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Assessing potential limitations when characterising the epibiota of marine megafauna: Effect of gender, sampling location, and inter-annual variation on the epibiont communities of olive ridley sea turtles

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ABSTRACT

The epibionts of marine megafauna can serve as valuable indicators of the host's health or behaviour; however, only a few studies have attempted to determine how and why epibiont communities vary between host individuals, populations, or even species. Further complicating efforts to compare epibiont communities of marine megafaunas is that measures of epibiont abundance and diversity may be influenced by the sampling methods and timing of the assessment. Here, we examined how host gender, geographic location, and sampling year affect measures of epibiont community structure in olive ridley sea turtles, *Lepidochelys olivacea*, in the East Pacific Ocean. To achieve this, we identified, enumerated, and then statistically compared the epibiont communities of (1) nesting female turtles sampled over different nesting seasons, (2) female turtles sampled on nesting beaches and at sea, and (3) female and male turtles, both sampled at sea. We did not discover statistically significant differences between the epibiont communities of nesting female turtles sampled on different years nor between females sampled on nesting beaches and at sea. However, we did observe a statistically significant difference between the epibiont communities of female and male turtles. Thus, we conclude that while sampling epibionts from nesting sea turtles may be an accurately and more logistically straight-forward method than sampling turtle at sea, it should not be assumed that epibiont communities of male and female hosts are identical. We also suggest that knowledge of the factors that drive intra-specific variation in the epibiont communities of marine megafauna, be it biological or methodological factors, is necessary before broader-scale meta-analyses are made to determine spatial and temporal patterns in the distribution of epibiont communities worldwide.

1. Introduction

The bodies of marine megafauna, including sea turtles, cetaceans, and sirenians, often serve as a colonisable substratum for epifaunal organisms or epibionts (Mignucci-Giannoni et al., 1998; Morales-Vela et al., 2008; Frick and Pfaller, 2013). Epibionts form a polyphyletic grouping, ranging from vertebrates to invertebrates (Caine, 1986; Guerrero-Ruiz and Urban, 2000) and from microscopic algae to bacteria (Apprill et al., 2014; Robinson et al., 2016a), with new epibiont species frequently being described (Ogawa et al., 1997; Thurber et al., 2011; Majewska et al., 2015a). To characterise the diverse epibiota of marine megafauna, many studies have created annotated checklists of the epibiont taxa that have been identified from different hosts (Lazo-Wasem et al., 2011; Hayashi, 2013). However, few studies have attempted to determine how and why these epibiont communities vary between host individuals, populations, or even species (Domènech et al., 2015).

Epibiont communities tend to vary among hosts for three major

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reasons. Firstly, variation in the hosts' behaviour may expose them to different epibiont taxa. Indeed, for epibiosis to occur there must be spatial and temporal overlap between the epibiont and the basibiont at some point in their life cycles (Frick and Pfaller, 2013). In other words, the host must inhabit or traverse the specific habitat where the colonizing stage of the epibiont occurs. Animals with varying migratory strategies or foraging habitats may therefore host different epibiont communities (Casale et al., 2004; Reich et al., 2010). Similarly, some epibionts can only be transferred by direct physical contact between hosts, and so might be found only with highly social host species (Balbuena et al., 1995; Domènech et al., 2017).

Secondly, factors influencing episodic epibiont population growth and distribution alter the probability of encounter with hosts. For example, epibionts that thrive under specific oceanographic conditions, such as warmer waters, will be more prevalent when those conditions are present. In addition, many epibiont taxa, such as barnacles, have planktonic larval forms and thus depend on ocean current patterns to disperse them for a chance meeting with a suitable host. Shifts in current patterns, on short or long time scales, could also result in new host and epibiont combinations (Pascual et al., 2007).

Thirdly, hosts may differ in their suitability as a colonisable substratum. Indeed, variation in host morphology or physiology, such as the differences between adults and juveniles or between males and females, could alter the suitability for epibiont settlement and colonization. In turn, this could lead to intra-specific variation in epibiont communities. Indeed, mature male long-finned pilot whales, *Globicephala melas*, host greater abundances of the whale-louse, *Isocyamus delphini*, relative to females or juveniles of either sex (Balbuena and Raga, 1991), potentially due to differences in the morphology of the genital slit, where the epibionts tend to congregate. Differences in the texture of the skin or carapace between hosts could also lead to differential patterns of epibiont settlement, as seen in male and female horseshoe crabs *Tachypleus gigas* (Patil and Anil, 2000).

It is also important to remember that a host's suitability as a colonisable substratum may also change temporally or with the behaviour of the host. A dead or stranded host may provide a much less hospitable habitat than a living host whereas a debilitated host may offer opportunity for greater than usual levels of colonization (Sloan et al., 2014). Moreover, many epibionts are mobile and can rapidly abandon their host if needed. It has thus been hypothesised that abundance of mobile epibionts on stranded animals or, in the case of sea turtles, nesting females, would be far fewer than those on free-swimming animals (Lazo-Wasem et al., 2011). This has been born out indirectly with epibiotic crabs on sea turtles. When olive ridley turtles *Lepidochelys olivacea* were sampled at sea in the East Pacific Ocean, 42% of 181 turtles hosted crabs (Pfaller et al., 2014). In comparison, aggregating data from three separate studies, only 3% of nesting olive ridley turtles in the eastern Pacific hosted *Planes* spp. (Lazo-Wasem et al., 2011; Majewska et al., 2015b; Robinson et al., 2016b).

Sea turtles are an ideal model species to investigate the factors influencing intra-specific variation in epibiont community structure. This is because sea turtles are arguably the marine taxa with the most diverse epibiont communities (Caine, 1986; Pfaller et al., 2008; Corrêa et al., 2014), including many epibionts that are found on a variety of other marine megafauna (Suarez-Morales et al., 2010; Zardus et al., 2014). Sea turtles are also highly mobile, with large variation between individuals both in terms of movement patterns and foraging preferences (Vander Zanden et al., 2010; Robinson et al., 2017). Thus, they are suited for examining how variation in individual behaviour can influence epibiont communities. Lastly, female sea turtles routinely nest on tropical beaches around the world. Thus, they provide a unique opportunity to examine how mobile epibionts respond to hosts that leave the water. This may not only provide insights into whether epibiont communities sampled on sea turtles in-water or on nesting beaches are comparable, but also whether the epibiont communities of stranded animals are comparable to their free-swimming counterparts.

Here, we assessed whether the epibiont communities of olive ridley sea turtles in the East Pacific differed between genders, sampling years, and geographic location. We achieved this by sampling and comparing the epibiont communities of (1)nesting female turtles sampled over different nesting seasons, (2)female turtles sampled on nesting beaches and at sea, and (3)female and male turtles, both sampled at sea. Understanding the impact of each of these factors helps assess whether short-term sampling (e.g. during a single nesting season) of epibionts from nesting sea turtles is representative of long-term epibiont communities for the entire population. More importantly, this information can also provide information about how and why epibiont communities may vary between hosts. In turn, this information can help us to understand spatial and temporal variation in epibiont communities, may reveal interesting insights into the evolutionary history of both epibiont and host, and facilitate the use of epibionts as ecological indicators.

2. Methodology

To collect epibionts, we sampled olive ridley turtles on the Pacific coast of the Guanacaste Province of Costa Rica between 2015 and 2016. Turtles were sampled from two locations: male and female turtles encountered at-sea between Playas Del Coco and Cabuyal (within 3 km of the shore) and female turtles nesting on the beaches of Parque Nacional Marino Las Baulas (Fig. 1).

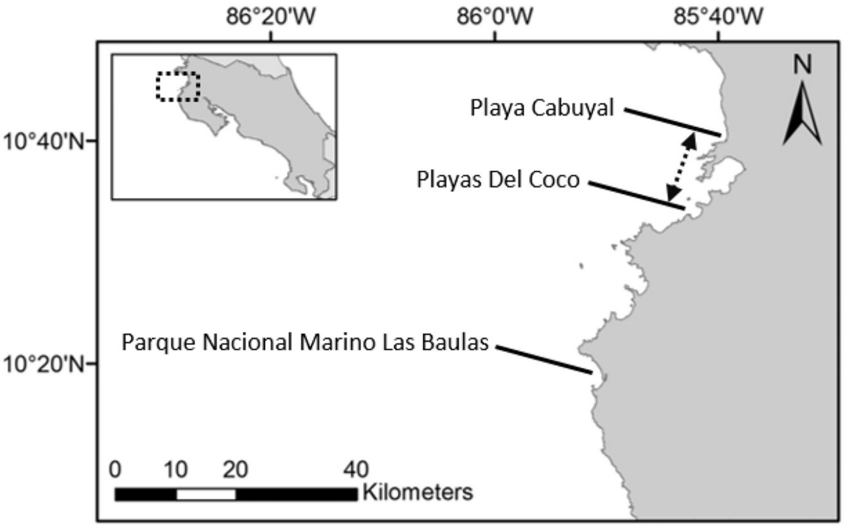

Fig. 1. Map indicating the study sites along the Pacific coast of Costa Rica, Central America. At-sea sampling of olive ridley turtles for epibionts was conducted in the waters between Playas Del Coco and Cabuyal as indicated by the dotted arrowed line. Sampling of nesting female olive ridley turtles was conducted on Cabuyal and the beaches within Parque Nacional Marino Las Baulas.

Table 1
Epibionts taxa and frequency per host-group (males in water, females at-sea, and nesting females sampled in 2014/15, and nesting females sampled in 2015/16) for olive ridley turtles *Lepidochelys olivacea* sampled in northwest Costa Rica.

Systematic Group	Epibiont Taxon	Mean and Standard Deviation of Epibionts			
		In water: males $n = 27$	In water: females $n = 22$	Beach: 2014/15 $n = 19$	Beach: 2015/16 $n = 13$
Annelida: Hirudinea	*Ozobranchus branchiatus*	0.04 (0.19)	0.41 (1.14)	1.47 (3.47)	0.08 (0.28)
Annelida: Hirudinea	*Ozobranchus branchiatus* (eggs)[a]	0.07 (0.27)	0.05 (0.21)	0	0
Annelida: Polychaeta	Syllidae	0	0	0	0.08 (0.28)
Chlorophyta	Algae/Hydroid[a]	0.11 (0.32)	0.18 (0.39)	0.15 (0.38)	0.15 (0.38)
Cnidaria: Actiniaria	Unknown sp.	0.15 (0.77)	0	0	0
Crustacea: Amphipoda	*Podocerus chelonophilus*	18.48 (70.74)	8.95 (20.04)	25.47 (60.74)	23.69 (59.83)
Crustacea: Brachyura	*Planes* spp.	0	0.18 (0.39)	0	0
Crustacea: Cirripedia	Balanamorpha	0.04 (0.19)	0	0	0
Crustacea: Cirripedia	*Chelonibia testudinaria*[b]	1.52 (3.12)	1.32 (3.77)	0.84 (1.57)	1.00 (1.29)
Crustacea: Cirripedia	*Conchoderma sp.*	0	0.14 (0.64)	0	0
Crustacea: Cirripedia	*Conchoderma virgatum*	1.93 (3.85)	21.41 (30.76)	7.63 (24.25)	2.08 (5.36)
Crustacea: Cirripedia	Lepadomorpha	0	0.59 (2.77)	1.05 (4.58)	0
Crustacea: Cirripedia	*Lepas* spp.	1.11 (5.21)	7.5 (26.14)	5.53 (15.25)	0
Crustacea: Cirripedia	*Platylepas decorata*	0	0	0.11 (0.46)	0
Crustacea: Cirripedia	*Platylepas hexastylos*	2.00 (3.56)	0.90 (3.83)	1.95 (4.19)	1.23 (2.49)
Crustacea: Cirripedia	*Stomatolepas elegans*	14.29 (26.47)	7.36 (12.35)	1.05 (1.68)	5.85 (13.38)
Crustacea: Cirripedia	Unknown sp. (Cyprids)	0.04 (0.19)	38.22 (123.17)	0	0
Crustacea: Copepoda	*Balaenophilus manatorum*	12.37 (37.89)	5.04 (10.11)	0.26 (1.15)	12.08 (25.29)
Crustacea: Isopoda	*Corallanidae*	0	0	0	0.15 (0.38)
Foraminifera	Unknown sp.	0	0	0	0.08 (0.28)
Mollusca: Gastropoda	*Crepidula sp.*	0	0	0.05 (0.23)	0
Mollusca: Gastropoda	Muricidae	0	0	0.05 (0.23)	0
Mollusca: Polyplacophora	Unknown sp.	0.04 (0.19)	0	0	0
Platyhelminthes	Unknown sp.	0	0.05 (0.21)	0	0

[a] Taxon represents presence only. Individual counts were not undertaken.

[b] The count only includes ‘adults’ and not complemental males.

To locate host turtles, we undertook a combination of day-time boat and night-time beach patrols. The boat patrols were conducted in August 2015 and involved at-sea capture of turtles by hand. When a turtle was spotted, it was approached by a swimmer who held the animal and guided it to the boat. Upon reaching the boat, the turtle was lifted out of the water by its carapace and placed onto a rubber tyre. The turtle was tagged using metal identification tags, its carapace was measured, and all visible epibionts were removed. To ensure thorough collection of epibionts from all the turtles' surfaces, each turtle was briefly ($<$ 5 mins) flipped onto its carapace to allow inspection of its underside. Similar methods were used to collect epibiont loads from nesting turtles, except these animals were encountered during night-time patrols on the beaches of Parque Nacional Marino Las Baulas. The beaches of Parque Nacional Marino Las Baulas were patrolled between October and March over two consecutive nesting seasons: 2014/15 and 2015/16. To avoid interrupting the nesting process, epibiont sampling was always carried out after oviposition was completed. Epibiont abundance data from the 2015/16 nesting season are already published in Robinson et al. (2016b).

Epibionts were removed using a knife or forceps and subsequently preserved in vials containing 75% ethanol (denatured ethanol up until March 2016, and non-denatured alcohol afterwards). Samples were exported to the Yale Peabody Museum of Natural History, USA. On their arrival at the museum, all epibionts were counted and identified to the lowest taxonomic level by consulting appropriate literature. Finally, the samples were catalogued for curation into the museum's collection and an identified series of voucher representatives will be sent to the Universidad de Costa Rica, Costa Rica. Further information and images of selected specimens are available at http://peabody.yale.edu/collections.

No attempt was made to investigate any micro-epibionts, such as diatoms, even though it is known that they exist in large number on olive ridley sea turtles (Majewska et al., 2015b; Robinson et al., 2016a).

2.1. Statistical analyses

Epibiont samples were separated into four groups: those sampled from (Anderson and Walsh, 2013) males sampled at sea, (Apprill et al., 2014) females sampled at sea, (Balbuena and Raga, 1991) nesting females sampled in 2014/15, and (Balbuena et al., 1995) nesting females sampled in 2015/16. To determine whether the sample sizes we employed were sufficient to adequately characterise epibiont species richness for each turtle grouping, we calculated rarefaction curves following the Bernoulli product model (Colwell et al., 2012). We also used these curves to estimate how many unique epibiont taxa would be found if sample sizes were increased to 30 for each turtle group. Sample-based rarefaction and extrapolation curves were calculated using EstimateS V.9 (Colwell, 2013).

To test for statistical differences in the epibiont communities between the four turtle groupings, we used a one-way PerMANOVA. For the PerMANOVA test we employed the Bray–Curtis index of dissimilarity as it is suitable comparing raw abundance data. We choose a PerMANOVA test over other methods for examining difference in community structure, such as ANOSIM or Mantel tests, because PerMANOVA tests are considered more robust to difference in multivariate dispersion (Anderson and Walsh, 2013). Significance was computed through permutation by group membership with 9999 replicates and applying the Bonferroni correction to account for biases associated with multiple comparisons.

To investigate patterns in abundance of each epibiont taxon between the four turtle groupings, we used one-way ANOVAs with Tukey's pairwise comparisons. PerMANOVA and ANOVA tests were conducted using PAST V.3.13, considering significance if $p \leq 0.05$.

3. Results

We sampled epibionts from 81 olive ridley turtles, 32 of which were encountered on nesting beaches and 49 sampled at-sea. Of the female turtles sampled on nesting beaches, 19 and 13 were sampled in the

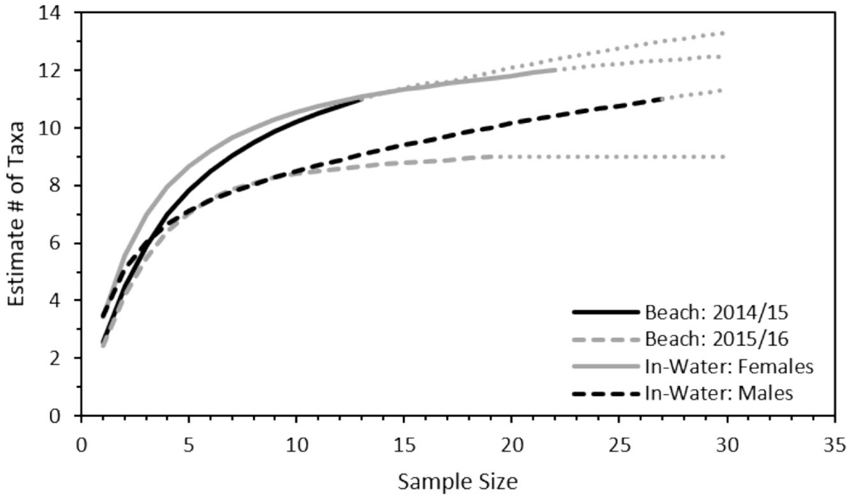

Fig. 2. Species-richness rarefaction curves for the number of unique epibiont species found on both male and female olive ridley turtles that were sampled at-sea as well as females that were sampled on nesting beaches. The solid and dashed lines represent the modelled rate of increase for species richness with increasing sample size, while dotted lines represent extrapolated data to predict species richness up to 30 samples.

2014/15 and 2015/16 nesting seasons respectively. Of the turtles encountered at-sea, 22 were female and 29 were male. We collected a cumulative total of 4918 epibionts, representing at least 19 unique taxa. For a full list of these taxa see Table 1. The presence of many epibionts under 1 mm in size (e.g. *Balaenophilus manatorum*) in our samples suggests that we thoroughly collected most non-microscopic epibionts. Epibionts were present on every turtle sampled.

We found a total of 11, 12, 9, and 11 unique epibiont taxa on at-sea males, at-sea females, nesting females sampled in 2014/15, and nesting females sampled in 2015/16 respectively. Extending the rarefaction curve to sample sizes of 30 for each group, the number of estimated unique taxa did not increase by > 2 for each turtle grouping (Fig. 2).

The PerMANOVA test identified a statistically significant difference in the epibiont communities of the four turtle groupings (F = 2.564, $p < 0.001$). Furthermore, post-hoc pair-wise PerMANOVA tests demonstrated that the epibiont communities of males sampled in water were statistically different from females sampled in water ($p < 0.001$), nesting females sampled in 2014/15 ($p < 0.001$), and nesting females sampled in 2015/16 ($p = 0.012$). However, the post-hoc pair-wise PerMANOVA tests did not reveal any statistically significant differences among females sampled in water, nesting females sampled in either 2014/15, nor nesting females sampled in 2015/16 (Table 2).

ANOVA analysis indicated that there were no statistically significant differences in the abundance of any epibiont taxa between turtle groupings with the sole exception of *Conchoderma virgatum* ($F_{3,76} = 4.37$; $p = 0.006$; Fig. 3). Interestingly, the Tukey's pairwise comparisons indicated that this was due to significant differences between the abundance of *Conchoderma virgatum* on females sampled at sea with both males sampled at-sea ($p = 0.022$) and females on the nesting beach in 2015/16 ($p = 0.024$).

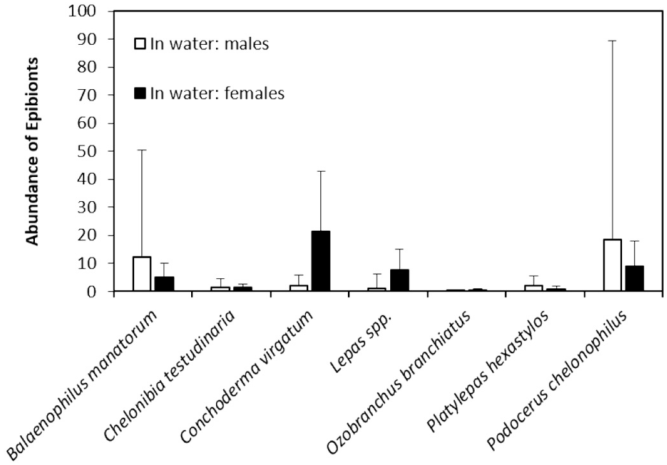

Fig. 3. Abundance of the major epibiont taxa found on males and female olive ridley turtles that were sampled in water. The only statistically significant difference between genders was in the abundance of *Conchoderma virgatum*. Error bars are 1 SD.

3.1. Taxonomic considerations and limitations

Several taxa, such as *Lepas* spp. and *Concoderma* spp., could not be unequivocally identified to a species level, yet they clearly represented unique taxa and so were considered as such. Other epibionts, such as those listed as Balanamorpha and Lepadomorpha barnacles as well as the barnacle cyprids, were likely to be representatives from previously observed taxa, even though this could not be confirmed. Consequently, these epibionts were not considered unique taxa and were not incorporated into the PerMANOVA analysis nor the rarefaction curves.

Table 2
P values for the PerMANOVA test comparing the epibiont communities of olive ridley turtle groupings (males in water, females at-sea, and nesting females sampled in 2014/15, and nesting females sampled in 2015/16).

	In water: females	In water: males	Beach: 2014/15	Beach: 2015/16
In water: females		0.0006	0.2358	0.1206
In water: males	0.0006		0.0126	0.0012
Beach: 2014/15	0.2358	0.0126		1
Beach: 2015/16	0.1206	0.0012	1	

Several turtles had mats of filamentous algae attached either directly to the carapace or indirectly to basal epibionts. Although the alga was often conspicuous and easily observed, it was also present in smaller microscopic quantities that only became apparent after inspecting the basal epibionts under a microscope. Thus, we did not quantify the alga but instead simply noted its presence. Furthermore, the algae were often intertwined with hydroids and could not be separated without damaging the samples. Thus, both algae and hydroids were combined into a single functional grouping.

A single foraminiferan was found in a sample from a nesting turtle. These organisms are often found in sandy sediments and have not previously been recorded as epibionts on sea turtles (though individuals attached to large barnacles such as *Chelonibia* may potentially have been overlooked). Thus, we presume that this organism was present in the beach sand that the turtle had thrown on its carapace during nesting but, though probably not a true epibiont, is included for completeness (Table 1).

4. Discussion

The epibiont communities of several marine megafauna populations worldwide are well characterised. Yet only rarely have studies attempted to define both how and why these epibiont communities vary between populations. Furthermore, a holistic understanding of how epibiont communities vary between populations requires knowledge of why individual in the same population may have variable epibiont communities. Here, we compared variation in the epibiont communities of a single population of olive ridley sea turtles. Specially, we examined how epibiont communities varied between male and female turtles sampled in-water, female turtles sampled either in-water or on the nesting beach, and nesting females sampled during different nesting seasons.

4.1. Characterising the epibiota of olive ridley turtles in the East Pacific

From the 81 olive ridley turtles sampled in this study, we identified 19 unique epibiont taxa. A total of 11 of these taxa, including *Ozobranchus branchiatus*, *Podocerus chelonophilus*, *Chelonibia testudinaria*, *Conchoderma virgatum*, *Lepas* spp., *Planes* spp., *Platylepas hexastylos*, *Stomatolepas elegans*, *Balaenophilus manatorum*, and algae/hydroid, were present on multiple hosts and have been recorded in other epibiont studies on olive ridley turtles in the region (Lazo-Wasem et al., 2011; Pfaller et al., 2014; Majewska et al., 2015b; Robinson et al., 2016a). Thus, it appears that these taxa constitute the typical epibiont fauna of olive ridley turtles in the East Pacific Ocean. In contrast, all other epibiont taxa were encountered in on very few hosts (< 2 individuals) and in relatively low numbers. We suspect that most of these taxa are likely opportunistic, and not obligate, epibionts. Moreover, many of these rarer species reflect novel examples of epibiosis for sea turtles. Indeed, there are no previously published reports of chitons (Polyplacophora) as epibionts and almost all other reports of flatworms (Platyhelminthes) have been as internal parasites (Dailey et al., 1992; Innis et al., 2009) rather than external epibionts.

4.2. Effect of sampling turtles on nesting beaches or at sea

We identified several mobile taxa, such as *Podocerus chelonophilus* and *Balaenophilus manatorum*, within the epibiont fauna of olive ridley turtles. While it could be plausible that these taxa might abandon hosts that are about to spending significant periods of time outside of the water, as is the case during nesting, we found no statistically significant difference, as determined by a PerMANOVA, between the epibiont communities of nesting turtles and turtles sampled at sea. The rarefaction curves, although they do not allow for direct testing of statistical hypotheses concerning species richness of the different sea turtle groupings, also do appear subjectively very similar between females sampled at sea and nesting females sampled on the nesting beaches.

Our results therefore suggest that most epibiont taxa do not abandon hosts even if they emerge out of the water to nest. Indeed, large clusters of the amphipod *Podocerus chelonophilus* were even observed attached to the carapace of daytime nesting turtles when temperatures were presumably high enough to be fatal for these epibionts (NJ Robinson, personal observation). The high numbers observed for the small copepod *Balaenophilus manatorum* on nesting turtles also suggest that this species, and probably most other nestling crustaceans, remain attached to a host even after it leaves the water. It should be noted, however, that even the turtles sampled at-sea had to be removed from the water, albeit briefly (< 10 min), to collect all the epibionts. In our analysis, we are not able to consider epibionts that might immediately flee their host as it is removed from the water. Moreover, when sampling turtles at sea, we often observed that crabs, presumably *Planes* spp., immediately abandoned their host as the turtle was removed from the water. Any epibiont study that involves removing the animal from the water, even when conducted at sea and animals are only out of the water for short lengths of time (< 10 min), is therefore likely to underestimate the true abundance of some highly mobile epibionts, such as *Planes* spp.

Why some mobile epibionts immediately abandon host turtles if they leave the water while others do not could simply be a product of the limited swimming ability of some mobile epibionts e.g. *Podocerus chelonophilus* (Domènech et al., 2017). Alternatively, it may reveal some interesting evolutionary relationships between epibiont and host. It has been generally noted that most epibiotic crustaceans found on floating debris, such as sea weed, will abandon the substrate after sensing that conditions are no longer favourable, e.g. as the substratum leaves the water (Lazo-Wasem et al., 2011). However, our observations suggest that obligate epibionts, such as *Podocerus chelonophilus* or *Balaenophilus manatorum*, have adapted behaviourally to turtle behaviour by remaining on the host even after it has left the water. This could be highly beneficial trait for obligate epibionts as sea turtles generally only leave the water to nest and will thus return to the water within a few hours. In contrast, more generalist epibionts that are not obligate on vertebrate hosts and are commonly associated with floating debris, such as *Planes* spp., would be expected to abandon any substratum as soon as it leaves the water, if it is unlikely to be returning the water soon.

4.3. Sex-specific differences in epibiont communities

The PerMANOVA analysis identified statistically significant differences between the epibiont communities of male and female turtles. While there multiple reasons why male and female sea turtles might be expected to host different epibiont communities, e.g. differential morphologies, immune-responses, migratory behaviors etc…., it is not clear what is the ultimate reason for this difference. The individual ANOVAs used to compare the abundance of each epibiont taxa between male and female turtles sampled at sea (as well as female turtles sampled on land) identified a statistically significant difference for only a single epibiont taxa, *Conchoderma virgatum*. In addition, even though pair-wise Tukey tests identified differences in abundance of *Conchoderma virgatum* on male and female turtles sampled at sea ($p = 0.022$), no significant differences were observed between male turtles and females sampled on their nesting beaches (2014/15: $p = 0.614$; 2015/16: $p = 0.285$). It also does not appear that the statistically determined differences in the epibiont communities of males when compared to the other groupings was determined by differences in species richness (see the rarefaction curves in Fig. 2) or the presence of unique epibiont taxa (see Table 1). We therefore exercise caution in interpreting our finding that the epibiont communities of male and female olive ridley turtles are different and recommend further studies to answer this question.

4.4. Stability of epibiont communities between nesting seasons

We did not discover a statistically significant difference between the epibiont communities of nesting turtles sampled in either 2014/15 or 2015/16. Although this could be interpreted to mean that epibiont communities are relatively stable with respect to inter-annual variation, and thus it is possible to compare the epibiont communities between populations sampled over different years, this is most likely an over simplification. One of the reasons that we were interested in determining whether the epibiont communities of olive ridley turtles in the East Pacific differed between years is that this population has been observed to switch foraging habitats between years from coastal or offshore habitats depending on the prevailing environmental conditions, specifically the state of the ENSO (Plotkin, 2010). If turtles with different foraging habitats or migratory behaviour have different epibiont communities as predicted (Frick and Pfaller, 2013; Robinson et al., 2016b), then epibiont communities would therefore also be expected to vary with the prevailing environmental conditions. Similarly, variation between years would also be expected if the prevailing environmental conditions also affect epibiont recruitment. However, both the sampled years coincided with positive phases of ENSO. While our data suggest that epibiont communities can be stable between years, more research is required to fully assess the range of potential inter-annual variation in epibiont communities.

4.5. Implications

Most studies attempting to characterise the epibiont communities of sea turtles have focused on nesting individuals (e.g. Caine, 1986; Frick et al., 1998; Pfaller et al., 2008; Lazo-Wasem et al., 2011). Though it is generally more practical to sample sea turtles on their nesting beaches rather than at sea, never have epibiont loads of sea turtles sampled on their nesting beaches been compared to those of turtles sampled at sea. Without such information, it is not possible to determine whether assessments of turtles sampled on nesting beaches accurately represent the full epibiont community and make valid comparisons with other studies that were not conducted on nesting beaches. In this study, we demonstrate that the epibiont communities of olive ridley sea turtles in the East Pacific are not significantly different between animals sampled at sea or on their nesting beaches. Furthermore, epibiont communities can remain stable between years. Thus, we conclude that sampling nesting sea turtles is a reliable method for assessing epibiont communities of female sea turtles. However, it should not be assumed that the epibiont communities of male and female turtles are the same, though this requires more research.

Knowledge of how biological factors, such as gender, lead to intra-specific variation in epibiont communities as well as logistical factors, such as whether an animal was sampled on land or at sea, is essential if we are to compare epibiont communities in a standardised manner. For example, if it is found that epibiont communities of turtles sampled at sea differ from those on nesting beaches, it would complicate direct comparisons of epibiont communities already in the published literature. Similar limitations would also be apparent when comparing the epibiont communities from stranded animals, be they sea turtles or other taxa, to those from free-swimming taxa. Thus, as other studies improve our understanding of the patterns behind intra-specific variation in epibiont communities, it will facilitate the development of larger meta-analyses that compare the epibiont communities from populations sampled around the world. In turn, such analyses have a wide-range of potential uses, ranging from tracking the spread of epibionts that may serves as vectors for disease transmission (e.g. Greenblatt et al., 2004; Lazo-Wasem et al., 2007) to understanding co-evolutionary interactions between epibionts and their hosts.

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