kasparek, 1989). There are 25 nesting beaches hosting large populations identified in Turkey both for loggerhead and green turtles (Türkozan and Kaska, 2010). It has been estimated that the annual number of loggerhead turtle nests in Turkey range between 769 and 3521, and the annual number of green turtle nests in Turkey range between 452 and 2051 (Türkozan and Kaska, 2010). Please see Table 1.2.1.1. for summary of existing data for protected nesting beaches.

Common threats for terrestrial sea turtle breeding habitats in Turkey are human presence, coastal construction, pollution (marine debris and chemical), artificial lighting, beach restructuring, vehicle driving (terrestrial and marine), erosion and predation. Most of those problems are caused by the big holiday villages and hotel complexes. They use heavy machinery for beach cleaning and maintenance, and carry sand from one place to another (Türkozan and Kaska, 2010). These hotels also cover the beach in front of their complexes with sunbeds and umbrellas, and put spot lights for the night activities. Other constructions near the sea may cause problems

too. There was an example of accidental Soda-chromium factory waste discharge occurred in Kazanlı in 2001. It resulted by the death of over 30 loggerheads (Aureggi, 2001). Furthermore, predation is one of the main threats on sea turtle populations of Turkey. The common predators on sea turtles hatchlings and adults are red foxes (*Vulpes vulpes*), badgers (*Meles meles*), golden jackal (*Canis aereus*) (for the eastern part of the Turkey), crabs, dogs and birds (Türkozan and Kaska, 2010). Lastly, collision with sea vehicles and incidental bycatch are threats on sea turtles in marine habitat. There are records of adult turtle deaths both accidentally and intentionally by fishermen after they become entangled in the fishing nets (Türkozan and Kaska, 2010).

Sea turtle meat or eggs are not consumed by human in Turkey, however from 1950 to 1970, sea turtle hunting was widespread on the eastern Mediterranean coast. In this period, a factory in İskenderun bought harvested sea turtles from the local people to export to Europe (Baran and Kasparek, 1989). Since 1973, after publication of the 1380th Water Products Circular, collecting and hunting sea turtles have been forbidden in Turkey (Laurent et al., 1998). There are also other national laws in Turkey for sea turtle protection such as 3621st Coastal Law, 2873 National Park Law, 2872nd Environment Law and 2863rd Law of Protection of Nature and Culture. Additional to the national laws, Turkey has been part of international conventions such the Paris Declaration (since 1983), Bern Convention (since 1984), Barcelona Convention (since 1988), Rio Convention (since 1996) and CITES (since 1996) (Sönmez, 2016).

Majority of the large nesting beaches in Turkey have been declared as "sea turtle nesting beaches" by the Ministry Forest and Water Affairs. Some parts of Dalyan, Dalaman, Belek, Göksu Delta, Patara and Fethiye beaches are specially protected areas. Additionally, some parts of Dalyan, Dalaman, Belek, Kale, Gazipaşa, Anamur, Akyatan beaches and whole Çıralı, Alata and Kazanlı are protected under the natural SIT status. Moreover, Göksu Delta and Akayatan are Ramsar areas. However Samandağ, Kumluca, Tekirova and Kızılat have no protection status.

Although Turkey has all the regulations and legislation for sea turtle conservation, they are poorly implemented on some beaches (Türkozan and Kaska, 2010). Additionally, it is difficult to regularly monitor all beaches along their complete coastal track for the entire nesting period to have a more comprehensive information about their status. Furthermore, more studies on marine areas on the interaction with fishermen have been needed to fill the gap about bycatch, foraging areas and genetic stock (Türkozan and Kaska, 2010).

Table 1.2.1.1. Summary of existing data of major nesting beaches in Turkey. (Retrieved from Türkozan and Kaska (2010))

Beach name	Length of the beach	Range of nests numbers	
		C. caretta	*C. mydas*
Ekincik	1	9-12	
Dalyan	4,7	57-330	
Dalaman	10.4	69-112	
Fethiye	8.3	72-191	
Patara	14	35-127	2-2
Kale	8.5	39-109	
Fenike	21	75-305	0-7
Çıralı	3.2	23-96	
Tekirova	3.7	4-23	
Belek	29.3	68-819	2-8
Kızılot	15.7	50-270	0-3
Demirtaş	7.8	41-137	
Gazipaşa	7	14-53	
Anamur	12.2	146-674	1-1
Göksu Delta	25.6	36-151	3-20
Alata	3	16-32	20-198
Kazanlı	4.5	2-26	73-403
Tuzla	25		4-9
Akyatan	22	3-31	108-735
Karataş	7		3-3
Ağyatan	8.5	2-2	0-3
Yelkoma	23.1		2-3
Sugözü	3.4		213-213
Yumurtalık	6	1-1	1-3
Samandağ	14.2	7-20	20-440
Total	289.1	769-3521	452-2051

1.3. Concept and Synthesis

Sea turtles captured the interest of researchers worldwide because of their interesting phylogenetic history, complex ecological interactions and the urgent need of conservation worldwide. This has led to variety of studies and abundant literature on their biology and conservation. However, six of the sea turtle species are still on the IUCN Red list globally categorized as vulnerable, endangered or critically endangered (Wallace et al., 2010). The threats are varied and have differential effect on different populations of these sea turtles. Therefore, there is still a need for advancement of our understanding of sea turtle breeding biology, their population structure in smaller scales and current anthropogenic impacts to lead for more effective conservation implementations and better management (Rees et al., 2016). Understanding the distinct populations of sea turtles and revealing their meta-community structure is crucial to develop population and site-specific monitoring and conservation methods. Overall, better conservation management of sea turtles requires varied implementation at different spatial scales covering both intra- and inter nesting populations and nesting sites.

The identification of sea turtle meta-populations is mainly done through genetic differentiation caused by natal homing. There has been great progress of knowledge about male and female gene flow incorporating bi-parentally inherited nuclear DNA (nDNA) and maternally inherited mitochondrial DNA (mtDNA) (Dutton et al., 2013) to define demographically independent populations. Mitochondrial DNA is useful to resolve female nest fidelity and homing behavior and identifying nesting populations. Sea turtle females show phylopatry to nesting areas and both females and males might show phylopatry to breeding areas adjacent to nesting areas (FitzSimmons et al., 1997). However, sea turtle males have also been observed not to show phylopatry to their ancestral breeding areas and can mate at feeding areas and migratory corridors where they encounter with females from different nesting populations (FitzSimmons et al., 1997). This male mediated gene flow that connect nesting populations can be quantified via nuclear DNA since, it shows contributions of both male and female parents (Bowen and Karl, 2007). Genetic studies also help to identify the structure within the meta-populations by grouping nesting sites with genetic similarity between populations (Rees et al., 2016). Additionally, genetic studies can give information about degree of dispersal and exchanges within the meta-populations that helps understanding of extinction-recolonization histories.

In the first chapter of this thesis, we tried to understand the genetic structure of the eastern Mediterranean loggerhead and green turtles to elucidate their meta-population genetic structure. Four different nesting sites were sampled and analyzed: Hatay, Mersin, Antalya and Northern

Cyprus. Two markers: nuclear intron R35 and mitochondrial COI were amplified on muscle and dermal tissues taken from dead hatchlings those found during nest excavations. For R35 marker, 131 loggerhead, 104 green and 4 softsheled Nile turtle samples were amplified in total and aligned together with 15 samples (2 loggerhead, 7 green, 6 softsheled Nile turtle) taken from NCBI database. For COI marker, 54 selected samples in total were amplified and aligned successfully. Analyses of the loggerhead and green turtle populations based on R35 marker revealed high level of nesting-site specific haplotypes. The results showed the Mersin loggerhead turtles have the highest amount of haplotypes among all the other nesting populations, which is represented by only METU Erdemli Campus. Furthermore, hybridization and multiple paternity detected among Antalya and Mersin samples. In total 30 individuals (14 Mersin, 16 Antalya) from 23 nests (8 from Mersin, 15 Antalya) revealed as hybrids crossing between female loggerhead and male green turtle. In total 9 multiple paternity detected among those 23 hybrid nests, with female loggerhead mating at least 2 males (one loggerhead one green turtle). According to study the eastern Mediterranean loggerhead turtle populations were clustered as North Cyprus and Antalya/Mersin groups while green turtle populations were clustered as Antalya and Hatay/Mersin/North Cyprus groups. Hybridization among sea turtle populations of the eastern Mediterranean has been reported the first time in the present study. Moreover, it is revealed that each nesting site should be considered as different management units according to high amount of site specific haplotypes. Especially, high haplotype diversity and genetic polymorphism among loggerhead populations in METU Erdemli Campus, despite the low annual number of nests and the small area of the nesting beach shows that the contribution of the small nesting sites to the metapopulation should not be underestimated. Overall, the first chapter emphasizes that genetic studies provides rapid and insightful knowledge about inter and intra population structure of sea turtle populations, which facilitates determining the management units and improving the present conservation plans.

One of the key elements for the ecology of the sea turtle populations is the factors affecting hatchling production. Recent studies have focused on biotic and abiotic factors influencing hatchling survival, embryonic development affected by changing temperatures (Kılıç and Candan, 2014), predators (Burger and Gochfeld, 2014), oxygen availability (Cheng et al., 2015), emergence timing and pattern (Glen et al., 2005). Especially, there is still a need for information on climate change effects on sea turtles and the available studies are mostly limited to the breeding populations of the loggerhead, green and leatherback turtles in the North Atlantic and Pacific (Rees et al., 2016). It is expected that sex ratio, incubation duration and hatchling survival might be greatly affected from the climate change directly (Özdilek et al., 2016).

Therefore, the need for further studies in the Mediterranean Sea, South Atlantic and Indian Ocean have been emphasized (Rees et al., 2016).

The great majority of the available knowledge is based on labor intensive breeding beach monitoring studies or opportunistic observations and thus most of the data is available as grey literature (Rees et al., 2016). It has been suggested that every beach along the sea turtles' nesting areas should be regularly monitored for at least 3 successive years to have an idea about the status of nesting beaches lacking data on sea turtle hatchling ecology (Türkozan and Kaska, 2010). Accordingly, Sönmez (2016) suggested that the efficiency of the sea turtle monitoring and conservation depend on long term studies. Therefore, using advancing technology for more effective and less costly hatchling monitoring is needed for better understanding sea turtle hatchling biology at higher spatial and temporal resolution. To answer these problems the second chapter of the thesis covers the implementation of IR camera systems for better understanding of sea turtle hatchling emergence and behavior. The study conducted in METU Erdemli Campus beach during 2014 and 2015 nesting seasons. IR cameras installed on four loggerhead and one green turtle nests and hatchling emergences recorded continuously. Video recordings were analyzed with automatic screen captures with 30 second-intervals in order to understand temporal patterns, incubation durations and group emergence patterns. According to the study, asynchronous emergence reported and 98.6% of hatchlings emerged during night with a peak activity between 21:00 and 00:00. Incubation period varied between 60 and 65 days (from egg deposition to last emergence). Emergence activity continued between 1 and 22 days after the first emergence. The present study provided a very detailed insight and accurate information on the hatchling behavior and emergence pattern of sea turtles in METU Erdemli Campus. We suggest that the IR cameras are useful, time saving methods and require less labor than traditional monitoring methods, if the logistics constraints allow their implementation.

The present thesis provides an improved understanding of sea turtle nesting population structure and provides the first records of hybridization and multiple paternity in the eastern Mediterranean. We also suggest that the usage of nuclear and mitochondrial markers together is essential to understand complex dynamics such as hybridizations and even multiple paternity with interbreeding. We also report that METU Erdemli Campus beach, although a small nesting site, is a very important nesting site with high genetic variance contribution to the loggerhead metapopulation as well as revealing hybrid and multiple paternity occurrence. Besides, location of the campus is a transition area between green turtle dominated on the east and loggerhead turtle dominated o the west nesting sites. Therefore, long term monitoring and conservation of

these small scattered nesting sites are important for the maintenance of healthy sea turtle metapopulations. Furthermore, IR camera can be an important tool for sea turtle conservation facilitating rapid and accurate assessment of hatchling dynamics and success. IR cameras remove the relativity of traditional hatchling monitoring with higher resolution. However, the present study conducted in restricted area which was suitable for IR camera monitoring thus location specific constraints should be considered for further implementation. Overall, this thesis provided further insights on the genetic structure of the Eastern Mediterranean sea turtle populations at larger scales and hatchling emergence patterns at smaller scales.

Further studies might be benefitted by taking into account both the novel findings and the limitations of this thesis. Recording of detailed morphological data and usage of different markers should be considered in case introgression is detected in the samples. In the future, the present study may be repeated with extended scope covering more area and wider sample sizes to estimate how common the hybridization among sea turtles is and how it will affect the future populations. Lastly, in addition to IR monitoring, temperature data loggers located within the nest could be complementarily used for further studies for sex ratio estimations in the nests.

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CHAPTER I

GENETIC STRUCTURE OF SEA TURTLE POPULATIONS IN THE EASTERN MEDITERRANEAN

2.1. Introduction

Genetic methods are quick to answer many questions and fill the gaps in the sea turtle conservation studies and that is why they have gained increasing attention in the last decades (Bowen and Witzell, 1996). Genetic studies are now essential to improve present knowledge about sea turtle biology and evolution, and to lead better management policies for sea turtle conservation (Bowen and Karl, 2007).

There have been many studies about sea turtle phylogeny, genetic structure of populations, hybridization, multiple paternity etc. (Encalada et al., 1996; Moore and Ball, 2002; Fujita et al., 2004; Vilaça et al., 2012), using different molecular markers. However, most of the conservation genetics studies have focused on mtDNA markers, since most of the population's reproductive output is driven by females due to their natal homing (Bowen and Witzell, 1996). Although there are many different mtDNA markers used by different studies, analyses on mtDNA control region provided similar conclusions indicating limited gene flow between Atlantic and Mediterranean green and loggerhead turtle populations (Encalada et al., 1996; Encalada et al., 1998; Laurent et al., 1998; Kaska et al., 2000). Those studies also showed that although spatially close nesting populations share same types of haplotypes, differences in haplotype frequencies have also been observed such as in Florida and Georgia (Encalada et al., 1998; Laurent et al., 1998), and Turkey and Greece/Cyprus nesting populations (Laurent et al., 1998).

Templeton et al. (1990) suggested that both mtDNA and nDNA markers are equally suitable for species with no sex differences in dispersal of gametes to determine the genetic structure. However, for species such as marine turtles with limited female-mediated dispersal and gene flow between rookeries (because of female natal homing) mtDNA and nDNA markers may provide different results on genetic structure and gene flow (Karl et al., 1992; Palumbi and Baker, 1994). According to studies conducted in the Mediterranean Sea using both nuclear microsatellites and mitochondrial control region markers, gene flow, polymorphism and genetic structuring between nesting populations were significantly higher in nDNA than mtDNA (Carreras et al., 2007; Bagda et al., 2012). Conversely, another study showed that mtDNA control region has higher haplotype variation than nDNA microsatellites among breeding populations (Bowen et al., 2005). Discordancy has also been observed between the two

rookeries nDNA and mtDNA data by Naro-Maciel (2014). Different genes have different evolutionary history thus using only one type of marker for conservation genetics studies may mislead the researchers or provide inadequate information about management units (Rubinoff, 2006). Moreover, it is known that there are inter generic turtle hybrids and using only mtDNA would be insufficient to document these complex processes and thus nDNA marker is necessary (Murphy et al., 2013).

The first suggestion of hybrid occurrence among sea turtle species was given by Carr (1952). The first green sea turtle hybridization event was reported in the Atlantic (Surinam) region using morphological character (Wood et al., 1983). Despite complementary mtDNA sequence may detect hybridization (morphological evidence comes from one species, mtDNA evidence comes from another species), additional nDNA usage is essential for accurate result in different cases and deeper knowledge such as introgression (James et al., 2004). For example, in a previous study 14 hybrids between loggerhead and olive ridley were reported based on morphological features of two groups: 9 individuals with the loggerhead morphology, 5 individuals with mixed of both species (Reis et al., 2010). This difference may be the clue of an introgression but using nDNA marker is suggested as necessary tool for the solution of problem (Reis et al., 2010). Another study pointed out the necessity of the use of single copy nuclear loci markers additional to mtDNA to reveal of second-generation individuals' potential existence (Karl et al., 1995).

Several nDNA markers including exon (coding) and intron (non-coding) markers for reveal hybridization processes were used by Vilaça et al. (2012) and Garofalo et al. (2012). Garofalo et al. (2012) reported the first hybridization event in the Mediterranean-Sicily. Additionally, a rescued juvenile loggerhead turtle that have three different species' morphology (loggerhead, green and hawksbill turtles) revealed as an F2 individual using mtDNA D-loop and nDNA markers (Garofalo et al., 2012). According to the study, both mtDNA and nDNA sequences were identical with loggerhead genotype and there was no clue for any other species. It is explained with backcross between F1 hybrid and loggerhead turtle (Garofalo et al., 2012). Vilaça et al. (2012) used 12 different nDNA markers (3 SCN, 4 microsatellites, 4 exons and 1 intron (R35)) to analyze 387 individuals that include 66 hybrid and involving three species those previously identified by Lara-Ruiz (2006) from Brazil. Fifteen introgressions were observed in total of 66 hybrids and a new hybrid class revealed as mixture of loggerhead x hawksbill x green turtle which was known as offspring of a loggerhead x hawksbill turtles F1 female that breed at least from two different male (Vilaça et al., 2012). They also found two additional hybrids which were not identified as hybrid previously. One of those individuals was identified as hawksbill

according to both morphologically and mtDNA genome evidence (RFLP), but revealed as introgressed individual with nDNA analysis (Vilaça et al., 2012).

There are also differences within the mitochondrial and nuclear markers in themselves, providing different benefits for different concerns about sea turtle conservation. For barcoding and taxonomy concerns, it is more appropriate to use a part of mtDNA Cytochrome Oxidase subunit I (COI) gene since it has low inter- and intraspecific variation than other gene region and provide more accurate taxonomic information about the species (Rubinoff, 2006; Naro-Marciel et al., 2010). According to a study conducted with all seven sea turtles using both mtDNA D-loop (public data from GenBank and Archie Carr Centre for Sea Turtle Research) and COI (L-turtCOI, H-turtCOIc), higher inter- and intraspecific divergence was observed in D-loop region than COI gene, on the other hand COI was defined more appropriate gene region for taxonomic concerns (Naro-Marciel et al., 2010). Also the big database of COI gene, allows to compare samples very quickly that collected from all around the world (Naro-Marciel et al., 2010). There are several studies on sea turtles barcode region those used different COI markers either designed for other organisms or for sea turtles (SOCOF1, H8121, LCO1490, HCO2198, BLCO1490F, BHCO2198R, FishF1,FishR1, VF2, VR1) (Vargas et al., 2009; Naro-Marciel et al., 2010; Elmeer et al., 2011; Daza-Criado and Hermandez-Fermandez, 2014; Caracappa et al., 2016). COI is maternally inherited so it is important to define hybrids' parents.

Nuclear DNA markers have some advantages for conservation genetics studies such as having exon (coding) and intron (non-coding) parts those have different evolution rates (Fujita et al., 2004). Introns are relatively free from exon regions' functional constrains and tend to have/accumulate higher amount of mutation (Fujita et al., 2004). Although there are several nuclear exon markers such as RAG-1 (Krenz et al., 2005) and C-mos (Saint et al., 1998), exon regions are appropriate for the deeper divergence analyses (Fujita et al., 2004). Fujita et al. (2004) used nDNA R35 finger print protein for turtles as an intron marker for the first time. According to the study, although R35 has an excellent potential as phylogenetic marker for turtles it was impossible to align turtle sequences with distant outgroups' sequences due to the large difference between them. Fujita et al. (2004) suggested that R35 intron is not an appropriate marker for deeper phylogenetic relationship concerns (e.g. Sea turtles and birds) but provide insight and consistent information about turtle phylogenetic and intraspecific variation. Garofalo et al. (2012) also suggested that R35 intron marker can be used with universal primers for sea turtles and have specific positions for green and loggerhead turtle.

In the present study, we have focused on the Eastern Mediterranean *green* and loggerhead turtle populations using both nuclear R35 intron and mitochondrial COI markers to resolve hybridizations and population structures and to help constituting an effective conservation management strategy for those three important nesting sites. While the COI gene can provide a geographic structure and divergence network for the species, nDNA intron markers can provide complementary information on inbreeding, male dispersal, hybridization, effective population size and related questions. Thus using COI and nuclear intron markers together can provide better information for decision makers to manage the conservation/management units.

2.2. Materials and Method

2.2.1. Study Site

In this study, samples were collected from selected nesting beaches of the Eastern Mediterranean; 1) METU-IMS campus, 2) Belek and 3) Samandağ and 4) Northern Cyprus during 2015 nesting season (Figure 2.2.1.1).

The METU Erdemli Campus beach has 1.2 km long coastal area, approximately 10 km away from the Erdemli city center and 40 km away from Mersin city center. The study site is in the middle of the urbanized places and adjacent to the public beaches. In spite of that, the site is well protected and it has very limited human activity and fishing is prohibited. The area hosts both green turtles and loggerhead turtles during the nesting season. In total 8 and 5 nests for green turtles, 2 and 18 nests for loggerhead turtles have been observed during the 2013 and 2014 season respectively (Cihan, 2015). Beside of those two sea turtle species, the harbor of the METU Erdemli Campus supplies suitable habitat for a brackish/freshwater species *Trionyx triunguis* (softsheled Nile turtle) for all seasons.

Breeding site Belek is located in Antalya city, expands 29.5 km in the border of Serik and Manavgat (Canbolat and Nalbantoğlu, 2001). Between 1987 and 2000 (Canbolat, 2004) period 345 loggerhead turtle and 6 green turtle nests were observed on average in Belek region. Even though Belek has legal protection status and partially as natural site, it is threatened by high tourism activity and, wrong tourism and coastal development plans (Türkozan and Kaska, 2010).

Samandağ beach has 14 km length nesting site that formed by Asi river sediments and located in Hatay-Samandağ area (Türkozan and Kaska, 2010). According to existing data, the number of nests varies between 7-20 and 20-440 for loggerhead and green turtle respectively (Türkozan and Kaska, 2010; Sönmez and Özdilek, 2013). Sand mining and erosion are the main problems

for this nesting sites (Türkozan and Kaska, 2010). Despite Samandağ area is declared as "marine turtle nesting site" it has not a legal protection status yet (Türkozan and Kaska, 2010).

There are also important nesting sites for both loggerhead and green turtles at Northern Cyprus. During 2015 nesting season, 235 green turtle nests and 364 loggerhead turtle nests were observed (Snape et al., 2015). The green turtle nesting sites are fewer than the loggerhead turtle nesting sites and mainly located Alagadi, South Karpaz and west coasts (Fuller et al., 2010). According to Broderick et al. (2002) the Northern Cyprus contains 30% of the green and 10% of the loggerhead turtle nests within the Mediterranean nesting sites. It is illegal to harm, collect or disturb sea turtles under local legislations in Northern Cyprus (Fuller et al., 2010).

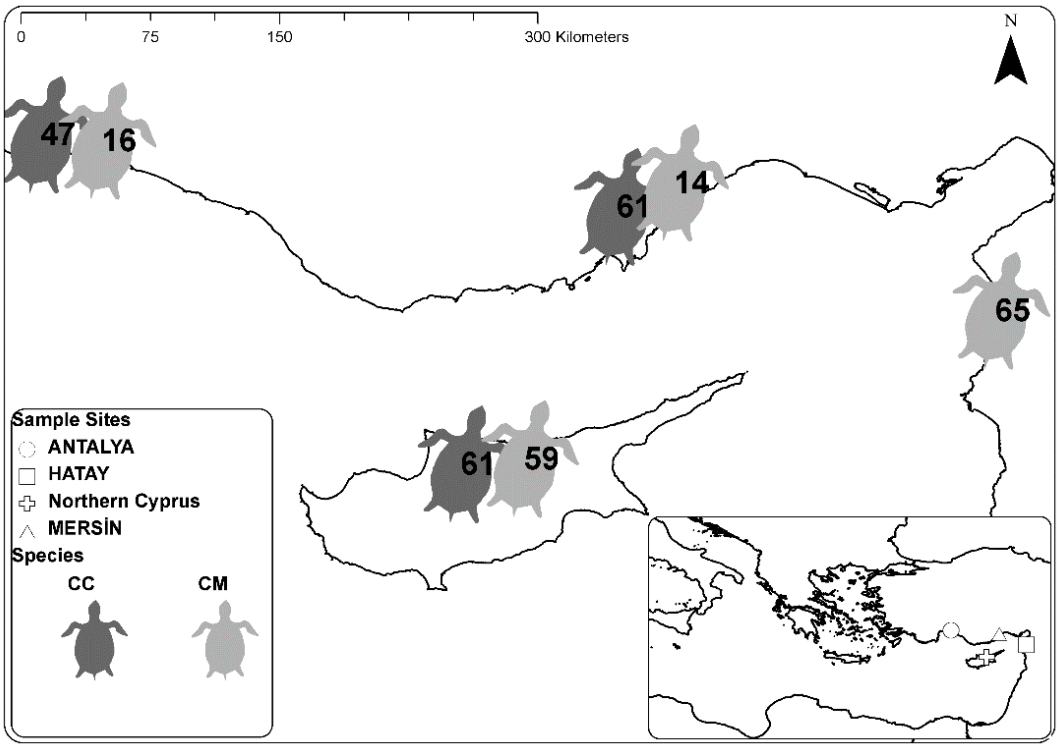

Figure 2.2.1.1. Study Sites and number of samples for each species

2.2.2. Sample collection

During 2015 nesting season, dead hatchlings were collected those found during the excavation in the nest chambers and patrolling along the beaches. Date, nest name, sample number, coordinates and species were labelled for each sample and restored in 70% ethanol. Species identified by mother crawling sign and plastron coloring of the hatchlings (dark coloring as

loggerhead, white coloring as green turtle). Detailed morphological identification were not implemented. For the hatchlings found on the beach that could not be certain about which nest those belong to, were not assigned to any particular nests. While complete body collection performed in Belek and METU Erdemli Campus; one front flipper collected for Samandağ, and small dermal tissue collected for Northern Cyprus. Three green and 20 loggerhead turtle nests have been recorded in 2015 nesting season in the METU Erdemli Campus beach. But samples collected from two green and 14 loggerhead turtle nests. Cyprus samples were taken from several nesting beaches along the Northern Cyprus coastline from north to the west (Balalan, Kaplıca, Kantara, Tatlısu, Smalls, Esentepe, Alagadi, Message, Lost, Monster, and Secret). Although loggerhead turtles nest along the entire sandy beaches along the coastline, the highest amount of loggerhead nesting occurs in three sites Alagadi, Akdeniz and Tatlısu.

Table 2.2.2.1. Tissue type and sample numbers for each species according to sampling sites. CC: Loggerhead turtle, CM: Green turtle, TT: Sofsheled Nile turtle.

Sampling Site	Sample Species	Sampled Nest Number	Sampled Hatchling Number	Sampled Adult Number	Tissue Type
METU Erdemli Campus	CC	14	61	3	Muscle
	CM	2	14	2	Muscle
	TT	-	-	2	Dermal
Northern Cyprus	CC	60	60	-	Dermal
	CM	59	59	-	Dermal
	TT	-	-	-	_
Belek	CC	33	48	-	Muscle
	CM	6	16	-	Muscle
	TT	2	5	-	Muscle
Samandağ	CC	-	-	-	_
	CM	20	65	-	Dermal
	TT	-	-	-	_

Muscle tissue extraction performed for those have complete body. While some of the nests were represented by multiple hatchlings, some of them were represented by only one hatchling. Details about the nest and samples were given in the Table 2.2.2.1. There was no specified interval between nesting dates of sampled clutches to prevent sampling of the same female's nests except Samandağ. Thus present samples might contain pseudo replication. A brackish water species softshelled Nile turtle was used as an out-group in the present study.

2.2.3. Genetic Analyses

2.2.3.1. DNA extraction

Genomic DNA was extracted from approximately 100 mg (North Cyprus samples were less) tissue using the CTAB protocol (Stewart and Via, 1993). After placement of 300 µL CTAB buffer (1 L CTAB buffer: 100 ml 1 M Tris HCl pH 8.0, 280 mL 5 M NaCl, 40 mL of 0.5 M EDTA, 20 g of CTAB {cetyltrimethyl ammonium bromide}, 580 ml ddH2O) in to the tissue tubes; sterilized pestles used for each sample to smash the tissue and homogenize the content. After the homogenization; 300 µL CTAB buffer, 50 µL Beta -mercaptoethanol, 2 µL Proteinase-K were added to each of the samples and mixed gently then incubated for 1 hour at 65°C, 650 rpm (TSS-2000 Turbo Thermo Shaker, INOVIA Technology). After incubation; samples were placed in ice and 500-µL chloroform: isoamyl alcohol (24:1 v/v) was added to the present content. The content was mixed until the mixture's color turn into milky white then centrifuged for 15 minutes at 13.000 rpm to separate the phases. The upper phase contains DNA of each sample was transferred to a new 1.5 ml Eppendorf tube and 300 µL isopropanol alcohol (-20^{o}C) was added on the samples then left in -20^{o}C overnight. The tubes were centrifuged for 10 minutes at 13.000 rpm to settle the DNA of the mixture. Settled DNA was washed via 70% ethanol for three times to purify; and waited for evaporate of ethanol completely. DNA pellet was resolved in 50 µL TE buffer. The amount of DNA from each sample was subsequently quantified by spectrophotometry. According to the results (varied 50-6000 ng/ml); samples were diluted in varied molecular biology grade water (A7398, AppliChem Panreac ITW Companies) depending on the concentration of DNA.

2.2.3.2. PCR Amplification

2.2.3.2.1. R 35 intron region

An intron part of the nuclear DNA (RNA fingerprint protein 35 markers; Table 2.2.3.2.2.1) was amplified for three species; green, loggerhead and softheled Nile turtles. Same conditions were used for amplification explained by Fujita et.al. (2004). Following the 5 minutes at 94°C enzyme activation step, 35 repeats of 30 seconds at 94°C for denaturation, 60°C 90 seconds for annealing, 120 seconds at 72°C for extension and 10 minutes at 72°C for last extension step performed. All PCR amplifications performed on T-100 thermal cycler (Bio-Rad). After PCR amplifications; R35 PCR products were displayed on 1.3% agarose gel to check the quality of the products (Figure 2.2.3.2.1.1-A).

Table 2.2.3.2.1.1. Primers names and types.

Primer Name	Sequence (5'-3')
FishF1	TCAACCAACCACAAAGACATTGGCAC
FishR1	TAGACTTCTGGGTGGCCAAAGAATCA
R35_F	ACGATTCTCGCTGATTCTTGC
R35_R	GCAGAAAACTGAATGTCTCAAAGG

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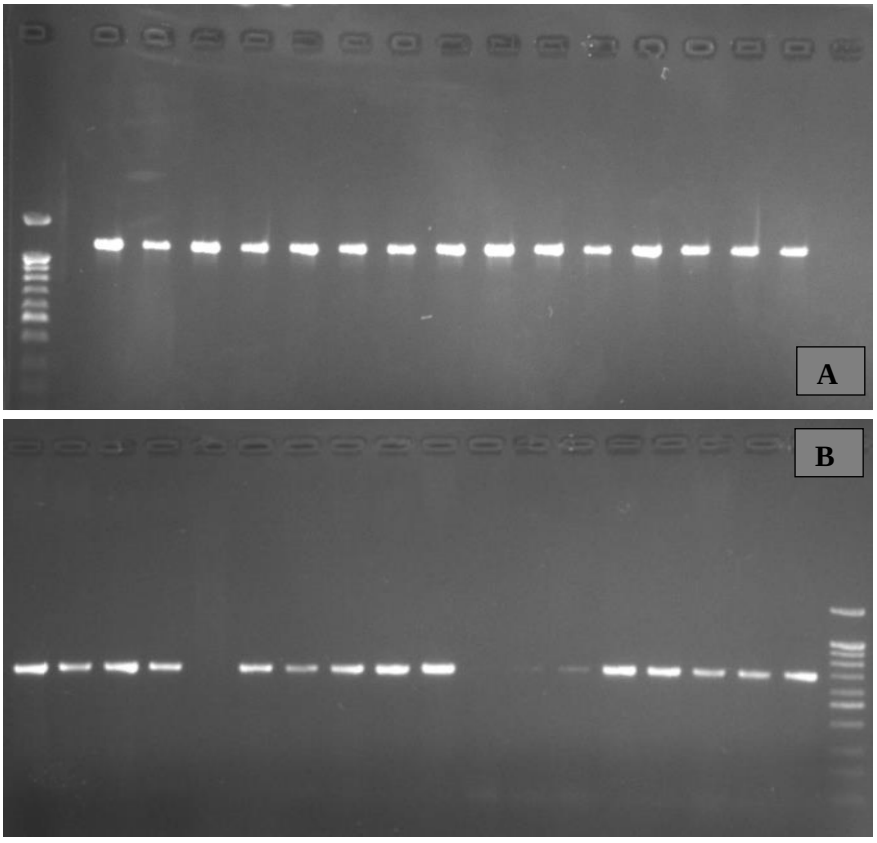


Figure 2.2.3.2.1.1. Agarose gel electrophoresis images of R35 (A) and Fish1 (B) PCR products.

2.2.3.2.2. Cytochrome oxidase subunit I (COI) gene

Mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using primers FishF1 and FishR1 (Ward et al., 2005) (Table 2.2.3.2.2.1) for the selected samples. Ward et al. (2005) PCR conditions were used. Following the 2 minutes at 95°C enzyme activation step, 35 repeats of 30 seconds at 94°C for denaturation, 54°C 30 seconds for annealing, 1 minute 72°C for extension and 10 minutes at 72°C for last extension step performed T-thermal cycler (Bio-Rad). To control the quality of the Fish1 PCR products were displayed on 1.2 % agarose gel (Figure 2.2.3.2.1.1.-B). Sequencing was performed by Macrogen Inc. (Seul-South Korea and Amsterdam-Netherlands) for both directions for all of the PCR products

2.2.4. Bioinformatics analyses

2.2.4.1. Sequencing, alignment, editing

The both directions R35 gene sequences were aligned using BIOEDIT version 7.0.9.0 (Hall, 1999) Clustal X (Thompson et al., 1997) software further refining the alignment by eye. Because several indels and repeating elements confounded assessments of homology, we removed these regions before performing any phylogenetic analyses.

Demographic history and neutrality tests were performed using the DNAsp version 5.0 software (Rozas and Rozas, 1999; Rozas et al., 2003). The number of polymorphic sites (Np), the number of haplotypes (Nh), nucleotide diversity (Pi), haplotype diversity (Hd) and the gene flow parameter (Nm) between the samples were estimated (Nei, 1973). The signatures of populations demographic changes in all species and populations were investigated with a test of neutrality, Tajima's D-test (Tajima, 1989;using 1000 simulated samples), and compared with Fu and Li's D* and F* (Fu and Li, 1993) test (1000 simulations), the former was considered as a more sensitive metric (Fu, 1997). The distribution of pairwise nucleotide differences (mismatch distribution) of the samples was calculated as an additional test for demographic expansion, using the Raggedness Index (r; Harpending, 1994).

Distances between haplotypes were estimated using the median joining algorithm with default settings for constructing the network (weight = 10 e = 0) in the program NETWORK version 4.6.1.2. (Bandelt et al., 1999).The maximum likelihood analysis was conducted using Mega 6 (Tamura et al., 2013). Kimura 2-parameter model was used with gamma distribution.

2.2.4.2. COI data submission and alignment

The sequence data, trace files and primer details for specimens were submitted to the Barcode of Life Data System [BOLD, http://www.boldsystems.org, (see Ratnasingham & Herbert, 2007)], which is available within the project file 'IMS-METU-Turtles'. The collection data and specimen images were listed in the same project folder. Sequence alignment was performed using both the Multiple Sequence Comparison by Log-Expectation (MUSCLE vs.3.8.31, Edgar, 2004) implemented on the BOLD system and BioEdit v.7.0.9.0 (Hall, 1999) software. Distance and barcode gap analyses were carried out using the tools implemented on the BOLD system. The divergences within and between species were calculated using the Kimura's two-parameter (K2P, Kimura, 1980) tool available in BOLD. The Barcode Gap analysis provides the distribution of the distances within each species and the distance to the nearest neighbor of each species. Haplotypes analyses were performed using the DNAsp version 5.0 software (Rozas & Rozas, 1999; Rozas, Sánchez-DelBarrio, Messeguer, & Rozas, 2003). And distances between haplotypes were estimated using the NETWORK version 4.6.1.2 program (Bandelt et al., 1999).

2.3. Results

2.3.1. Sequencing Success

All of the DNA samples (n= 328) were successfully amplified using R35 primers and sent to Macrogen Inc. (Seoul,South Korea and Amsterdam, Netherland) for sequencing. Eighty eight bad sequences quality products were removed and in total 239 sequences (73% of all samples; 131 loggerhead, 104 green and 4 softsheled nile turtle) were successfully aligned and trimmed for further analyses (Table 2.3.1.1) Additional 2 loggerhead, 7 green, 6 softsheled Nile turtle sequences those taken from NCBI database aligned together with present sequences and used for further analyses (EU787159.1; FJ039952.1;FJ039945.1; FJ039938.1; FJ039931.1; FJ039924.1; AY339635.1; HQ020481.1; HQ020480.1; HQ020479.1; HQ020478.1; HQ020477.1; AY259589.1; FJ009031.1; FJ009024.1).

Cytochrome Oxidase I (COI) gene region of 57 samples (including 30 hybrids) were amplified using FishF1-R1 primers. Two of them did not give any PCR product and one of them had bad sequence quality. In total 54 sequences, with high quality score, were successfully aligned.

Table 2.3.1.1. Total number of R35 sequences those aligned successfully for each sample site. Total number of samples are given within the parenthesis. CC: Loggerhead turtle, CM: Green turtle, TT: Sofsheled Nile turtle.

Sampling Site	Sample	Sequenced	Sequenced Hatchling	Sequenced Adult
METU Erdemli Campus	CC	14 (14)	50 (61)	3 (3)
	CM	2 (2)	13 (14)	2 (2)
	TT	-	-	2 (2)
Northern Cyprus	CC	38 (60)	38 (60)	-
	CM	34 (59)	34 (59)	-
	TT	-	-	-
Belek	CC	30 (33)	40 (48)	-
	CM	4 (6)	12 (16)	-
	TT	2 (2)	2 (5)	-
Samandağ	CC	-	-	-
	CM	17 (20)	43 (65)	-
	TT	-	-	-

2.3.2. Nuclear marker: R35 intron region Analyses

2.3.2.1. Network Analyses for R35

According to the haplotype analyses 115 haplotypes were revealed from the 240 sequences. In total 53 haplotypes for loggerhead, 59 haplotypes for green and 3 haplotypes for softsheled Nile turtles were recorded. Almost 97% (n=111) of the haplotypes reported for the first time in the present study. In total of 50 haplotypes for loggerhead turtles were private for nesting beaches: 26 for Mersin (n=21), 15 for Antalya (n=12), 9 for North Cyprus (n=11). While one haplotype was reported previously from Atlantic (FJ009031.1) and Pacific (FJ009024.1) found as major haplotype in common for the present study with 69 homozygote 4 heterozygote individuals. Moreover two common haplotypes were found between Mersin *loggerhead* -North Cyprus *loggerhead* and Antalya loggerhead - Eastern Pacific green turtle samples (FJ039938.1, FJ039931.1) with 9 Mutations distance from loggerhead major haplotype (Figure 2.3.2.1.1). Additionally, 7 loggerhead turtles (4 individuals from Antalya, 3 individuals from Mersin) showed green turtle structure on R35 region with 8 private haplotypes (5 haplotypes for Antalya, 3 haplotypes for Mersin).

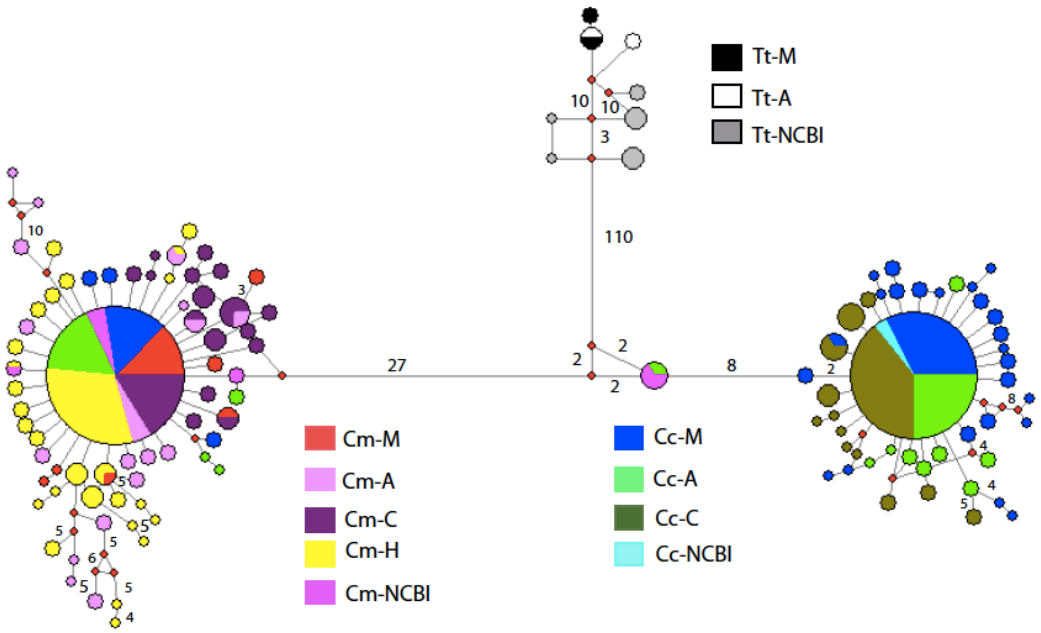


Figure 2.3.2.1.1. The median-joining network of loggerhead, green and softsheled nile turtle for the R35 intron region genotypes. The pie size is proportional to the number of samples, and colors indicate different samplings/populations/haplotypes (the numbers indicate mutations >2).

In total of 54 haplotypes of green turtle are private for nesting beaches: 22 for Hatay (n=20), 15 for North Cyprus (n=20), 12 for Antalya (n=10) and 5 for Mersin (n=4). Furthermore 5 common haplotype were found including one major common haplotype. In total of 4 haplotypes common between Hatay- EU787159.1 (California, Davis), Hatay-Mersin, Mersin-North Cyprus, Hatay-Antalya. The major haplotype in common for present study was reported previously from California, Davis (EU787159.1), Atlantic (FJ039952.1, FJ039945.1) and unknown source (AY339635.1). Including 23 loggerhead turtle hatchlings (12 individuals from 11 nests from Antalya, 11 individuals from 8 nests from Mersin) 61 homozygote and 3 heterozygote individuals clustered under this major green turtle haplotype. According to adult track morphology and hatchling colouring (when it is available), all of the species of individuals were identified before genetic analyses. However those 23 loggerhead hatchlings show green turtle genetic structure on R35 region and clustered under the main haplotype of green turtle populations. Therefore additional barcoding efforts were applied (see Mitochondrial marker: COI) for tin total of 30 hybrid individuals. Additional samples taken from 9 hybrid individuals' nests (5 nests from Antalya and 4 nests from Mersin) out of 23 nests (15 nests from Antalya and 8 nests from Mersin) in total showed loggerhead turtle characteristic unlikely their hybrid

siblings. This result indicate that the nesting females of these 9 clutches mated at least two different male one of loggerhead and one of green turtle (Table 2.3.2.1.1.).

For the shoftsheled Nile turtle; all of the samples showed heterozygosity and 3 haplotypes were found those not reported previously. In total 2 private haplotypes for Antalya and Mersin and one common haplotype between Antalya and Mersin (one allele of each samples) were revealed. The samples of the present study did not cluster with the haplotypes neither of Lake Kükürtlü (HQ020481.1; HQ020480.1; HQ020479.1) nor Mersin (HQ020478.1) samples taken from Turkey from database.

Table 2.3.2.1.1. The number of hybrids and multiple paternity observations according to nests and genetic evidence. Cc and Cm denote loggerhead and green turtles respectively. NA: Additional sample from the nest is not available to have an idea about multiple paternity.

Site	Nest Name	Total Hybrid numbers	Morphology Of Nest	nDNA (R35) of hybrids	mtDNA (Fish1) of	Number of Siblings	nDNA (R35) of siblings	Multiple Paternity
Mersin	E12F	2	Cc	Cm	Cc	2	Cc	Yes
Mersin	E12R	2	Cc	Cm	Cc	19	Cc	Yes
Mersin	E1R	2	Cc	Cm	Cc	1	Cc	Yes
Mersin	E3R	1	Cc	Cm	Cc	NA	Cc	NA
Mersin	E4R	2	Cc	Cm	Cc	NA	Cc	NA
Mersin	L1F	1	Cc	Cm	Cc	NA	Cc	NA
Mersin	E5R	3	Cc	Cm	Cc	3	Cc	Yes
Mersin	E7R	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	KD5	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	KD7	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	NY1423	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	NY250	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	NY351	1	Cc	Cm	Cc	1	Cc	Yes
Antalya	NY369	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	NY383	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	NY45	1	Cc	Cm	Cc	1	Cc	Yes
Antalya	NY487	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	NY493	1	Cc	Cm	Cc	1	Cc	Yes
Antalya	NY509	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	NY698	1	Cc	Cm	Cc	1	Cc	Yes
Antalya	NY791	1	Cc	Cm	Cc	2	Cc	Yes
Antalya	NY911	1	Cc	Cm	Cc	NA	Cc	NA
Antalya	NY937	1	Cc	Cm	Cc	NA	Cc	NA

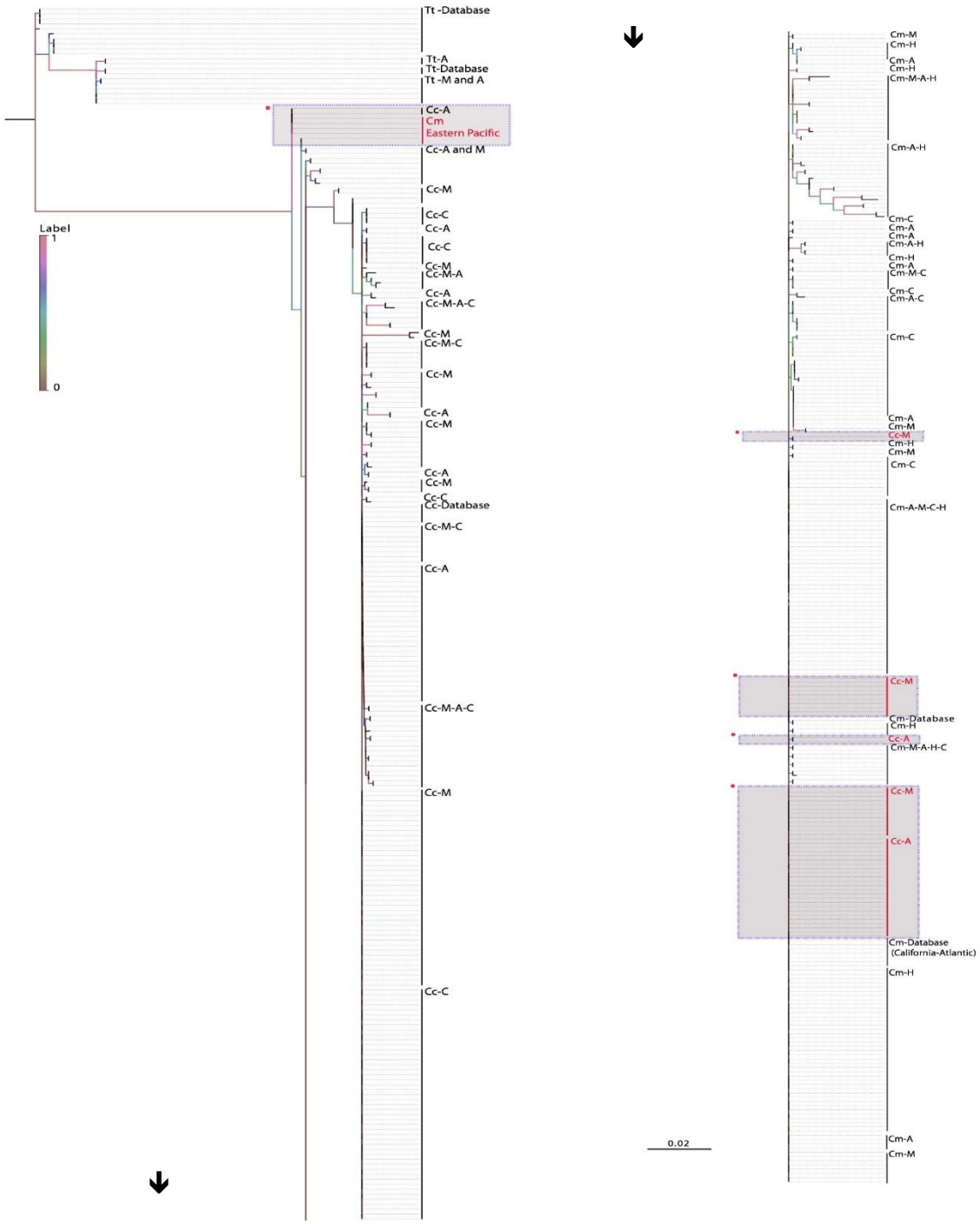


Figure 2.3.2.1.2. Maximum Likelihood tree of the R35 gene of loggerhead, green and softsheled Nile turtle and NCBI data set analysis shows the relationship between species and population of turtles. The branches' coloration is length encoded (brown for the shortest branch and red for the longest). Individuals that clustered in different species are colored in red. Kimura 2-parameter model used with gamma distribution.

2.3.2.2. Pairwise genetic distance and Gene flow

The final length of the R35 gene fragment after alignment and trimming was about 810 bp. The main parameters describing populations, namely the number of polymorphic sites (Np), haplotypes (Nh), haplotype diversity (Hd) and nucleotide diversity (Pi), are summarized in Table 2.3.2.2.1.

Table 2.3.2.2.1. R35 gene diversity parameters calculated for the 9 marine turtle populations North Eastern Mediterranean sites. Cc: Loggerhead turtle; Cm: Green turtles; Tt: Shoftsheled Nile turtles; M: Mersin: C: North Cyprus: A: Antalya: H: Hatay. N, sample size; Np, number of segregation sites (polymorphic sites); Nh, number of haplotypes; Pi, nucleotide diversity; Hd, haplotype diversity. D* test statistic is based on the differences between the number of mutations appearing only once among the sequences (singletons), and the total number of mutations (Fu and Li, 1993). The F* test statistic is based on the differences between the number of singletons and the average number of nucleotide differences between pairs of sequences (Fu and Li, 1993). Window length: 100 Step size: 25, P < 0.01**, P < 0.05*, NS: not significant.

Populations/	N	Np	Nh	Pi	Hd	F&LD*	F&LF*	Taj. D
Tt-M	2	1	2	0.001	0.67	1.63 ^{NS}	1.28 ^{NS}	1.63 ^{NS}
Tt-A	2	6	2	0.002	0.67	1.89 ^{NS}	1.61 ^{NS}	1.89 ^{NS}
Cc-M	53	77	29	0.008	0.80	1.08 ^{NS}	1.19 ^{NS}	-1.17 ^{NS}
Cc-A	41	59	18	0.009	0.76	0.97 ^{NS}	0.86 ^{NS}	0.32 ^{NS}
Cc-C	38	17	11	0.001	0.54	0.48 ^{NS}	-0.46 ^{NS}	-1.98*
Cm-H	45	50	26	0.004	0.74	-0.44 ^{NS}	-1.43 ^{NS}	-2.32**
Cm-A	12	31	14	0.001	0.96	1.19 ^{NS}	0.70 ^{NS}	-0.70 ^{NS}
Cm-M	15	10	8	0.002	0.60	0.33 ^{NS}	-0.35 ^{NS}	-1.71 ^{NS}
Cm-C	35	10	13	0.002	0.76	0.82 ^{NS}	0.13 ^{NS}	-1.26 ^{NS}

To test the neutrality of mutations Tajima's D (Tajima, 1989) and Fu and Li's tests (Fu and Li, 1993; Fu, 1997) were applied. Both of the method are used to test the assumption of all mutations are neutral while Fu and Li's test based on coalescent and Tajima's D did not. If the value of Tajima's D is negative, it means there is high level frequency of rare alleles than expectation, showing expansion of population size (after a selective sweep or bottleneck) and negative selection. If the value of Tajima's D is positive, it means there is low level frequency of rare alleles showing balancing selection and decreasing population size (Tajima, 1989). For Fu and Li's D tests, significant values showing background selection and population growth (Fu, 1997). Based on Tajima's D and Fu's Fs tests, the null hypothesis for the R35 gene neutral

evolution was not rejected for most populations, except for the North Cyprus loggerhead and Hatay green turtle populations, which showed significant negative values, indicating population expansion, natural selection, demographic and/or geographic expansions.

According to analyses the highest amount of haplotypes and polymorphic site among loggerhead and green turtle populations observed in Mersin (Nh=29, Np=77) and Hatay (Nh=26, Np=50) respectively. North Cyprus loggerhead turtles have the least amount of haplotype (Nh=11) and polymorphic site (Np=17) among loggerhead turtle populations with lowest haplotype diversity (Hd=0.54) among all populations. Antalya green turtle population has the highest haplotype diversity (Hd= 0.96) among all populations (Table 2.3.2.2.1).

Except for Hatay green turtle population (Cm-H), all distributions displayed a non-significant raggedness index (r > 0.03) (Figure 2.3.2.2.1.j). These results were confirmed by mismatch distributions. Multimodal distributions were observed for all samples, fluctuating between 0.05-0.17 for loggerhead and 0.02-0.2 for green turtle. This may suggest population subdivision and a stable population size (Figure 2.3.2.2.1.a-j).

Demographic analyses showed evidence of range expansions in especially green turtle populations. Tajima's D significantly negative for Hatay green turtle (Table 2.3.2.2.1) population, indicating that this population experienced a demographic expansion event under a neutral model. To characterize the expansion pattern further, a model of sudden demographic growth was fitted to the pairwise sequence mismatch distribution. This outcome was supported by the low Harpending's Raggedness index (r = 0.002) (Figure 2.3.2.2.1.j).

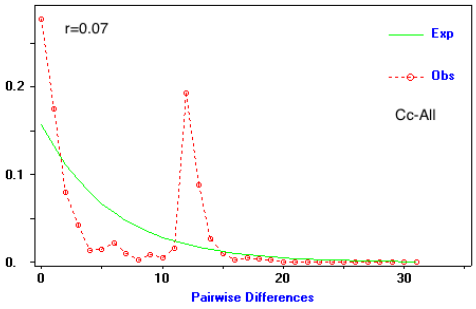
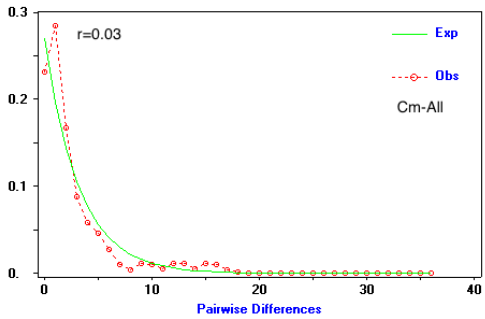


Figure 2.3.2.2.1.a



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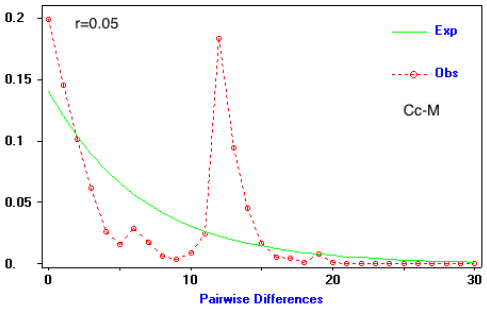

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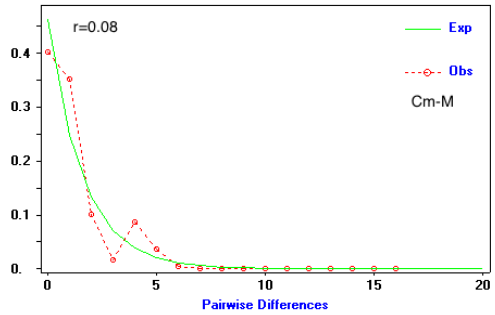

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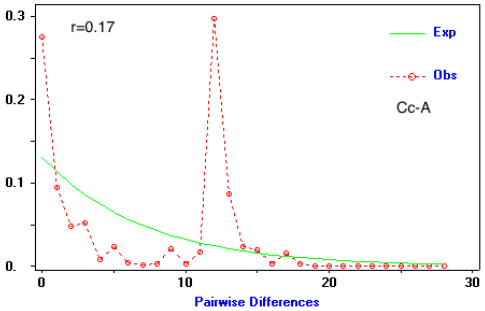

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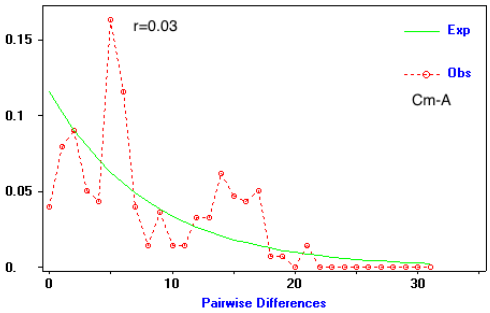

Figure 2.3.2.2.1.f

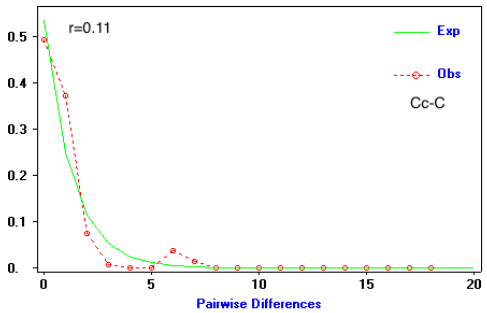

Figure 2.3.2.2.1.g

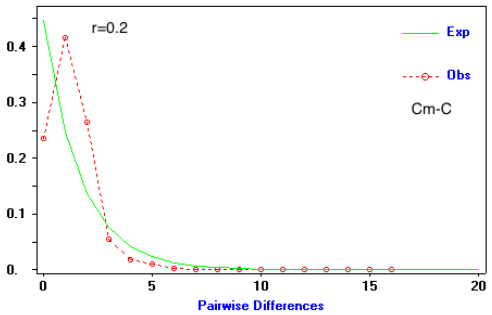

Figure 2.3.2.2.1.h

Figure 2.3.2.2.1. (a-j) Mismatch distribution based on the R35 gene for the Mersin (M), Antalya (A), Hatay (H) and North Cyprus (C) populations of loggerhead (Cc) and green (Cm) turtles. The x-axis represents the number of uncorrected pairwise differences and the y-axis represents frequency. Exp: Expected value, Obs: Observed value for constant population size.

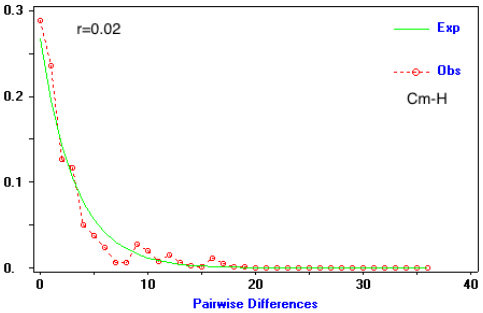

Figure 2.3.2.2.1.j

In the present study, beside of the intra-population gene flow, we calculate inter-population gene flow because some of the loggerhead turtle individuals (n=30) were clustered in green turtle cluster. According to gene flow estimation analyses, the highest gene flow (Nm) recorded between Mersin and Antalya loggerhead turtle populations (Cc-M and Cc-A) as 202 (Table 2.3.2.2.2). Although Nm values between Antalya-Mersin green turtle populations (Cm-A and Cm-M, Nm=2.70) within the limits to negate the genetic drift affects it still very low comparing the other values. All of the Nm values between North Cyprus loggerhead turtle population (Cc-C) and other populations are relatively lower than the other values. Additionally, there is significantly low gene flow (Nm<1) between North Cyprus loggerhead turtle population and both Hatay (Nm=0.90) and Mersin (Nm=079) green turtle population, which is expected. On the other hand, there are significantly high gene flows between North Cyprus and Turkey (Hatay, Mersin, Antalya) green turtle populations.

Pairwise analyses are in congruent with the gene flow estimations. According to present study Antalya green turtle population shows significant genetic differentiation from other green turtle populations while they are not significantly different from each other (Table 2.3.2.2.3.). In another words green turtle populations grouped as Antalya and Hatay/Mersin/North Cyprus populations. Moreover the genetic difference between Antalya and Mersin loggerhead populations and softsheled turtle populations are estimated as non-significant.

Table 2.3.2.2.2. Gene flow estimations (Nm) between populations/species (Nei 1973). Nm > 4: local populations belong to one randomly mating population (insufficient to prevent genetic differentiation); Nm > 1; there is enough gene flow to negate the effects of genetic drift. Cc: Loggerhead turtle; Cm: Green turtles; M: Mersin: C: North Cyprus: A: Antalya: H: Hatay.

Nm	**Cc-M**	**Cc-C**	**Cc-A**	**Cm-H**	**Cm-A**	**Cm-M**	**Cm-C**
Cc-M	-	5.75	202	3.08	6.42	3.11	3.95
Cc-C		-	3.87	0.90	1.72	0.79	1.06
Cc-A			-	3.73	5.25	3.32	4.39
Cm-H				-	6.01	33.4	21.8
Cm-A					-	2.70	8.36
Cm-M						-	11.45

Table 2.3.2.2.3. Pairwise genetic differentiation (Fst) estimates between the all populations. Pairwise Fst were calculated using the method of Weir and Cockerham (1984), and were tested using 10.000 permutations. NS: Not significant. Cc: Loggerhead turtle; Cm: Green turtles; Tt: Shoftsheled Nile turtles; M: Mersin: C: North Cyprus: A: Antalya: H: Hatay. * P<0.0033 (after Bonferroni correction).

Fst	Tt-A	Tt-M	Cc-M	Cc-C	Cc-A	Cm-H	Cm-A	Cm-M
Tt-A								
Tt-M	0.111^{NS}							
Cc-M	0.239*	0.239*						
Cc-C	0.429*	0.429*	0.081*					
Cc-A	0.264*	0.264*	0.002^{NS}	0.114*				
Cm-H	0.284*	0.284*	0.140*	0.359*	0.118*			
Cm-A	0.143*	0.143*	0.104*	0.285*	0.114*	0.104*		
Cm-M	0.383*	0.383*	0.180*	0.436*	0.155*	0.010^{NS}	0.159*	
Cm-C	0.226*	0.226*	0.116*	0.321*	0.103*	0.023^{NS}	0.067*	0.043^{NS}

2.3.3. Mitochondrial marker: COI

Present study COI assignment is straightforward. Genetic analyses clustered all individuals in accord with species assignment based on nest and body morphology. Relationships and the geographical distribution of the 4 haplotypes (1 loggerhead, 2 green and 1 softsheled Nile turtles) revealed a single mtDNA clade for each species, loggerhead turtle separated by 50 mutations from the green turtle and 102 mutations from sofsheled Nile turtle (Figure 2.3.3.1.). There are 106 mutation steps between green and softsheled Nile turtle (Figure 2.3.3.1.) according to COI data.

In total 30 loggerhead turtle hatchlings, which are clustered in the green turtle for R35 region and in the loggerhead turtle for COI data. These data indicate that mothers of these samples are loggerhead turtle but father of them are green turtle. Together with nuclear intron region (R35) and COI region results indicate that those 30 hatchlings are hybrids of female loggerhead and male green turtle.

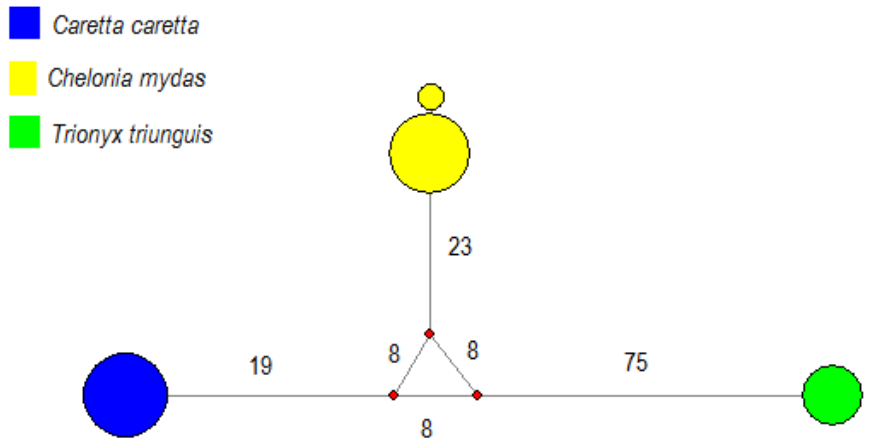

Figure 2.3.3.1. The median-joining network of loggerhead, green and softsheled Nile turtles for the COI gene region haplotypes. The pie size is proportional to the number of samples, and colours indicate different samplings/populations/haplotypes (numbers indicate the number of mutations.

2.4. Discussion

In the present study eastern Mediterranean green and loggerhead turtle population structure, hybridization profile and multiple paternity presence were revealed using nuclear DNA intron region and mitochondrial cytochrome c oxidase subunit I (COI). The analyses were conducted in four different important nesting beaches Samandağ-Hatay, Erdemli-Mersin, Belek-Antalya and North Cyprus (Balalan, Kaplıca, Kantara, Tatlısu, Smalls, Esentepe, Alagadi, Message, Lost, Monster, and Secret). In total of 239 sequences (131 loggerhead, 104 green and 4 softsheled nile turtle as outgroup) analyzed with additional 15 samples (2 loggerhead, 7 green, 6 softsheled Nile turtle) taken from database (NCBI). Although there are similar genetic studies those include Mediterranean region on different region of nDNA and mtDNA (Carreras et al., 2007; Yılmaz et al., 2011; Bagda et al. 2012), the present study is the first to use R35 and Fish1 markers in this region. The present study provides complementary data with comprehensive results such as hybridization, multiple paternity and genetic structuring among eastern Mediterranean loggerhead and green turtle populations.

There are several genetic studies previously conducted in the Mediterranean, however the related data is not available to compare with the present study. Most of these studies are conducted

using mtDNA (control region, D-loop, and RFLPs analyses) and nDNA (microsatellites, RAPD's). Although there are differences between previously used genomic regions and nuclear intron R35 region, which makes a full comparison difficult, most of the previous studies with different resolutions came into the similar conclusions: Turkey should be considered as an independent population unit along entire Mediterranean (Carreras et al., 2007; Yılmaz et al., 2011; Bagda et al. 2012). Carreras et al. (2007) suggested eastern and western coast of the Turkey differ from the entire Mediterranean coast according to mtDNA D-loop analyses. Yılmaz et al. (2011) conducted a study in the Mediterranean using mtDNA d-loop and microsatellite regions, and revealed that there are 5 main management units as western, eastern and middle Turkey and Dalyan, Dalaman nesting beaches. Bagda et al. (2012) suggested that although the mtDNA D-loop analyses reveal nesting site fidelity of the female green turtles indicating a different structure between North Cyprus and Turkey, they are not suitable for the population structuring concerns. On the other hand according to nDNA microsatellites allele size analyses, more polymorphisms revealed for green turtle populations (Bagda et al., 2012).

According to present study in total of 115 Eastern Mediterranean haplotypes were revealed for R35 gene region. The distribution of private haplotypes according to nesting sites are: 26 for Mersin (n=21 samples), 15 for Antalya (n=12 samples), 9 for North Cyprus (n=11 samples) for loggerhead turtles; and 22 for Hatay (n=20 samples), 15 for North Cyprus (n=20 samples), 12 for Antalya (n=10 samples) and 5 for Mersin (n=4 samples) for green turtles. In total of 15 database samples (n= 7 green, 2 loggerhead, 6 soft-sheled Nile turtles) showed 6 regional haplotypes (5 for softs-heled Nile turtle, 1 for Pacific Green turtle) and 4 common haplotypes. The results show that Eastern Mediterranean populations should be considered as different management units because of the high level of haplotype diversity, which is a well-known phenomenon (Karl et al., 1992; Bowen and Karl, 2007).

Antalya green turtle population showed significant divergence between the other populations. According to Nm and Fst estimations, the eastern Mediterranean green turtle populations grouped as Antalya and Hatay/Mersin/North Cyprus nesting colonies. However, Bagda et al. (2012) reported that there is significant structuring between Samandağ and North Cyprus and Alata green turtles according to microsatellite analyses. This conflict may be due to marker differences. Microsatellites are short (1-10) repeat regions mostly occur within intron, while R35 intron marker has approximately 1200 bp (810 bp for the present study). Although both microsatellite and R35 are intron markers and have high mutation rates, microsatellites tend to have insertion and deletion of repeat units or motives affecting the length. Additionally, it is

known that the longer the microsatellites the higher the mutation rates they have (Vieira et al., 2016). The conflict between the results of two significantly different markers is expected considering the microsatellite mutation rate variance within the marker itself.

According to Nm (gene flow) and Fst (genetic differentiation) estimations, North Cyprus loggerhead turtles have the lowest values among all the other populations and differentiate from Turkey populations. However one common haplotypes between North Cyprus and Mersin loggerhead populations and relatively higher Nm value (Nm= 3.87) indicate that the Mersin loggerhead population is relatively closer to North Cyprus population comparing other nesting population. On the other hand results showed that the gene flow between Antalya and Mersin loggerhead turtles is very high (Nm=202) and there is no significant difference between those populations. In other words, the grouping of those populations is between North Cyprus and Antalya/Mersin loggerhead turtle populations. This result is in coherent with previous mtDNA control region and RFLP's analyses (Laurent et al., 1998). However, Yılmaz et al. (2011) reported that Antalya and Mersin loggerhead turtles grouped differently according to both microsatellite and mtDNA d-loop analyses. There are two possible reasons for this conflict, one is the difference between microsatellite and R35 markers which is explained above. The other reason is male mediated gene flow that can be demonstrated by nuclear markers. Maternally inherited mtDNA is a good agent to show natal homing effect on population structuring, however fails to detect male mediated gene flow. The interpretation of this conflict is; the estimated gene flow between Mersin and Antalya loggerhead turtles of the present study is mostly derived by male interaction of those populations.

In the present study although Mersin loggerhead turtles grouped with Antalya loggerhead turtles, the highest number of haplotype number (Nh=29) and polymorphic sites (Np=77) were observed in Mersin (METU Erdemli Campus), which differentiates Mersin loggerhead population from the others. METU Erdemli Campus is one of the last protected and restricted area to public access along highly urbanized eastern Turkey Mediterranean coast. Additional to loggerhead and green turtles, it hosts critically endangered species softsheled Nile turtle for the entire year. The results indicate that despite the limited length or annual nesting activity, METU Erdemli Campus beach has high potential of genetic diversity, and very important for eastern Mediterranean sea turtles. Mersin is also an important link between west and east nesting beaches of Turkey those have significantly different green/loggerhead nest ratio. However telemetry studies (Snape et al., 2016) show that Mersin offshore is used as migration pathway actively by loggerhead turtles which explains the high polymorphism of Mersin population. On

the other hand it is known that sea turtle females can change their nesting beaches to more suitable beaches due to disturbance or destruction (Sönmez et al., 2017). Thus, METU Erdemli Campus beach is providing a protected nesting area as a secondary beach against disturbance along highly urbanized coastline.

The demographic analyses showed evidence of population expansions for North Cyprus loggerhead turtles and Hatay green turtles with significantly negative value of Tajima's D especially in comparison to Hatay green turtle population. These results are confirmed by the mismatch distributions showing that they might have coincided with a recent bottleneck. The recent bottleneck of Hatay green turtle's effect cannot be seen from Fst estimations. Although it did not divided the Hatay population from others, further studies are needed to see the ecological and biological effect of bottleneck among the population. Multimodal distribution and demographic analysis indicate that the other populations' sizes are stable and may be subdivided.

In total of 3 haplotypes with one common haplotype between Antalya and Mersin among 4 individuals of softheled Nile turtle are found. No common haplotype found between other Turkey samples, which are found in previous studies. Although the samples of shoft-sheled Nile turtle showed that there is no significant difference between Antalya and Mersin populations it should be noted that sampling size is too low, yet the haplotype diversity is high. Therefore, further studies with larger sample sizes are needed to provide a full understanding of the population structure of soft shelled Nile turtle.

Inter-specific gene flows are also calculated because of the observation of hybrid individuals. The majority of the inter population gene flow estimations are found significantly high ($Nm>1$) indicating that hybridization and possible introgression is present within the eastern Mediterranean. The results show that the highest gene flow is between Antalya green and Mersin loggerhead turtle population followed by Antalya loggerhead turtle population. This result is indicating that Antalya green turtles may be the main source of hybridization within this region. The gene flow estimations of loggerhead populations between Antalya and Mersin ($Nm= 202$) and overlap of loggerhead and green turtle migratory corridors (Stokes et al., 2015; Snape et al., 2016) supports that there may be an interaction between Antalya and Mersin sea turtle populations. Additionally, the lowest gene flow estimations ($Nm= 0.90$) between North Cyprus loggerhead turtles and green turtle populations of all nesting sites may show that the North Cyprus loggerhead turtles do not tend to interbreed with Turkey green turtles.

2.4.1. Hybridization profile

The present study revealed the first hybridization record for the North Eastern Mediterranean and Turkey covering four important nesting sites using both mitochondrial COI (Fish1) and nuclear intron (R35) markers. In total 30 hybrid individuals (nest=23) were observed between loggerhead female and green turtles male from Mersin (n=14, nest=8) and Antalya (n=16, nest=15) regions. The first interbreeding between loggerhead female and a green turtle male reported by Karl et al. (1995) in Brazil using mtDNA restriction profile and scnDNA loci. James et al. (2004) also revealed a hybrid of loggerhead female and green turtle male in Canada. However, so far there is only one record for the Mediterranean Sea that given by Garofalo et al. (2012) in Sicily (Italy).

Seasonal and temporal overlap suggested as a possible reason to two different species mating (Wood et al., 1983; Karl et al., 1995). It is well known that the migratory corridors and foraging habitats of both loggerhead and green turtles overlaps in Eastern Mediterranean (Stokes et al., 2015; Snape et al., 2016). The gene flow estimations of the present study also show that there are significant gene flows between nesting populations and species (Table 2.3.2.2.1), which support the suggestions.

The gender bias is one of the main interest of hybridization. It is suggested that the hybrids' mother are usually from rarer species among sunfish in Georgia (Avise and Saunders, 1984). Wirtz (1999) also suggested that hybrid mating occur between female of rare species and male of common species under scarcity of conspecific male. However this suggestion is not coherent with the hybrid between green turtle male and loggerhead turtle female in the eastern Mediterranean. It is known that green turtles (1500 nests/year) are rarer than loggerhead (7200 nests/ year) turtles in the Mediterranean (Casale and Margaritoulis, 2010). Karl et al. (1995) also reported four hybrids those mothers coming from more abundant species and emphasize that conversely the common phenomenon among other species, sea turtle females are more discriminant in mate selection. Thus, there might be a constant error of mate choice leads hybridization with a mother of abundant species. Karl et al. (1995) also suggested that, because of mechanical reasons; males of smaller species may not be able to copulate females of bigger species which is in line with green (SCL=120) and loggerhead (SCL=90) turtles. However, there are some reports that revealed hybridization between females of bigger species and males of smaller species (Seminoff et al., 2003; Lara-Ruiz et al., 2006). Lack information about adult

male sizes is a limitation for gender bias of hybridization studies. Those findings can be explained by an interbreeding between an exceedingly large male of smaller species and a newly mature female of larger species (Seminoff et al., 2003). The hybridization of the present study occurred between smaller and more abundant species female – loggerhead turtles and larger and rarer species male –green turtles. It should be also noted that Antalya (Belek) green turtle annual nesting activity is significantly lower than loggerhead turtle's, and relatively lower than other sites'. This ratio may leads the male green turtle of Antalya population to mate with loggerhead females due to lack of green turtle females in Antalya.

Multiple paternity is known as a common phenomenon among sea turtles (Pearse and Avise, 2001). In the present study revealed at least 9 nests (4 Mersin, 5 Antalya) showed multiple paternity. The nuclear intron region (R35) analyses of the additional samples from the same clutch of hybrids clustered under loggerhead haplotypes unlikely their hybrid siblings. That means, the nesting females of those 9 clutch, loggerhead females mated at least two males; one loggerhead one green turtle. Multiple paternity with at least one interbreeding was reported previously by Vilaça et al. (2012). Additionally, it should be noted that it is unknown if the nests were laid by the same female (pseudo replication). Since there is no available data for other hybrids' nests, it is not possible to make a comment about multiple paternity on other nests.

Allendorf et al. (2001) suggested that hybrid individuals tend to be sterile. However, it is known that there are some introgression reports (Karl et al., 1995; Seminoff et al., 2003). Vilaça et al. (2012) suggested that the introgression (>F1) can be considered when one or more loci have the alleles derived from the same species even if they are different haplotypes. As an example, Garofalo et al. (2012) revealed a backcross individual between a female loggerhead turtle and green x hawksbill hybrid using mtDNA control region and nuclear markers including R35. In the study the individual showed a mixture morphology of green, loggerhead and hawksbill turtles yet all of the genetic analyses resulted as loggerhead turtle. As a similar result of present study, a loggerhead individual (from Antalya region) clustered within Eastern Pacific green turtles (taken from NCBI: FJ039938.1, FJ039931.1; Naro-Maciel et al., 2008) under a haplotype, which was closer (10 mutations) to loggerhead turtle major common haplotype. Although the mtDNA COI gene analysis was not applied to this loggerhead sample, this clustering can be explained with introgression for Eastern Pacific green turtle and Antalya loggerhead turtle sample.

The results of the present study show presence of significantly high gene flow between different species, which may facilitate higher genetic diversity and can increase survival capacity of the populations. Additionally the presence of possible introgression with loggerhead individual

(from Antalya region) clustered within Eastern Pacific green turtles, may be a sign of the hybrid individuals fitness and survival success. On the other hand, all of the tissue samples of the present study taken from dead hatchlings. Although 30 hybrids (12.5% of all samples) are found within 240 samples, still the cause of deaths cannot be totally related with hybridization. Besides all of the samples collected from either emerged-dead hatchlings or completely developed-dead eggs (late- late stage). However, report for other species indicate that the hybridization causes lower fitness and higher mortality (Wirtz, 1999). But there is still a big gap about introgressed individuals mating and surviving processes. Although gene flow is a normal process of evolution, hybridization and introgression; it also can be a threat for rare species (Allendorf et al., 2001). Further information about hybridization is needed to get effective measurements for sea turtle conservation. In the present study for the first time hybrids between loggerhead and green turtle are reported for the North Eastern Mediterranean and parental resolution of them were given by using both nuclear an mitochondrial markers.

2.4.2. Phylogeny

Although proportion of the database samples (comparing present study, n= 6 database/131 present samples) is very low, 2 major common haplotypes are reported from Pacific and Atlantic Oceans. That shows that the major common haplotypes of both loggerhead and green turtles are assumed the oldest haplotype and originated from Pacific Ocean. Bowen et al. (1993) suggested that according to climatic history while the Mediterranean loggerhead population originated from the Western Atlantic population the western Atlantic loggerhead population originated from Florida more recently than the Pacific Ocean (Bowen et al., 1993). A similar scenario was suggested as, there is a deep separation between Pacific and Atlantic/Mediterranean green turtles according to mtDNA restriction fragment length polymorphisms (RFLPs) by Bowen et al. (1992) for green turtle populations. Encalada et al. (1998) underlined that the Mediterranean Sea colonization is laid back to Wisconsin glaciation (approximately 10 thousand years ago).

Bowen et al. (1994) observed two primary mtDNA linages for loggerhead turtle in both Indian-Pacific and Atlantic-Mediterranean basins and explained it as transplantation between rookeries though Southern Africa. Additionally Reis et al. (2010) reported significantly high gene flow in mtDNA D-loop region among Brazil Turkey and southeastern USA samples. Despite the small sampling size from Atlantic and Pacific Oceans and different mutation rate and evolutionary history between mtDNA and nDNA, our results are in line in this aspect with previous mtDNA studies.

In the present study while the mtDNA results (COI) did not support a pattern of genetic differentiation connected with geography, it provided valuable information regarding the Eastern Mediterranean populations' maternal border. On the other hand, the neutral intron marker (R35) revealed valuable genetic information about populations. The study showed that nuclear intron RNA finger protein region (R35) has species specific sites that facilitate to collect extensive information about species and very suitable for population structuring. In the present study 30 hybrid individuals between loggerhead and green turtles revealed for the first time in the eastern Mediterranean using both nDNA (R35) and mtDNA (COI) markers. Additional to hybrid individuals, multiple paternity was detected among 9 nests (4 Mersin, 5 Antalya) hybrid nests and revealed that the mothers of the clutches mated with at least two males as one of loggerhead, the other one of green turtle. The structure of groupings within the Mediterranean revealed as; North Cyprus and Mersin/Antalya for loggerhead, Antalya and Hatay/Mersin/North Cyprus for green turtle populations. In total 115 haplotypes revealed among 240 individuals and 4 nesting sites thus every nesting site should be considered as a management unit. Moreover, the importance of METU Erdemli Campus was emphasized with high genetic diversity contribution to the meta-population. In the second chapter, I will focus on a new monitoring method IR camera usage in METU Campus beach, which facilitate a better conservation management by providing better understanding on hatchling behavior and incubation duration.

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CHAPTER II

HATCHLING BEHAVIOR OF SEA TURTLE POPULATIONS IN THE EASTERN MEDITERRANEAN

3.1. Introduction

Two marine turtles, the loggerhead turtle *(Caretta caretta, Linnaeus, 1758*) and the green turtle (*Chelonia mydas, Linnaeus, 1758*) frequently nest along the Mediterranean coast of Turkey such as Belek, Fethiye, Anamur, Alata, Kazanlı, Samandağ, Akyatan (Canbolat, 2004; Başkale and Kaska, 2005; Yalçın-Özdilek and Sönmez, 2006; Casale and Margaritoulis, 2010; Uçar et al., 2012; Türkozan et al., 2013, Ergene et al., 2013). During the last few decades, sea turtles have experienced significant threats through direct and indirect human activities (Canbolat and Nalbantoğlu, 2003; Donlan et al., 2010; Sönmez and Özdilek, 2013) and have had dramatic population declines (National Research Council, 1990) in many regions. However, the Mediterranean subpopulation of loggerhead sea turtle has experienced a recent population increase as a result of conservation activities (Casale, 2015). Breeding success is very important for sea turtle conservation (Musick and Limpus, 1997; Hamann et al., 2010; Rees et al., 2016). Hatchling success has been largely studied (Fowler, 1979, Hays et al., 2001; Taşkın and Baran, 2001) and several pressures have been identified (Kasparek et al., 2001; Tomás et al., 2002), which lead to implementation of better conservation measures.

Regular nesting beach monitoring has long been conducted in order to determine daily emergence patterns of sea turtle hatchlings (Witherington et al., 1990; Adam et al., 2007). These studies documented a strong preference for nocturnal hatchling emergence (Witherington et al., 1990; Glen et al., 2005). Studies aimed at understanding emergence group formations frequently observed intra-nest synchrony in hatchling emergences. Intra-nest synchrony in the hatchling emergence is defined with respect to the hatchling group sizes, i.e. the whole clutch emerging together as a single group indicates perfect synchrony (Moriya and Moriya, 2011). Complete synchrony (single emergence) is rare, however, moderate synchrony is documented (Peters et al., 1994; Adam et al., 2007). Additionally, asynchrony with a large emergence group and several smaller groups has also been documented (Glen et al., 2005; Moriya and Moriya, 2011). Furthermore, diurnally emerged groups were smaller in size than those which nocturnally emerged for green turtles in North Cyprus, however, no significant difference in group sizes was found for the loggerhead turtles (Glen et al., 2005). Carr and Hirth (1961) suggested that the

single emergences lower the chance of hatchling emergence and reaching to the sea. However, Houghton and Hays (2001) proposed that the asynchronous emergence is driven by the temperature differences in the nest and the resulting differential egg development. Therefore, the synchrony in the hatchling emergence might reflect the duration of incubation period. The incubation period for sea turtles varies substantially. For loggerhead turtles in Greece, Turkey and North Cyprus, the incubation period varied between 39 and 89 days (Godley et al., 2001; Ilgaz and Baran, 2001; Taşkın and Baran, 2001; Margaritoulis, 2005; Fuller et al., 2013). Whereas, for green turtles in North Cypru, the incubation period varied between 43 and 70 days (Broderick et al., 2000; Ilgaz and Baran, 2001).

One of the main factor affecting the sea turtle incubation period is temperature. Within a species specific scale, higher temperature increase the embryo development rate and lower the incubation duration (Booth, 1998). Temperature is depended on varied environmental factor such as season, sand type (Milton et al., 1997) and albedo (Hays et al., 2001), nest depth (Booth and Astill, 2001), moisture (McGehee, 1990) and metabolic heat (Zbinden et al., 2006). Van de Merwe et al. (2006) suggested that the metabolic heat has more influence on nest temperature than nest depth. Furthermore, it is reported that the location and the shading of nests do not have effect on nest temperature (Van de Merwe et al., 2006) but deeper nests tend to be cooler than the shallower nests (Booth and Astill, 2001).

Hatchling orientation is primarily determined by visual cues (Osovsky and Shettleworth, 1968; Kingsmill and Mrosovsky, 1982; Salmon et al., 1992) and artificial lights is a common reason for hatchling mortality due to disorientation (Peters and Verhoeven, 1994; Lorne and Salmon, 2007). Furthermore, sea turtle hatchlings might be differentially affected by different wavelengths of light (Kawamura et al., 2009; Fritsches, 2012). Although moonlight has been observed to aid hatchling orientation, no effect of moonlight and moon phase on hatchling emergence have been detected (Salmon and Witherington, 1995; Kawamura, 2009).

Detailed observations on sea turtle hatchlings are essential for more efficient conservation management. However, observing sea turtle hatching biology via common patrolling methods are not always very accurate, as well as demanding for labor, time and money. Therefore, new observation methods based on available technologies should be utilized. Only a few studies have employed technological tools for hatchling monitoring including radar for hatchling traps (Hays et al., 1992) and camera for hatchling response to different wavelengths of light (Kawamura et al., 2009; Fritsches, 2012). We could not find any use of camera or video recordings in order to understand the pattern of hatchling emergence in literature. To have a detailed understanding of the green and loggerhead turtle hatching emergence patterns we used camera monitoring on 5

nests in a protected beach, METU Erdemli Campus in the eastern Mediterranean (southern coast of Turkey). Between 2013 and 2015, 2-20 loggerhead turtle and 3-8 green turtle nests and more than 2600 emerged hatchlings were recorded (Cihan, 2015). The study site hosts both species during nesting season and it can be considered as an important breeding ground because it is a link between larger breeding sites distributed within a highly urbanized coastal area. This study aimed at testing the use of observations from camera recordings in hatchling monitoring as well as obtaining more information on hatchling emergence pattern, duration and behavior that can be missed from direct visual observation.

3.2. Material and methods

3.2.1. Study site

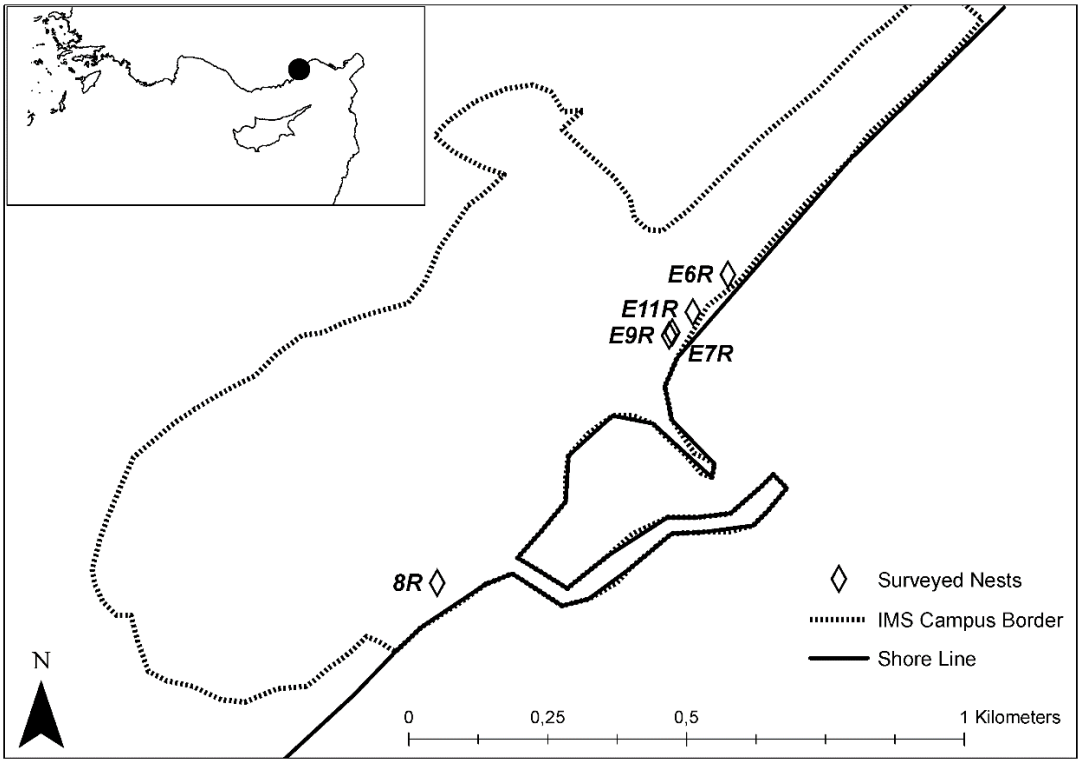

Figure 3.2.1.1. Location of the study site and camera monitored nests on the beach of the Institute of Marine Sciences (IMS) of the Middle East Technical University (METU) in Erdemli, eastern Mediterranean.

The study was conducted at the beach of the Institute of Marine Sciences (IMS), Middle East Technical University (METU) in Erdemli, southern Turkey. The Institute beach stretches along a 1.2 km long coast and is located in a heavily urbanized area of the eastern Mediterranean (Figure 3.2.1.1). The study site has restricted access to public and human activity is limited, however, its adjacent beaches are heavily used by the general public. The beach is mostly sandy and spans approximately 15-25 m width and consist of natural sand dunes approximately 0.5-3 m above sea level. The sand dunes host natural coastal vegetation dominated by sand lily (*Pancratium maritimum, L.*) (Cihan, 2015).

3.2.2. Video monitoring of sea turtle hatchling emergence

To understand the emergence patterns of the sea turtle hatchings, video monitoring surveys were conducted on one green turtle nest in 2014 and ten loggerhead turtle nests in 2015. Conventional infrared (IR) security cameras (BALITECH BL-6150) with 200 meter range, 8 millimeter stable lens and 650 TVL resolution were installed on wooden poles connected to uninterrupted power supply (Figure 3.2.2.1). The cameras were placed approximately 1.5-3 m away and 1-1.5 m above the nests after the egg deposition. The nests were selected according to logistical constraints (i.e., proximity to electricity source). The data was stored on a digital video recorder (SAMSUNG SRD-1650D) with 16 channels and 1 TB memory. Video recorder was placed in an air-conditioned cabinet and recordings were transferred every second day to an external 1 TB hard drive. All video recordings were analyzed with automatic screen captures with 30 second-intervals. When emergency activity is detected in photos; the corresponding video clip is examined and the details on the date, time, crawling duration, orientation, behavior and the number of hatchlings as well as any predation event were noted. Emergence activity starts with the first hatchling movement noticed on the nest surface, ends with disappearance of the last hatchling from camera angle. Furthermore, we calculated the orientation of hatchlings in reference to the circular area (i.e. 360°). We expected that any hatchling crawling in 140° portion of the circle towards sea (i.e. 70° on each side facing towards sea) is taken as orientation toward sea. Additionally, the hatching success of nests was analyzed by dividing the total eggs laid in the nest to the total number of hatchlings emerged from the nest. Camera data were used for the number of emerged hatchlings, instead of the empty eggs number acquired from excavation data.

Figure 3.2.2.1. An example photo of nest and camera set up.

3.2.3. Field research

The study is a part of sea turtle breeding monitoring program initiated in 2013 at METU IMS. Surveys conducted since 2013 demonstrated that the study site is used by loggerhead turtles and green turtles for breeding activity. During 2013 and 2014 nesting season, both day and night patrol were conducted by varied numbers of researchers and volunteers. Only day patrolling was conducted during 2015 nesting season by me and one volunteer. The aim of the program is to monitor female nesting activity, hatchling success and conservation management. The activity of females from May to August and the success of the hatchlings from July to September have been monitored each year. Information on the female turtle morphology and crawling track were taken during day and night patrols. The date, time, nest's characteristics were recorded for each nesting activity. Nest distances were measured between nest chamber and the sea (FEW, from nest to the end of the waves), dry (FDS, from nest to the dry sand) and wet sand (FWS, from nest to the wet sand), and vegetation (FV, from nest to the start of continuous vegetation). If nest is observed on the line of vegetation and dry sand FV recorded as zero. During the hatching period, hatchling numbers (predicted from the tracks) and the emergence orientation were recorded. Nests were excavated seven days after the last emergence. Eggs in their different

developmental stages were counted and recorded according to the methods explained previously (Bell et al., 2004; Alava et al., 2006). The depth of the nests were measured from the top of dry sand to the bottom of nest chamber at the end of the excavation. Additionally 10 temperature measurement devices placed on the top of eggs in to the selected nests. In total 4 nests temperature during 2014 and 5 nests temperature during 2015 recorded with 30 minutes intervals starting on the first day of their incubation period till the excavation. Temperature measured nests were different nests from the camera monitored nests except E11R.

3.2.4. Data analyses

Emergence from five nests, one green and four loggerhead turtle nests were analyzed to understand the emergence patterns (i.e. group sizes, nocturnal emergence, temporal emergence span and moon phase effect). Friedman rank sum test (non-parametric test for unreplicated blocked data) were implemented on incubation duration, clutch size, emergence group sizes and numbers in order to see the difference between loggerhead and green turtle nests and to make the decision of analyzing together. Emergence groups were categorized by the number of individuals; 1-3 as single-, 4-10 as small-, more than 11 as large-groups. Those groups are assigned according to hatchling accumulation on the nest chamber. Those individuals appear at the same time or subsequently were taken as one group even there is a lag they commence crawling. The emergence groups were designated as day or night activity according to the timing of sunrise and sunset. Sunrise and sunset occurred between 05:50-06:23 and 18:51-19:46 respectively during study period and thus, 6:23 and 18:51 were taken as sunrise and sunset time respectively for the analyses. Emergences that occurred between 10 minutes before the sunset and sunrise were accepted as a night activity. The temporal emergence span analyses were conducted to evaluate any activity differences between nests. Total days until first emergence, peak activity (with respect to both egg deposition and first emergence) and last emergence were calculated from the night of nest deposition. The day with largest emergence was determined as the peak activity day for each nest. We examined whether the emergence patterns correlated with the moon phases. Quick Phase Pro 4 (http://www.quickphase.com/) was used to determine moon phases, and percentage of moon fullness data was exported for each emergence date. Moonrise and moonset times were not considered for the analyses. To understand the effect of nest location (FV, FEW, FDS, FWS) and depth on emergence and incubation period; correlation, multiple (Ridge Regression; Endelman, 2011) and linear regression tests were implemented on the five nests data of the present study. Linear regression tests were implemented on both emergence and incubation duration for each parameter one by one.

The mean of day and night temperatures calculated for each temperature measured nests and correlation, multiple (Ridge Regression; Endelman, 2011) and linear regression analyses implemented in order to understand the relation between temperature differences (mean of day and night temperatures) within the each clutch and nest parameters (FV, FEW, FDS, FWS, nest depth, and clutch size). Additionally the relation between hatching success and other nest parameters tested with linear regression analyze one by one. Hatching success is the number of completely developed hatchlings including the hatchlings those unable to crawl out of the nest. The temperature measurements cover the period of nest first day till excavation day and the information of first and last emergence day (emergence period) or incubation period were not considered. All of the statistical tests implemented on R (R Development Core Team, 2008).

3.3. Results

3.3.1. Video Recordings and temperature measurements

Six of ten monitoring efforts in 2015 survey failed due to technical malfunction (4 nests) and rescue excavation (two nests). The main reason for malfunction was corrosion, especially in cable joints, and thus the subsequent connection problems and the loss of data resulted in failure in the monitoring of four nests. Furthermore, hatchlings in two nests could not emerge due to obstruction by stones and rescue excavation was performed for on those nests. Therefore they were not included for further analyses.

Friedman rank sum test showed that there is no significant difference between one green turtle and four loggerhead turtle camera monitored nests according to emergence group sizes and numbers, incubation durations and clutch sizes (Friedman chi-squared = 1.1321, p-value = 0.8892). Therefore further analyses conducted accordingly and results represent green and loggerhead nests together. There is a negative correlation between incubation duration and emergence duration of camera monitored nests (8R, E6R, E7R, E9R, E11R), the longer incubation period the shorter emergence activity occur (Table 3.3.1.1.). The tests of multiple and linear regression analyses on camera monitored nests, resulted as none of the nest parameters (FV, FEW, FDS, FWS, nest depth) affect emergence and incubation duration (p-value > 0.05). Regression plots are presented in Appendix A and B.

Table 3.3.1.1. The correlation coefficients of emergence (emdur), incubation (indur) durations of camera monitored nest (8R, E6R, E7R, E9R, and E11R) and nests distance from vegetation (FV), from dry sand (FDS), from wet sand (FWS), from sea (FEW) and nest depth.

	emdur	indur	FV	FDS	FWS	FEW	depth
emdur	1	-0.98	-0.64	-0.28	-0.51	-0.49	0.25
indur	-0.98	1	0.59	0.34	0.59	0.57	-0.13
FV	-0.64	0.59	1	0.62	0.69	0.56	-0.01
FDS	-0.28	0.34	0.62	1	0.44	0.17	0.77
FWS	-0.51	0.59	0.69	0.44	1	0.96	0.06
FEW	-0.49	0.57	0.56	0.17	0.96	1	-0.17
depth	0.25	-0.13	-0.01	0.77	0.06	-0.17	1

Table 3.3.1.2. Information of temperature device placed nests. Night-T: Monthly mean temperature of nests during nights, Night-MT: Total mean temperature of nests during nights, Day-T: Monthly mean temperature of nests during days, Day-MT: Mean temperature of nests during days, FV: Nest distance from vegetation, FDS: Nest distance from dry sand, FWS: Nest distance from wet sand, FEW: Nest distance from sea.

Year	Nest	Species	Date	Night-T	Night-MT	Day-T	Day-MT	FV (m)	FDS (m)	FWS (m)	FEW (m)	Clutch size	Nest depth (cm)
2014	9R	CM	Juli	31.1	31.0	30.6	30.7	9	4	7.1	20	74	37
			Aug	30.8		30.9							
	4R	CC	Juli	31.3	30.4	30.7	30.5	7	2.8	7.9	16	99	34
			Aug	29.5		30.2							
	1R	CM	Juli	31.0	32.1	30.5	36.3	2.2	6.5	14.2	18	89	43
	2R	CC	Juli	32.1	31.3	36.3	33.9	6.2	6.1	8.7	16	59	36
			Aug	30.4		31.5							
2015	E2R	CC	June	30.0	31.5	29.2	30.7	4	5.4	14	17	106	41
			Juli	33.1		32.2							
	E11R	CC	Juli	31.6	31.9	34.5	34.4	1	2	5	8.5	104	46
			Aug	32.3		34.4							
	E14R	CC	Aug	31.6	31.1	31.0	30.5	0	8	15	20	46	65
			Sep	30.6		30.0							
	E15R	CC	Aug	30.4	30.1	30.2	29.9	0	8	15	20	68	39
			Sep	29.8		29.7							
	E16R	CC	Aug	29.9	28.9	29.3	28.9	1.5	3.9	6.6	10	82	50
			Sep	29.5		29.0							
			Oct	27.2		26.8							

According to temperature measurements data, temperatures varied among nests although the nest parameters and active periods are the similar or the same (e.g. E14R and E15R). Additionally monthly temperatures are in coherent with seasonal expected temperatures. There is no regular diel fluctuation pattern can be observed among the nests. Detailed nest information on the temperature measured nests are given in Table3.3.1.2. Furthermore, again multiple and linear regression analyses on temperature data showed that temperature is not effected by none of the nest parameters (p-value > 0.05). According to correlation analyses, there is a strong positive correlation between day and night temperatures as expected (Table 3.3.1.3). The linear regression test of the hatching success resulted significantly only two parameters; FDS (R squared= 0.68, p value=0.006) and FEW (R squared=0.48, p value=0.038). Regression plots of the temperature measured nests are presented in Appendix C-G.

Table 3.3.1.3. The correlation coefficients of mean temperature within the nests (9R, 4R, 1R, 2R, E2R, E11R, E14R, E15R, E16R). Night-MT: Total mean temperature of nests during nights, Day-T: Monthly mean temperature of nests during days, Day-MT: Mean temperature of nests during days, Day T- Night T: Mean temperature difference of day and night, FV: Nest distance from vegetation, FDS: Nest distance from dry sand, FWS: Nest distance from wet sand, FEW: Nest distance from sea.

	Night MT	Day T	Day T-Night T	FV	FDS	FWS	FEW	Clutch size	Nest depth
Night MT	1	0.8	0.54	0.07	0	0.2	0.14	0.19	-0.03
Day MT	0.8	1	0.94	-0.01	-0.07	-0.02	-0.12	0.18	-0.14
Day T-Night T	0.54	0.94	1	-0.06	-0.1	-0.14	-0.25	0.14	-0.18

3.3.2. Temporal patterns in emergence activity including its duration

A total of 357 hatchling emergences were captured by video camera from five nests with 42-94 hatchlings per nest. 221 hatchlings (62%) emerged in large groups, 61 hatchlings (17 %) emerge in small groups and 75 hatchlings (21%) emerged individually (Table 3.3.2.1). At least one large group emergence was observed for each nest (Table 3.3.2.1). Additionally, 68 of the 72 emergence groups (95.8%) accounting for 352 of 357 hatchlings (98.6%) were nocturnal emergences. (Figure 3.3.2.1). All of the group emergences and 49 of the 51 single emergences occurred during night. The highest emergence activity (60% of the group emergences) occurred between 21:00 and 00:00.

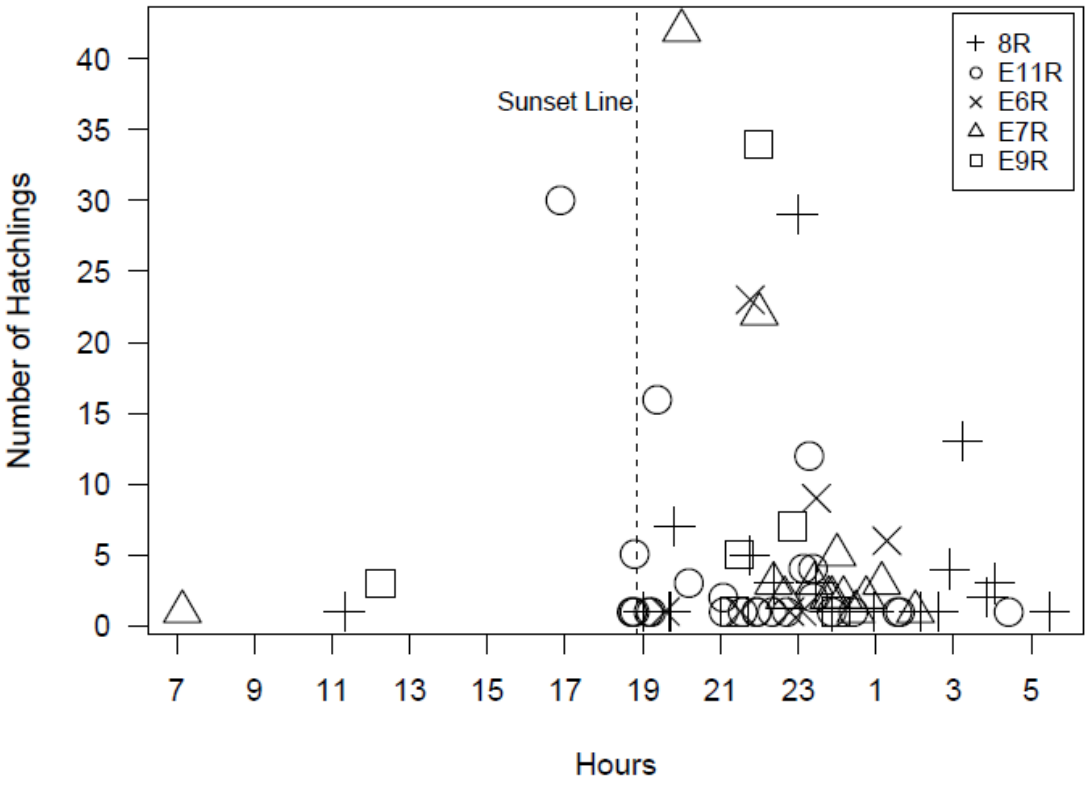

Figure 3.3.2.1. The daily temporal patterns of hatchling emergence activity. Each symbol represents a different nest and the dashed line represents the approximate sunset time.

Table 3.3.2.1. Total number of categorized emergence groups and hatchlings for each nest. CM and CC denote to green and loggerhead turtle respectively. NG and NH denote to number of groups and total number of hatchlings respectively.

Nest	Species	Large Group Emergence		Small Group Emergence		Single Emergence	
		NG	NH	NG	NH	NG	NH
8R	CM	2	42	3	16	13	20
E11R	CC	3	58	3	13	19	23
E6R	CC	1	23	2	15	4	4
E7R	CC	2	64	1	5	11	22
E9R	CC	1	34	2	12	4	6
	Total:	9	221	11	61	51	75

The total number of days from egg deposition until the first emergence, peak emergence and last emergence were analyzed to understand the duration of emergence activity for each nest. The highest variation was observed for the day of first emergence. Total incubation period of the camera monitored nests was between 38-64 days (mean=52.6 days) (Table 3.3.2.2.). Total incubation period of eleven conventional monitored nests in the same year was between 31-72 days (mean=53.2 days). The day of peak activity was less variable and changed between 47 and 64 days with a 17 days difference among nests (Figure 3.3.2.2). In total, three nests' peak activity occurred on the first day and one nest's peak activity occurred on the second day of emergence period. The day of the last emergences was least variable among nests and occurred between 60 and 65 days from the egg depositions (mean=63 days) (Figure 3.3.2.2). Total emergence duration between first emergence and last emergence had a large variation, changed between 1 and 22 days (mean=10.4 days).

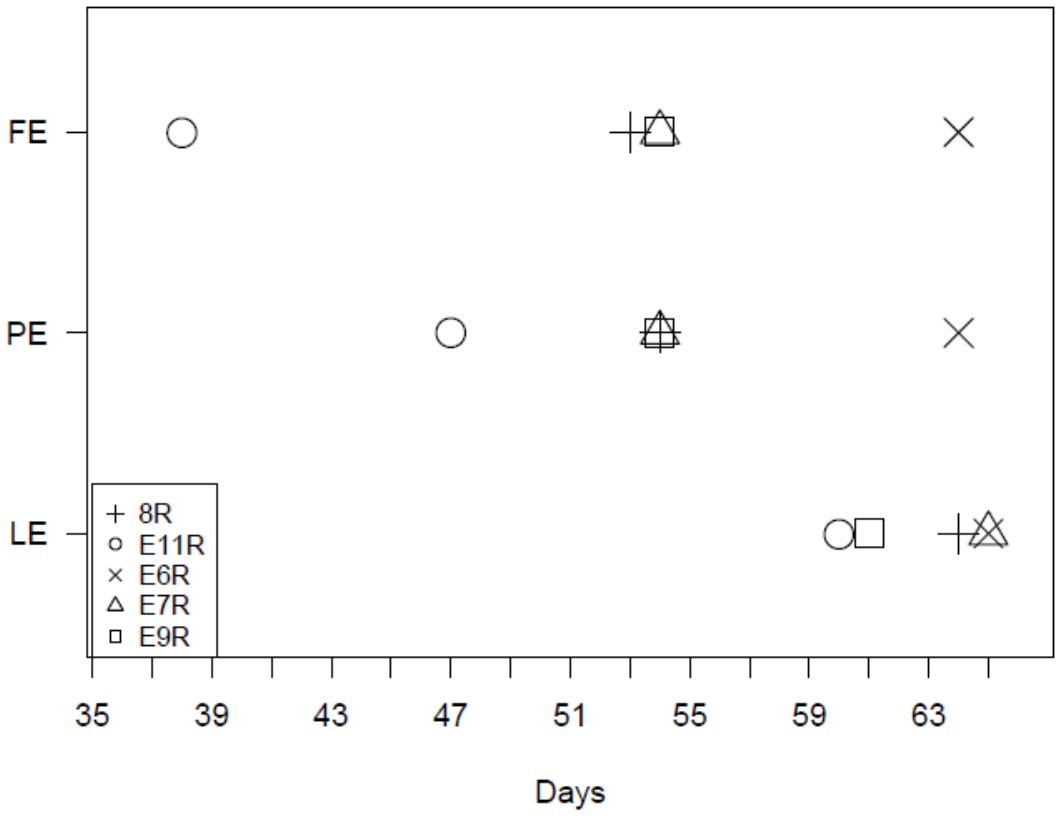

Figure 3.3.2.2. Duration of first emergence (FE), peak emergence (PE), last emergence (LE), calculated as the total number of days since egg deposition.

Table 3.3.2.2. Surveyed nests' characteristics and nest excavation records. FDS: Nest distance from dry sand, FWS: Nest distance from wet sand, FEW: Nest distance from sea, FV: Nest distance from vegetation.

Nest	8R	E6R	E7R	E9R	E11R
Species	CM	CC	CC	CC	CC
Egg deposition date	12/07/2014	6/06/2015	10/06/2015	16/06/2015	27/06/2015
Camera installation date	1/09/2014	12/07/2015	12/07/2015	12/07/2015	12/07/2015
First Emergence Date	3/09/2014	9/08/2015	3/08/2015	9/08/2015	4/08/2015
Last Emergence Date	14/09/2014	10/08/2015	14/08/2015	16/08/2015	26/08/2015
Incubation Duration	64	65	65	61	60
Excavation date	2/10/2014	23/08/2015	19/08/2015	21/08/2015	28/08/2015
Emergence Duration	11	1	11	7	22
Camera hatchling count	78	42	91	52	94
Clutch Size	97	50	104	71	103
Hatchlings reaching the sea	75	42	91	49	94
Hatchlings predated	1	0	0	0	0
Died hatchlings after emergence	2	0	0	3	0
Early stage embryos	9	4	2	12	2
Middle stage embryos	1	0	2	1	0
Late stage embryos	2	0	1	0	2
Unfertilized eggs	7	4	8	6	5
FDS (m)	6	3	6	5.4	2
FWS (m)	17.3	13	9.1	7.8	5
FEW (m)	20.6	18.3	10.4	9	8.5
FV (m)	4.2	3	2	4	1
Nest depth (cm)	55	39	64	49	46

Overall, 121 hatchlings out of 357 (33.9%) emerged during the first day and 221 (61.9%) hatchlings emerged over the first 5 days following the first emergence. On average, 24.2 hatchlings emerged in the first night in each nest. The average number of emerged hatchlings per day was 8.8 for the first five days in all nests. Additionally, the total duration of emergence activity tended to increase with decreasing incubation period until the first emergence (Spearman r: −0.921; p = 0.026). No relationship between emergence activity and the moon phases was observed and the emergence activity fluctuated regardless of the moon phases (Figure 3.3.2.3).

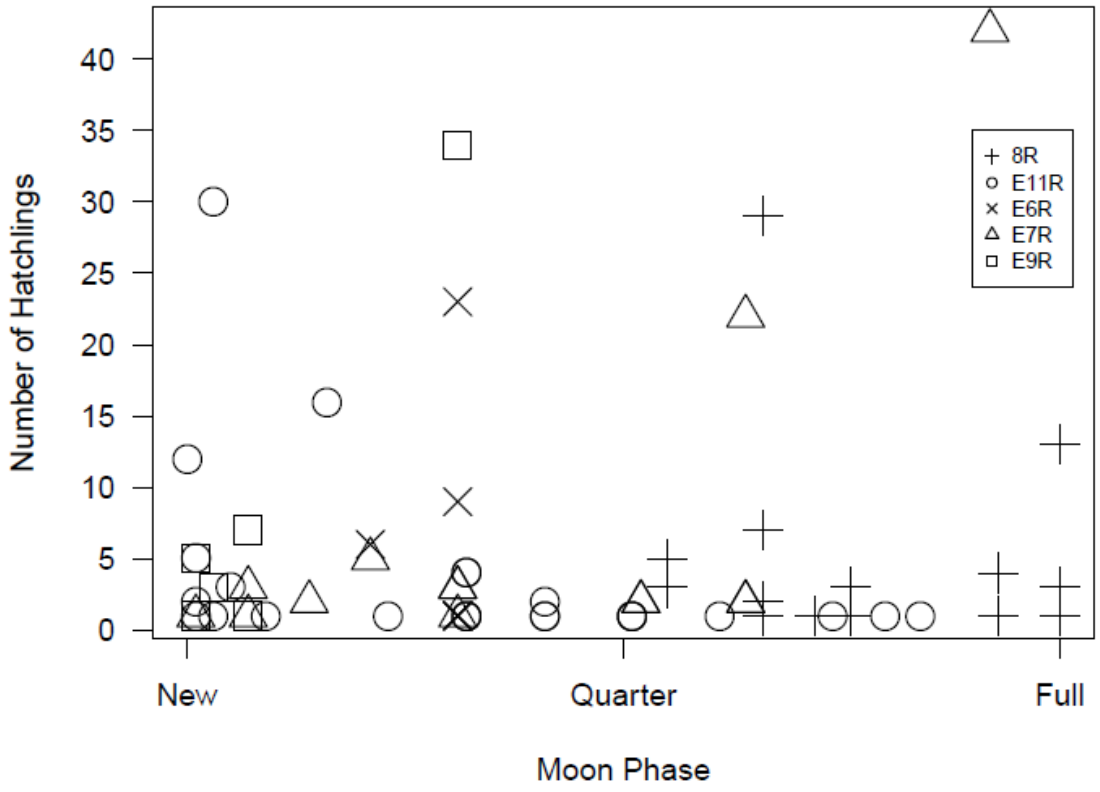

Figure 3.3.2.3. Emergence activity according to the moon phases. Each symbol represents an emergence group.

3.3.3. Predation and Conservation

In order to elucidate the effectiveness of the present conservation management and provide feedback for methodological improvements, predation activity and orientation behavior were monitored. A total of five dead hatchlings were observed due to overturning and subsequent heat shock (Table 3.3.1.2). Four of the deaths happened during the day, with only one happened during the night. Two of the dead hatchlings were later consumed by Hooded Crows (*Corvus cornix*, L.). Only one hatchling was predated by Hooded Crows during day. The majority of the hatchlings (88.8%) oriented towards the sea. Only three disorientated emergence groups (40 hatchlings) were recorded, but the predation cages re-directed them to the sea. The total number of hatchlings captured with camera recordings and the number of empty eggs found in excavations were largely consistent (only 1 to 3 differences have been found among nests, Table 3.3.1.2). We did not find any dead hatchlings within the nest chamber during excavation. In general, the hatchling emergence success of the nests varied between 66% and 92%.

3.4. Discussion

Direct visual observations that are common to sea turtle conservation monitoring have important limitations in temporal resolution, feasibility and man power. Although continuous camera recordings have been used in several organism groups, such as, wild boar (Huckschlag, 2008), deer (Scheibe et al., 2008) and birds (Pierce and Pobprasert, 2013), continuous camera recordings have not previously been used for sea turtle hatchling monitoring. Only in Florida Keys beach, a live-streaming webcam was installed on a sea turtle nest in 2014 in order to raise awareness on sea turtle conservation (http://www.fla-keys.com/turtlecam/). Our monitoring was based on continuous camera recordings on five nests in eastern Mediterranean and monitored sea turtle hatchling emergence patterns and behavior at a very high temporal resolution. The results provide important insights into patterns in emergence group sizes, timings and durations, as well as hatchling behavior and pressures on protected nests.

The majority of the hatchling events occurred nocturnally (98.6%) in the present study corroborating previous findings (Mrosovsky, 1968; Witherington et al., 1990). Studies on loggerhead turtles in Greece and green turtles in North Cyprus (Hays et al., 1992; Glen et al., 2005) documented that the hatchlings mostly emerge during night. In our study, nocturnal emergences occurred mostly in groups (~80%) and all diurnal emergences were single individuals. Glen et al. (2005) also found that 70% of all the group emergences occurred nocturnally and diurnal group sizes were smaller than the nocturnal group sizes, which is in line

with our findings. We found the peak emergence activity occurred between 21:00 and 00:00. Similarly, peak emergence activity of loggerhead turtles were between 23:00 and 24:00 in Florida (Witherington et al., 1990) and, 00:30 and 01:00 in Greece (Adam et al., 2007). The mean total incubation period (from egg deposition night to the first emergence) was 52.6 days (38-64 days) in the present study. This is in accord with previous observations in the study area (mean 53.2 days). Furthermore, the mean total incubation periods for green turtles were: 57.9 days in Northern Cyprus (Ilgaz and Baran, 2001) and for loggerhead turtles were: 52.1 days in Patara (Taşkın and Baran, 2001), 52.4 days in Dalyan (Ilgaz and Baran, 2001), 49 days in Northern Cyprus (Fuller et al., 2013) and 55.2 days in Greece (Margaritoulis, 2005). The duration of incubation and thus hatchling emergences are known to be affected by the sand temperature (Hays et al., 1992; Drake and Spotilla, 2002). Therefore, the difference between the incubation periods among different locations might be due to the ambient temperature differences (Glen, 2005; Adam et al., 2007). However, it should also be noted that the higher temporal resolution in the present study may provide greater accuracy in the estimation of emergence activity in comparison to the previous conventional monitoring methods.

We observed that hatchlings emerge asynchronously similar to some of the previous studies (Houghton and Hays, 2001; Moriya and Moriya, 2011). Adam et al. (2007) suggested that the temperature differences within the nest chamber caused asynchronous emergence, which reduce the terrestrial and aquatic predation risk. However, Pilcher et al. (2000) suggested that large group size may induce a predator dilution effect on land but increase predation risk in sea. These hypotheses might explain why hatchlings tend to emerge in groups, although the groups do not consist of the whole clutch and span across several nights (Glen et al., 2005). Larger groups of hatchlings are more motivated to reach the sea and more directional in their effort (Carr and Hirth, 1961; Burger and Gochfeld, 2014) and single emergences have less chance to reach to sea than group emergences (Carr and Hirth, 1961). Similarly, we found that all of the hatchling deaths occurred in single emergences in the present study.

The total emergence duration between first and last emergence spanned between 1 and 22 days in this study, which is longer than any of the previous studies (Glen et al., 2005; Moriya and Moriya, 2011). Emergence durations between 1 and 7 days in Cyprus, 5 and 11 days in Greece and 1 and 4 days in Turkey was previously reported for sea turtles (Glen et al., 2005; Hays et al., 1992; Witherington et al., 1990). The longest emergence duration was reported as 18 days for loggerhead turtles in Japan by Moriya and Moriya (2011). Four of the present emergence durations are in line with most of the previous observations (1-11 days) and the maximum duration of 22 days is not unexpected considering previous long records, like 18 days. However,

our results suggest that longer emergence durations might be more frequent than expected and continuous video recordings may provide a more reliable estimation for the duration of hatchling emergence activity in comparison to conventional beach monitoring.

Although it is known that the incubation duration affected directly by temperature and indirectly by nest depth and location of the nest (Özdemir et al., 2011), our results do not fit this phenomenon. There is no regression observed between nest temperatures and other locational parameters and depth of the nests. Although Van de Merwe et al. (2006) suggested that the depth is not influential as much as metabolic heat we could not find any relation with clutch size too. Temperatures varied among nests although the nest parameters and active periods are the similar or the same (e.g. E14R and E15R). According to nest depths (E14R: 65 cm; E15R: 39 cm) and clutch size differences (E14R: 46; E15R: 68) it is expected that E14R should have cooler temperature than E15R. However according to mean of night temperatures, E14R has approximately 1 °C warmer than E15R. The reason of this conflict may be the calculation scale of the temperature caused by disregarding the first and the last emergence date. Zbinden et al. (2006) reported that the metabolic heating detected only during the last third of the incubation period. The present temperature data including the period from the first day of nest till the excavation, thus residual days after last emergence might mislead the results. Besides, temperature of the sea turtle nest is derived from complex physical, chemical and biological interactions (Van de Merwe et al., 2006). A study conducted in Turkey shows that, loggerhead nests temperature is more determined than by sea surface than air temperature (Girondot and Kaska. 2015). The lack of complementary data on other parameter measurements and IR camera monitored nests hindered a better estimation of temperature effect on incubation period. Furthermore, linear regression analyses of hatching success (completely developed) of temperature measured nests showed that, FDS and FEW are related with hatching success. A previous study conducted in Turkey suggested that FV and wet depth of nest are the best predictors of hatching success (Özdemir et al., 2011).

The great majority of the hatchlings (98.6%) reached to the sea successfully in the present study, indicating a very high survival rate in comparison to other observations: 83.1% for Costa Rica green turtles (Fowler, 1979), 49.9% for Patara and Northern Cyprus loggerhead turtles (Ilgaz and Baran, 2001) and 43.5 % for Patara loggerhead turtles (Taşkın and Baran, 2001). The high success rate reflects the efficiency of the conservation efforts at METU IMS beach (i.e. artificial light and human use management). Only a single hatchling (in 357) was predated by Hooded Crows, which is very low in comparison with the other nesting areas (Carr and Hirth, 1961; Türkozan et al., 2011).

Sea turtle hatchlings are particularly susceptible to disorientation during the new moon (Salmon and Witherington, 1995). Bourgeois et al. (2009) and Berry et al. (2013) suggest that, if the moon is visible the effect of artificial lights decreases and hatchlings tend to better orient towards sea. This could be due to a decrease in the perceptibility of the horizon and the difficult discrimination between sky and silhouettes on land (Salmon et al., 1992). However, the present study clearly demonstrated that sea turtle hatchlings emerged without any correlation with the moon phases, corroborating previous findings (Salmon and Witherington, 1995; Witherington and Martin, 2000; Kawamura et al., 2009).

The high-resolution sea turtle hatchling monitoring in this study provided useful suggestions for conventional conservation activities. The peak emergence activity occurred between 21:00 and 00:00 and therefore, the night patrolling efforts could be prioritized accordingly, when man power is limited. Furthermore, nest cages were effective against potential light pollution by redirecting disorientated hatchlings. Moreover, the present study demonstrated that the natural emergence activity lasted between 60 and 65 days since the egg deposition with a very limited variability. However, the emergence activity lasted between 1 and 22 days since the first hatchling emergence with a remarkable variation. Accordingly, if the natural incubation and emergence process is preferred by the local conservation managers 65 days after nesting or 22 days after the first emergence may be waited until any excavation. However, for the nesting beaches under high predation pressure, conventional excavation methods might be more useful to reduce the predation risk.

Despite many advantages of using camera monitoring to record nesting and hatching behavior, some limitations were also observed. Due to the field of view of cameras, we could only monitor hatchling emergence immediately on the nest and not be able to follow the hatchlings to the sea. Therefore, the success rate of the hatchlings calculated from the camera monitoring only reflects the immediate survival of the hatchlings. Furthermore, four out of 11 cameras were malfunctioned due to technical problems (hardware and recording problems due to corrosion). Therefore, any further attempts on using continuous video recording for sea turtle nest monitoring should carefully select technical equipment for better spatial coverage and sufficient durability for harsh outdoor conditions. Additionally, METU Erdemli Campus has limited access to public and thus it is well protected from robbery or vandalism, which enabled us to install electronic equipment freely. However, using this method in larger or remote breeding beaches would require necessary security precautions.

Continuous video recordings with IR cameras provided valuable data with very high temporal resolution on sea turtle hatchling behavior with considerable advantages over direct visual

observation on effort, consistency and repeatability. Our results show that the hatchling emergence has significant patterns at different temporal scales that are difficult to detect with conventional beach monitoring. We found significant asynchrony in hatchling emergence and no relationship between moon phase and emergence activity. Emergence activity was overwhelmingly nocturnal and the emergence duration lasted up to 22 days. Overall, the present study demonstrates that the continuous camera monitoring can be an efficient alternative or complement to the conventional beach monitoring for sea turtle conservation. With combination of additional implementation on abiotic and biotic factors, this method will provide complementary knowledge for hatchling conservation.

4. CONCLUSION

Sea turtles are threatened by pollution, destruction of nesting and foraging habitats, climate change, by catch fisheries, hunting and egg collection, diseases and predation. Thus the sea turtles global populations have a decreasing trend. Many countries have legislation and regulations for sea turtle protection. However there are some information lack of those hinder the conservation efforts and effective implementation. One of the main problems is lack of long term and consistent monitoring along sea turtles complete coastal track. The threats and their reflection can be different for each nesting site. For a better conservation plan; first, the management units and their main characteristics and problems should be determined to develop and integrate better methods.

In the present study, genetic analyses applied to determine the Eastern Mediterranean management units for loggerhead and green turtle populations. The results revealed population structure among nesting sites in the Eastern Mediterranean and proved the high contribution of small units (METU Erdemli Campus) to meta-population. Moreover, interbreeding event detected which may affect population sustainability in the long term. Additionally, a new monitoring method (IR camera monitoring) was applied to understand the hatchling behavior. Continuous IR camera monitoring provides extensive and accurate insights on hatchling behavior and emergence pattern, which facilitate a better understanding on hatchling biology with less labor.

For a better conservation, further studies are needed that focus on the management units and their main threats. Genetic tools are very useful and can provide certain and quick answer for

conservation studies. Hybridization and introgression are relatively new concerns about sea turtles ecology and there is still a gap about long term effect on populations. Further applications should be done in wider sites including more genetic markers and samples for better resolution in long term in order to observe positive or negative effects on population structure. Morphological, behavioral and ecological data is necessary as complementary data to genetic data for integral understanding of sea turtle biology and reciprocal interaction. Integrating different methods will facilitate to fill this gap. Although IR camera monitoring is a good step to reply this concern, present study mostly focused on the response of the process. For the further implementation of IR camera, it can be combined with additional physical and chemical measurements to provide detailed understanding of the reasons of the response. Parallel monitoring methods such as temperature, moisture measurements synchronized data at different scales are needed for better estimations of the site specific interactions.

On the other hand, there is a huge gap about oceanic phase of sea turtles which hinders most of the other studies. Although there are significant efforts to fill this gap such as adult telemetry, collaboration with fisheries, stable isotope analyses; persistency and sustainability are very low due to hard conditions of implementation and lack of sources especially for the Mediterranean. Regular long term monitoring is essential to improve our present knowledge, thus developing and integrating a sustainable method into Mediterranean sea turtle studies should be one of the initial aim of the researchers.

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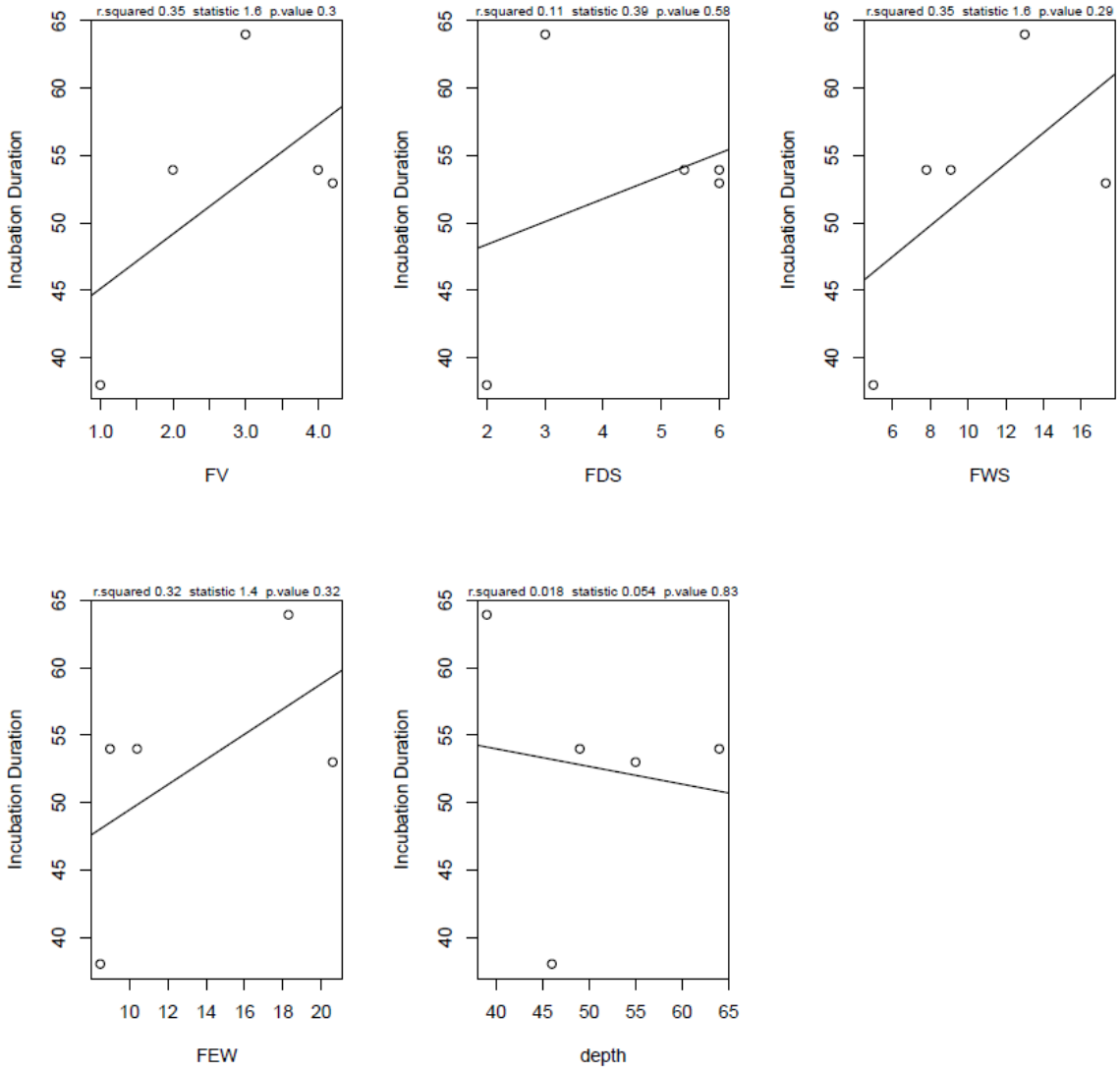
APPENDICES

APPENDIX A

Regression Plots between Incubation Duration and Nest Parameters of Camera Monitored Nests

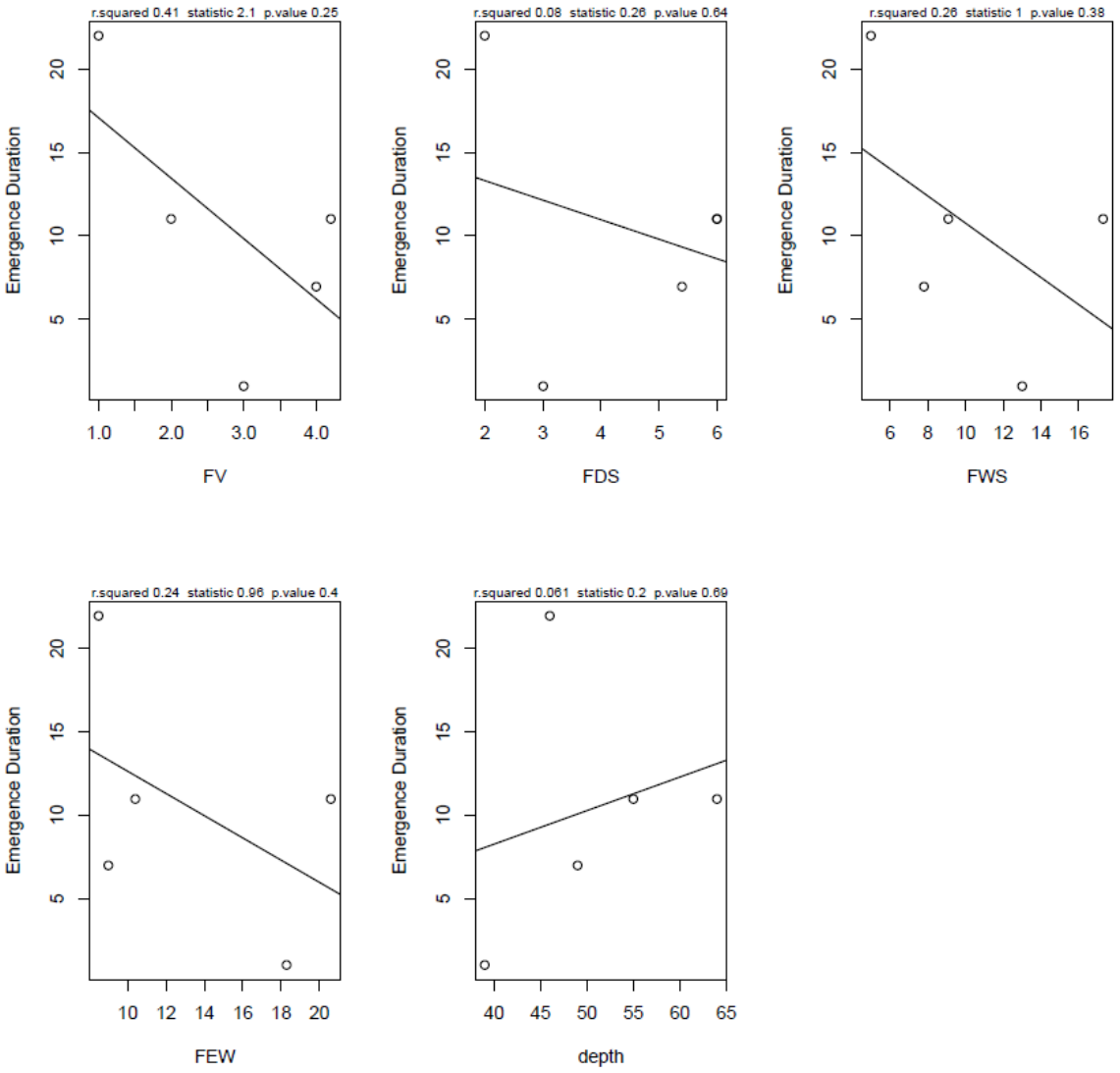


***** Nests distance from vegetation (FV), from dry sand (FDS), from wet sand (FWS), from sea (FEW) and nest depth.

Data represents following nests: 8R, E6R, E7R, E9R, and E11R.

APPENDIX B

Regression Plots between Emergence Duration and Nest Parameters of Camera Monitored Nests

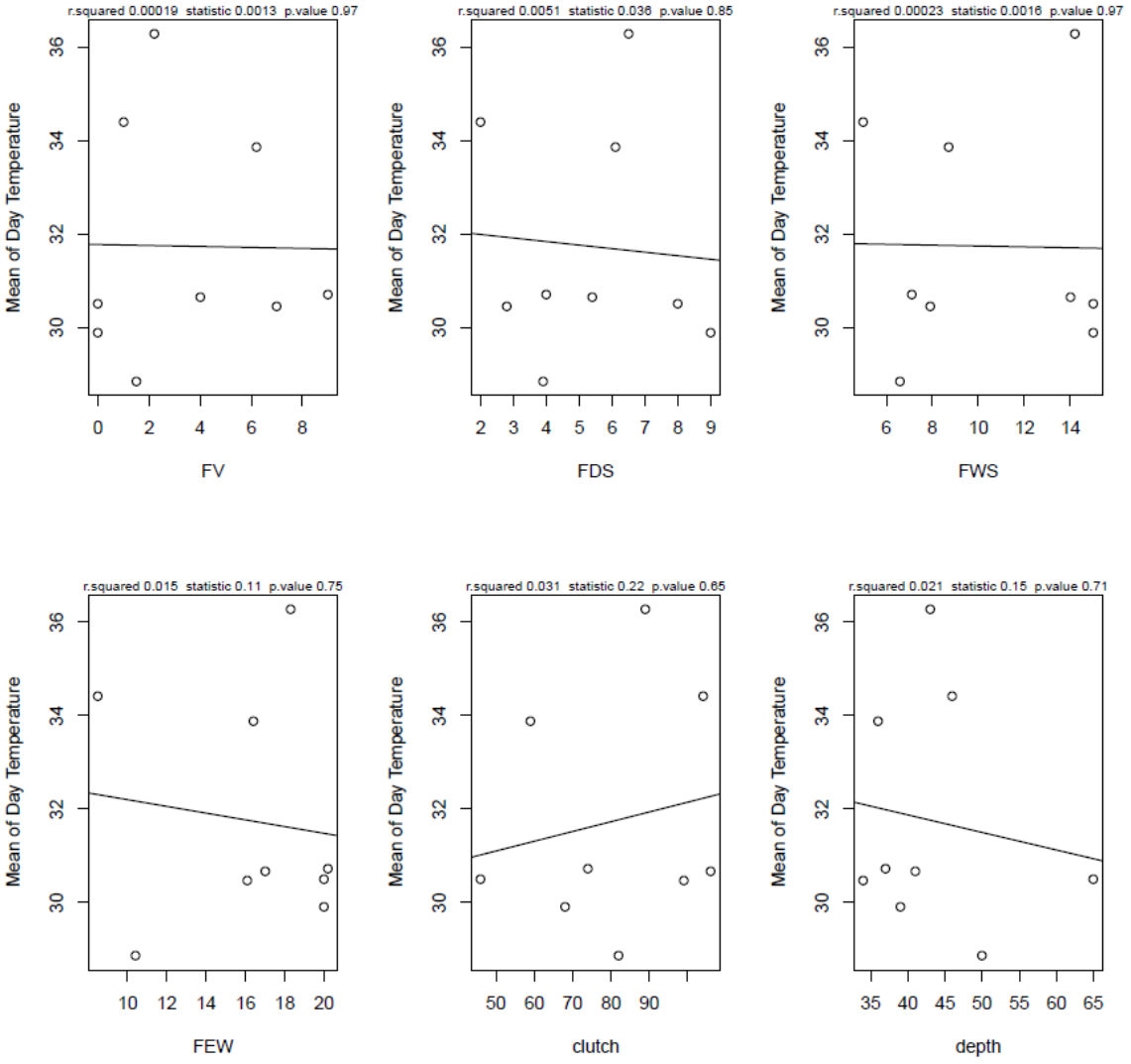


***** Nests distance from vegetation (FV), from dry sand (FDS), from wet sand (FWS), from sea (FEW) and nest depth.

Data represents following nests: 8R, E6R, E7R, E9R, and E11R

APPENDIX C

Regression Plots between Mean of Day Temperatures and Nest Parameters of Temperature Measured Nests

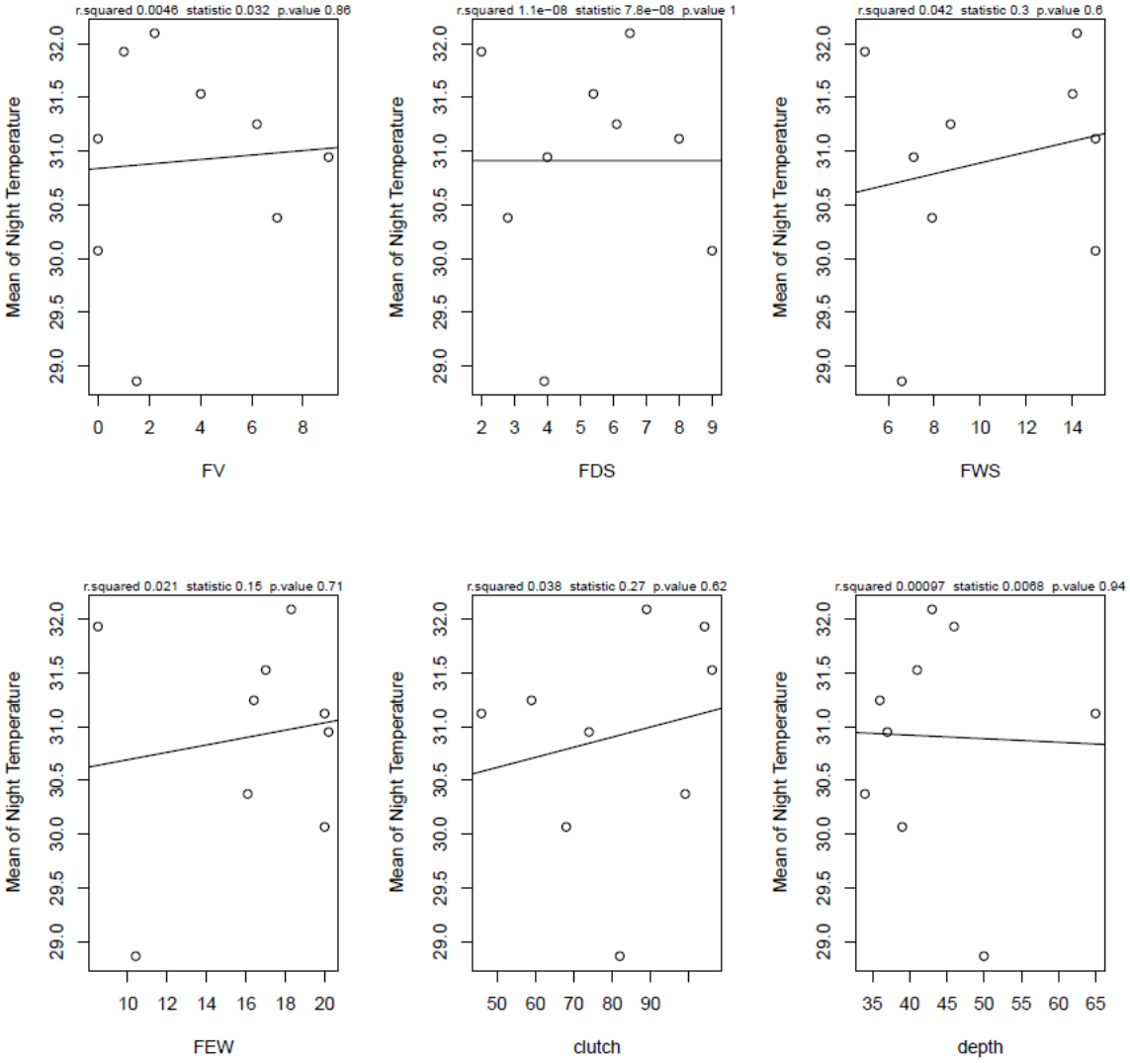


*FV: Nest distance from vegetation, FDS: Nest distance from dry sand, FWS: Nest distance from wet sand, FEW: Nest distance from sea, clutch: Clutch size, depth: Nest depth.

Data Represents following nests: 9R, 4R, 1R, 2R, E2R, E11R, E14R, E15R, E16R.

APPENDIX D

Regression Plots between Mean of Night Temperatures and Nest Parameters of Temperature Measured Nests

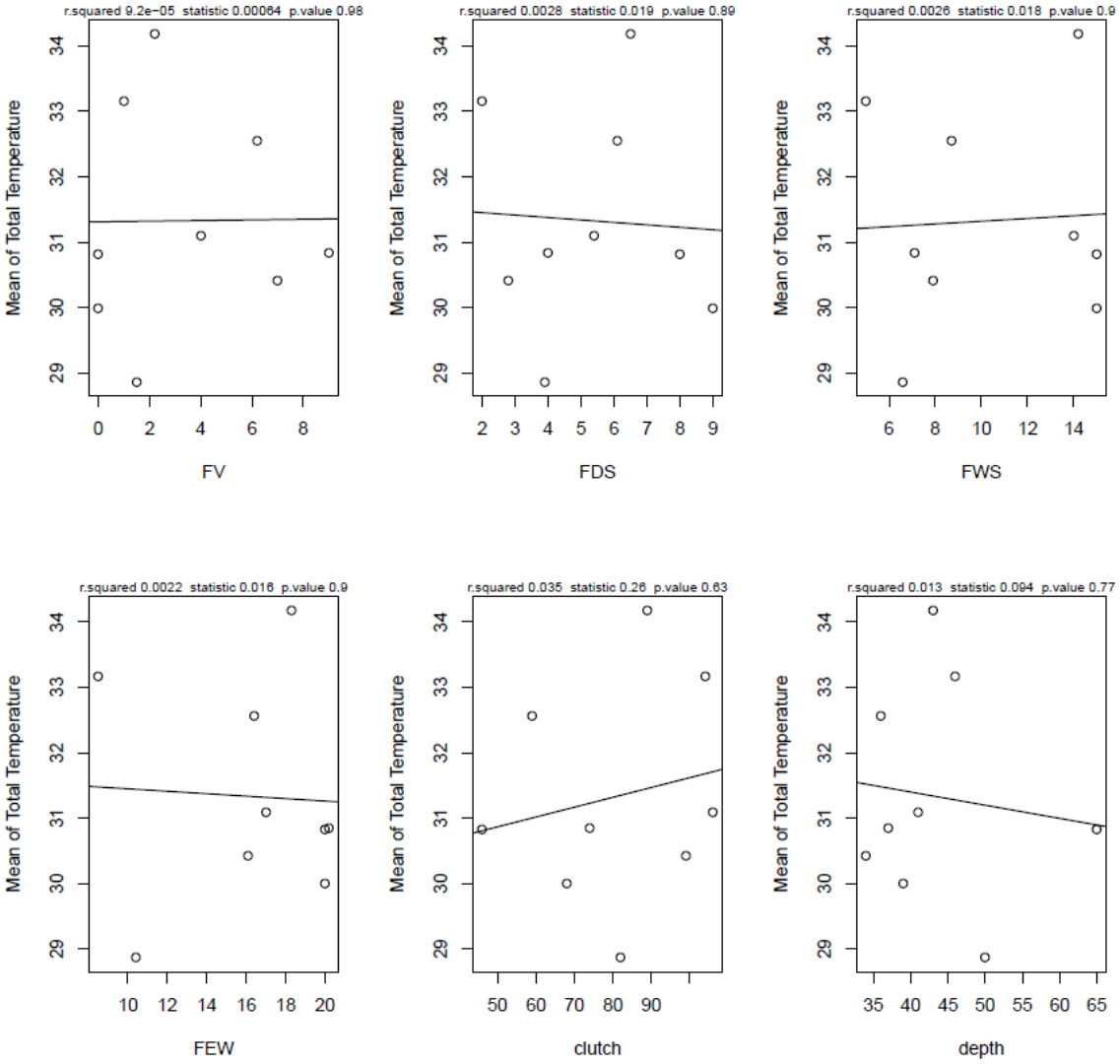


*FV: Nest distance from vegetation, FDS: Nest distance from dry sand, FWS: Nest distance from wet sand, FEW: Nest distance from sea, clutch: Clutch size, depth: Nest depth.

Data Represents following nests: 9R, 4R, 1R, 2R, E2R, E11R, E14R, E15R, E16R.

APPENDIX E

Regression Plots between Mean of Temperatures and Nest Parameters of Temperature Measured Nests

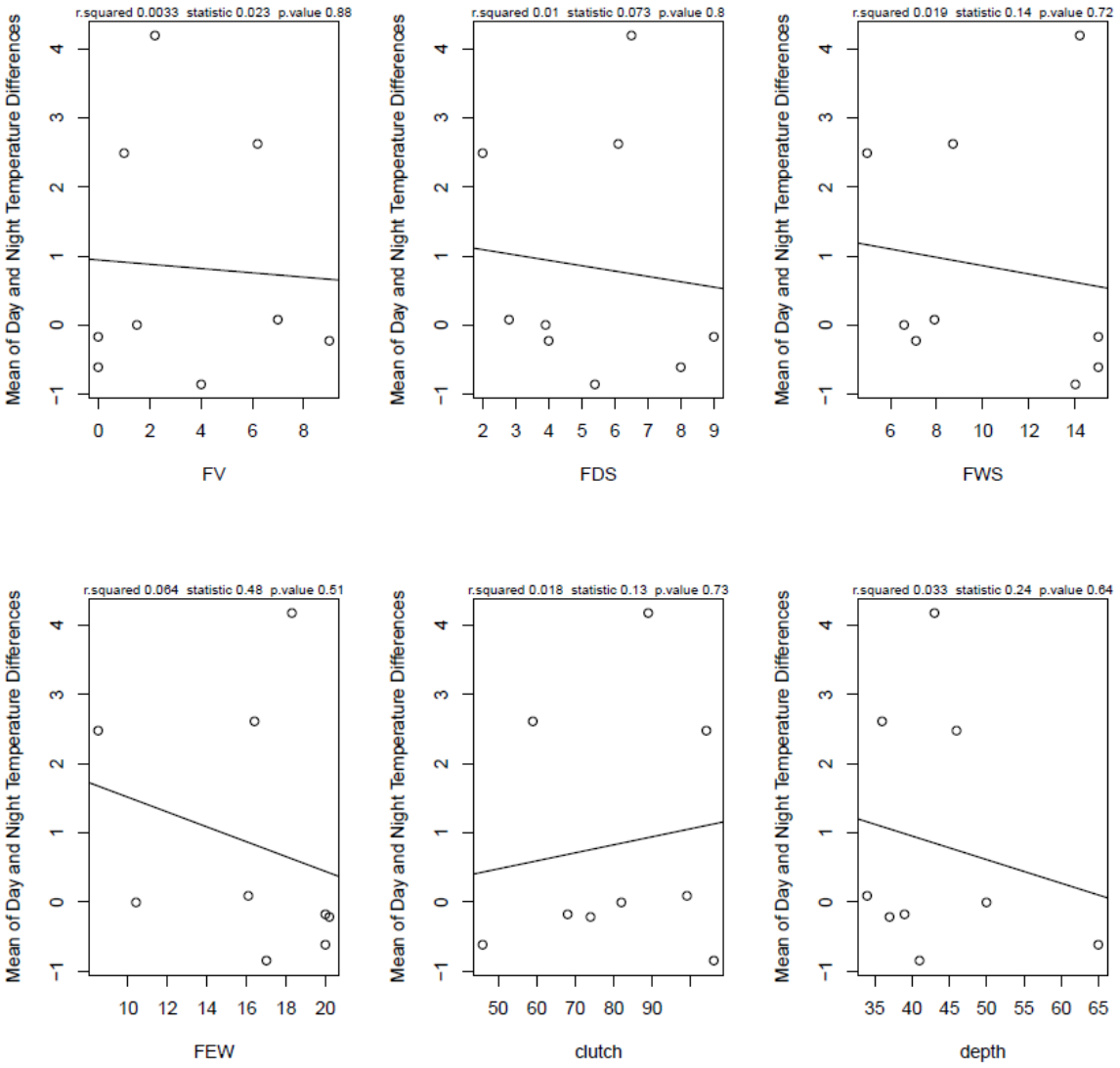


*FV: Nest distance from vegetation, FDS: Nest distance from dry sand, FWS: Nest distance from wet sand, FEW: Nest distance from sea, clutch: Clutch size, depth: Nest depth.

Data Represents following nests: 9R, 4R, 1R, 2R, E2R, E11R, E14R, E15R, E16R.

APPENDIX F

Regression Plots between Mean of Day and Night Temperature Differences and Nest Parameters of Temperature Measured Nests

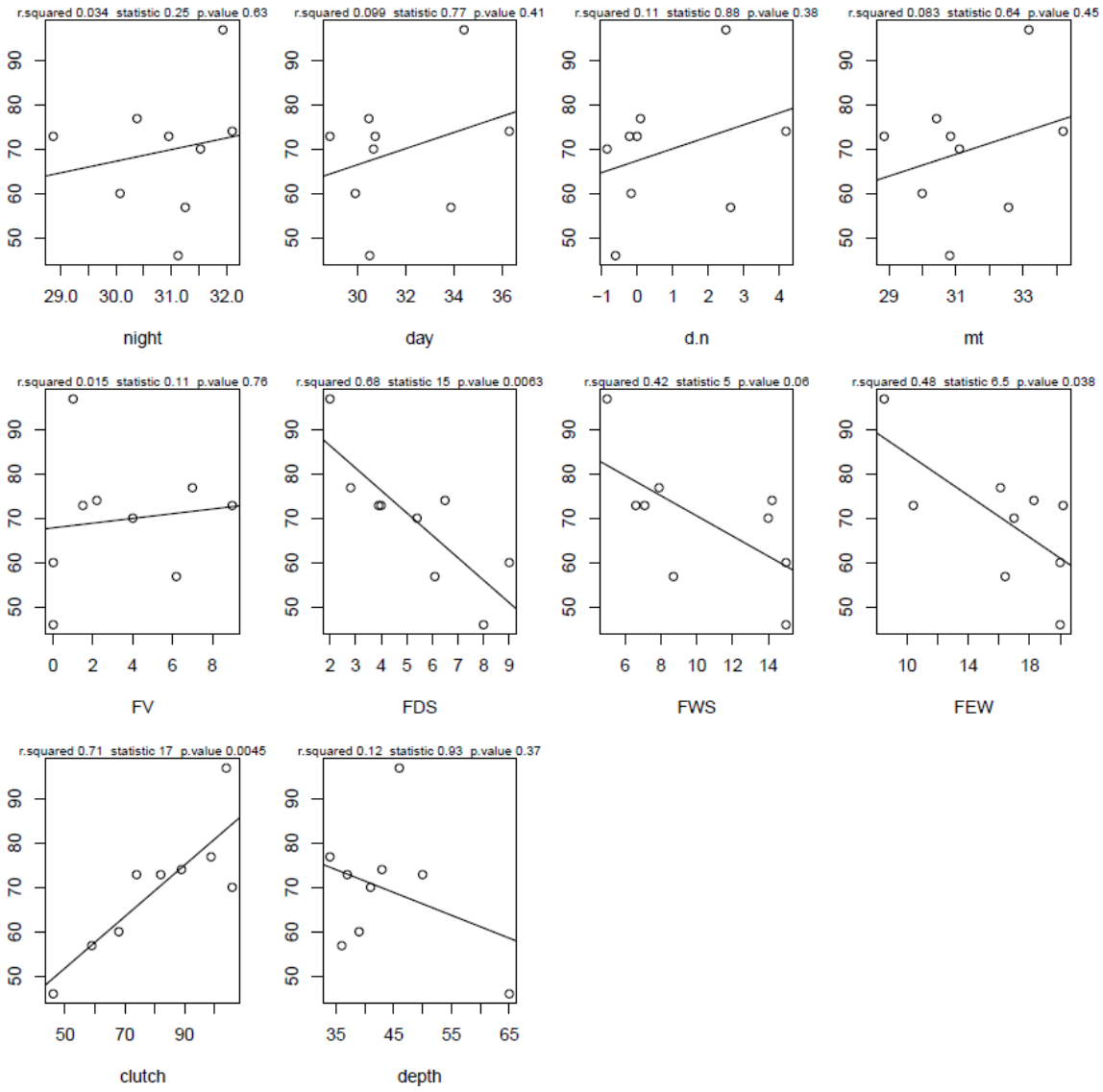


*FV: Nest distance from vegetation, FDS: Nest distance from dry sand, FWS: Nest distance from wet sand, FEW: Nest distance from sea, clutch: Clutch size, depth: Nest depth.

Data Represents following nests: 9R, 4R, 1R, 2R, E2R, E11R, E14R, E15R, E16R.

APPENDIX G

Regression Plots between Hatching Success and Nest Parameters of Temperature Measured Nests



*night: Mean of Night Temperatures, day: Mean of Day Temperatures, d.n: Mean of Day and Night Temperature Differences, mt: Mean of Temperatures, FV: Nest distance from vegetation, FDS: Nest distance from dry sand, FWS: Nest distance from wet sand, FEW: Nest distance from sea, clutch: Clutch size, depth: Nest depth.

Data Represents following nests: 9R, 4R, 1R, 2R, E2R, E11R, E14R, E15R, E16R.