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Operational sex ratio estimated from drone surveys for a species threatened by climate warming

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Abstract

Rising sand temperatures resulting from climate warming may cause the 'feminization' of sea turtle populations, which have temperature-dependent sex determination. In July and October 2021, we conducted surveys using a drone (also referred to as an unmanned aerial vehicle or UAV) and shore-based observations to assess the operational sex ratio (OSR) (number of males and females in breeding condition) for green turtles (*Chelonia mydas*) in the southern Great Barrier Reef, Australia (23.44 °S, 151.92 °E). Using drone-imagery, the length:width (L:W) ratio of a turtle's head was used to distinguish loggerhead turtles (*Caretta caretta*) from green turtles, and the L:W of the carapace was used to distinguish juveniles from adult-sized green turtles. The first breeding pair was observed in shore-based surveys on 14 September 2021 and the number of mating turtles then increased to a peak on 4 October, about 8 weeks before the peak in nesting. A total of 94 km of drone transects with associated video footage was analysed. In October, at the peak of the mating season, the ratio of adult turtles displaying breeding behaviours near the island was 0.51 female (95% CI ± 0.17), supporting previous conclusions that despite the female-biased (> 80%) hatchling sex ratio, this population likely has a fairly balanced OSR. These findings are likely explained by males breeding 2–3 times more frequently than females, which helps mitigate female-biased hatchling sex ratios. Assessing the OSR of populations with extreme female hatchling bias may help to inform whether intervention is needed to increase male hatchling production.

Keywords Breeding sex ratio · Climate change · Migration · Heron Island · Aerial survey · Remote sensing

Introduction

Climate warming research has burgeoned in recent years due to major concerns regarding how certain organisms will respond, particularly those already under threat from anthropogenic activities. Sea turtles are widely considered

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to be vulnerable to climate change (Poloczanska et al. 2009). One reason for concern is that all sea turtle species have temperature-dependent sex determination (TSD), whereby sex is determined by the nest temperature during the thermal sensitive period of development (reviewed in Wibbels 2003; Porter et al. 2021). Below the pivotal temperature (PT), a higher proportion of male hatchlings is produced and vice versa for females (Mrosovsky, 1994; Ackerman, 1997). There is a 2–4 °C transitional range of temperatures where hatchlings of both sexes are produced (Mrosovsky, 1994). Therefore, the 2–4 °C projected rise in atmospheric temperatures by 2100 (IPCC 2022) and the associated rise in sand temperatures may result in the 'feminization' of sea turtle populations globally (Poloczanska et al. 2009).

Operational sex ratios (OSR, the ratio of sexually active males to receptive females in a reproductive cohort) that are female-biased can increase competition between females to find a mate, reduce opportunities to breed, and decrease genetic diversity (Emlen and Oring 1977; Kvarnemo and

Ahnesjo 1996). Furthermore, a localised extinction event could occur if the production of male offspring declines to a level at which it is functionally zero (Poloczanska et al. 2009; Mitchell and Janzen 2010). For example, the northern Great Barrier Reef (nGBR) green turtle (*Chelonia mydas*) genetic stock is under considerable threat from feminization induced by climate change (Jensen et al. 2018). On Raine Island, where the majority of this population nests (also the largest green turtle rookery in the world), sand temperatures since the mid-1970s are estimated to have been well over the PT, and as a result the clutches produced are > 99% female (Jensen et al. 2018; Blechschmidt et al. 2020; Booth et al. 2020). A major concern for rookery managers and conservationists is if female-biased cohorts recruit to the breeding population and as the older males die out there will not be enough breeding males to fertilise the clutches. Recent modelling by Jensen et al. (2022) has shown that under an extreme climate warming scenario, the nGBR green turtle population will require each adult male to mate with 15–30 different females per season just to prevent population decline.

Approximately 1500 km southeast of Raine Island is Heron Island, a green turtle nesting site located within the Capricorn Bunker Group (CBG) in the southern Great Barrier Reef (sGBR) of Australia. Around September and October, sGBR green turtles that are in breeding condition migrate from their foraging grounds to mate and nest in locations such as the CBG (Limpus 2008). Despite inter-annual variation in the number of nesting turtles (Limpus 2008), the sGBR green turtle population has increased in recent decades (Chaloupka and Limpus 2001). There is also a foraging population of green turtles that are year-round residents of Heron Reef (Forbes 1996; Fitzsimmons 1997). The resident population in the lagoons of Heron Reef are historically reported as ~ 50% female (Forbes 1996), whilst the primary hatchling sex ratio for Heron island is estimated to be 80–97% female (Booth and Astill 2001; Booth and Freeman 2006). Data from experimental studies on Heron Island also suggest that average nest temperatures have likely increased in recent years (Ischer et al. 2009; Booth and Evans 2011; Booth et al. 2013; Smith et al. 2021; SI Figs. 1, 2). Despite a female-biased primary sex ratio predicted for Heron Island, sand temperatures in the CBG are still expected to be highly variable due to beach orientation and high vegetation cover, inferring complete feminisation is still not likely to occur soon (Booth and Astill 2001; Limpus 2008).

Previously, OSRs for the sGBR population have been estimated using both 'mark and recapture' and drone surveys in the lagoons surrounding Heron Island, with the conclusion that the OSR is balanced or slightly male-biased in October (Fitzsimmons 1997; Yaney-Keller et al. 2021). However, the extent of inter-annual variability in the OSR is largely unknown for sGBR green turtles and many other populations. Here, we used drone surveys to assess whether a balanced OSR has been sustained since it was first measured in the 1990s. Previous work by Yaney-Keller et al. (2021) using drone and boat surveys indicated a balanced OSR on Heron Reef in the 2016 breeding season; however, the presence of non-breeding adults and subadult turtles in these surveys was not considered in the analyses. In this study, we suggest and adopt methods to minimise the potential for counting non-breeding turtles and other turtle species when using drone surveys to assess OSR. Lastly, we documented the phenology of breeding activity around the island and in this way ensure that drone surveys to estimate OSR were conducted around the peak of breeding activity.

Materials and methods

Study site

Heron Reef (23.44 °S, 151.92 °E) is ~ 80 km east of Gladstone, Queensland and within the Capricorn Bunker Group (CBG) of reefs in the Great Barrier Reef Marine Park (Fig. 1a). Heron Island is a sand cay and is an important nesting island for the sGBR green turtle genetic stock (Limpus 2008). Heron Reef is ideal for surveying the breeding behaviours of green turtles because individuals aggregate in the shallow lagoon from September to November to court and mate (Fitzsimmons 1997). The tidal range at Heron Reef is ~ 3 m, which means that on most low tides the reef rim of this platform reef is exposed, isolating the shallow lagoon, and potentially limiting access to sea turtles.

Shore-based survey design

From 1 August 2021 to 17 January 2022, we conducted shore-based surveys around Heron Island to count adult green turtles close to shore. These surveys were used to estimate the start (when mating behaviour was first observed) and the peak (the observation periods when the frequency of mating behaviour was greatest) of the mating season. From August to November, these surveys were conducted weekly and opportunistically by the University of Queensland Research Station staff. During the final two months of the survey period, surveys were completed less regularly due to limited staff availability. However, courtship activity is known to substantially decrease by the beginning of December (Yaney-Keller et al. 2021), so this reduction in survey effort likely did not impact our findings. The observers walked around the island shore and counted the number of adult-sized green turtles and breeding pairs that were seen. Observers identified the mature turtles based on their relatively large size and speckled greyish-green carapace. Surveys took 30–45 min to complete and were conducted around high tide.
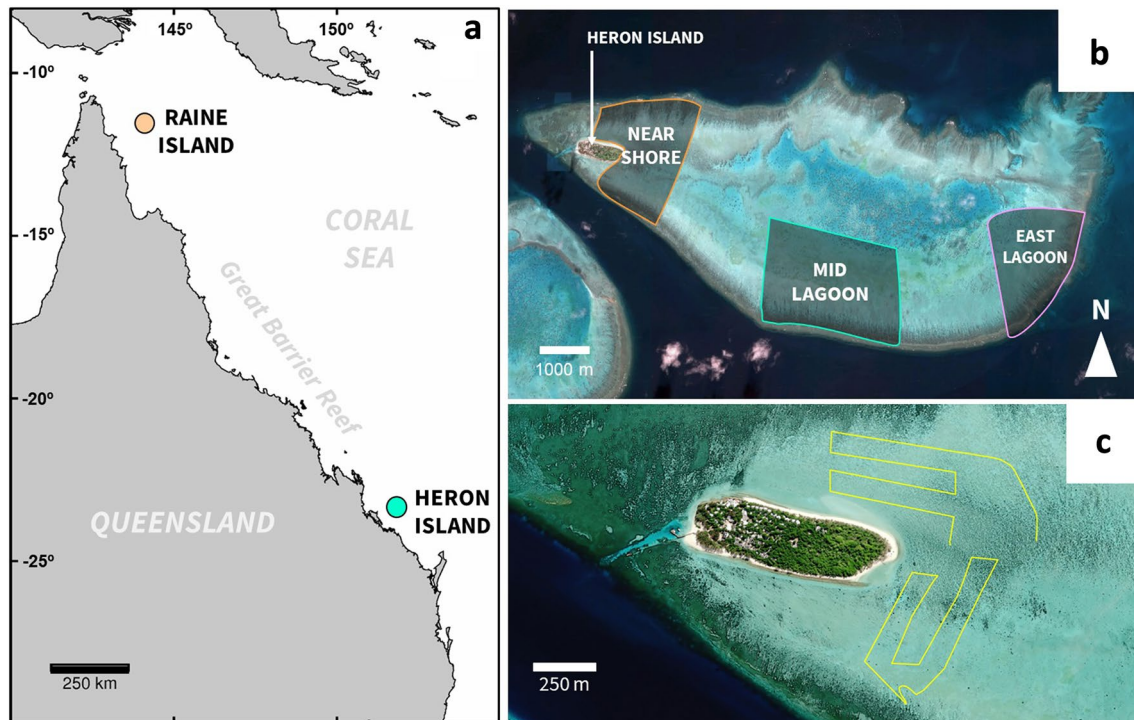
Fig. 1 **a** Map of Queensland, Australia, and the geographic locations of focal green turtle nesting locations; Heron Island (green circle) in the southern Great Barrier Reef (GBR), and Raine Island (orange circle) in the northern GBR. This is an 'equidistant cylindrical' map that was adapted from a projection created in the 'Maptool' program from seaturtle.org. **b** Satellite image shows Heron Reef, where adult green turtles aggregate to breed annually. Drone surveys in July and October 2021 were conducted within the 'nearshore' (orange), 'mid-lagoon' (blue), 'east-lagoon' (pink) areas of Heron Reef. **c** Examples of two transect lines (yellow) flown by a drone in the 'nearshore' survey area. Paths have been adapted to exclude observational detours from the transect. Satellite images were generated from ArcGIS Software and adapted

Survey equipment

A DJI Phantom™ 4 Pro drone (1375 g, SZ DJI Technology Co., Shenzhen, China) was used to conduct aerial surveys over several days in July and October. All flight paths were planned with the Litchi App™ on a tablet device (Apple Inc. iPad Mini 2™) and controlled by a licensed drone pilot. Each drone flight lasted 18–20 min depending on wind strength and direction. The drone carried a 4 K-quality video camera with a ND4 polarised filter added to reduce sun glare on the water's surface. The camera was attached to a three-axis gimbal system that stabilised the video and camera angles were controlled remotely. Flights were carried out with 4 K video recording, in some instances, video resolution was reduced to 2.7 K to assist with backing up video data on cloud storage whilst on the island.

Drone survey design

Drone surveys were completed from 14 to 20 July and 8 to 12 October 2021. On each day that data were collected, 2–3 flights were conducted (wind and tide dependent), and one of the three following areas were surveyed; the 'nearshore', 'mid-lagoon', 'east-lagoon' areas of Heron Reef (Fig. 1b). All drone flights in October and most drone flights in July were launched and landed on a research vessel, with some land-based launches occurring in July 2021. No drone flights launched or landed on the island during the October survey period to avoid disturbance to the large numbers of black noddy terns (*Anous minutus*) nesting and breeding in the trees throughout October.

Surveys were conducted on a rising tide, between 08:00 and 17:00 on days where winds were forecast to be < 15 kts and with no rainfall. The vessel was positioned within 50–100 m of the first transect line of a survey route. The drone always remained within the line-of-sight of the pilot's assistant (Civil Aviation Safety Authority regulations), so in some instances the drone was manually redirected to be within eyesight of the assistant. Transect lengths ranged from 500–700 m, were 50–100 m apart, with 2–6 lines per route and primarily in north–south oriented legs (Fig. 1c). Flight speed of each survey was 3–5 m/s and the altitude for the aerial surveys was 25–30 m (above the sea's surface). However, altitude was reduced to 20 m in moderate

wind conditions or to obtain closer observations of a turtle and assist with identification. The average flight altitude and camera angle were then used in the formulas for estimating the transect swathe (width), as provided in Burke et al. (2019). The total survey area of each transect was coarsely calculated by multiplying the horizontal distance travelled by the drone with the estimated swathe of each transect, restricting swathe width to 150 m. The total survey area covered was combined with the number of turtles observed during drone surveys to calculate standardised turtle density (turtles per km^2) per survey period.

Data processing and analysis

After the surveys were completed, the drone videos were reviewed in the Behavioural Observation Research Interactive Software (BORIS, https://www.boris.unito.it/) created by Friard and Gamba (2016). All turtle observations, species, sex, size class (juvenile or adult) and behaviour (breeding/courting or foraging) were recorded and annotated within the video projects on BORIS. A still image was exported from the video file in BORIS for each turtle observation and labelled with a code representing species, size class, sex and behaviour.

Adult male green turtles were distinguished by their long tails (> 25 cm beyond carapace), whilst adult female green turtles were identifiable by short tails (< 5 cm beyond the carapace) that are often not visible from above (Fig. 2) (Limpus 1993; Fitzsimmons 1997). Juvenile versus adult-sized turtles were distinguished primarily by carapace colouration (juveniles = orange/brown, adults = dark grey/green and speckled) (Fig. 2c). Additionally, the length to width (L:W) ratio of the carapace was measured using the pixels of the still images in ImageJ Ops Software (https://imagej.net/Ops) to distinguish juveniles from adult-sized turtles. While we explored a similar approach to distinguish adults from subadult turtles, it was not possible from these measurements alone. This is primarily because the carapace length (therefore, the carapace shape) of the largest subadults is too close to the smallest of the adult turtles (i.e., subadults = 65–85 cm, adults ≥ 85 cm; Limpus 2008; Jensen et al. 2016; Hof et al. 2017). Tail length is not a reliable indicator of sex in subadult turtles, therefore, methods such as laparoscopic examination of the gonads are required to confidently identify sex and maturity (e.g. Fitzsimmons 1997). Additionally, subadults make up a significant proportion (~ 42%) of resident turtles in the lagoons of Heron Reef (Forbes 1996). Therefore, the results from the July and October surveys do not include a sex ratio estimate for the resident population because subadult turtles could be incorrectly counted as adult females.

Breeding and courtship behaviours observed in the drone surveys included mounting, chasing and/or avoiding other turtles (males and females), flipper slapping, escorting a mounted pair (termed 'attendant' male behaviour) and directional swimming in the water column (Fig. 2; Supplementary
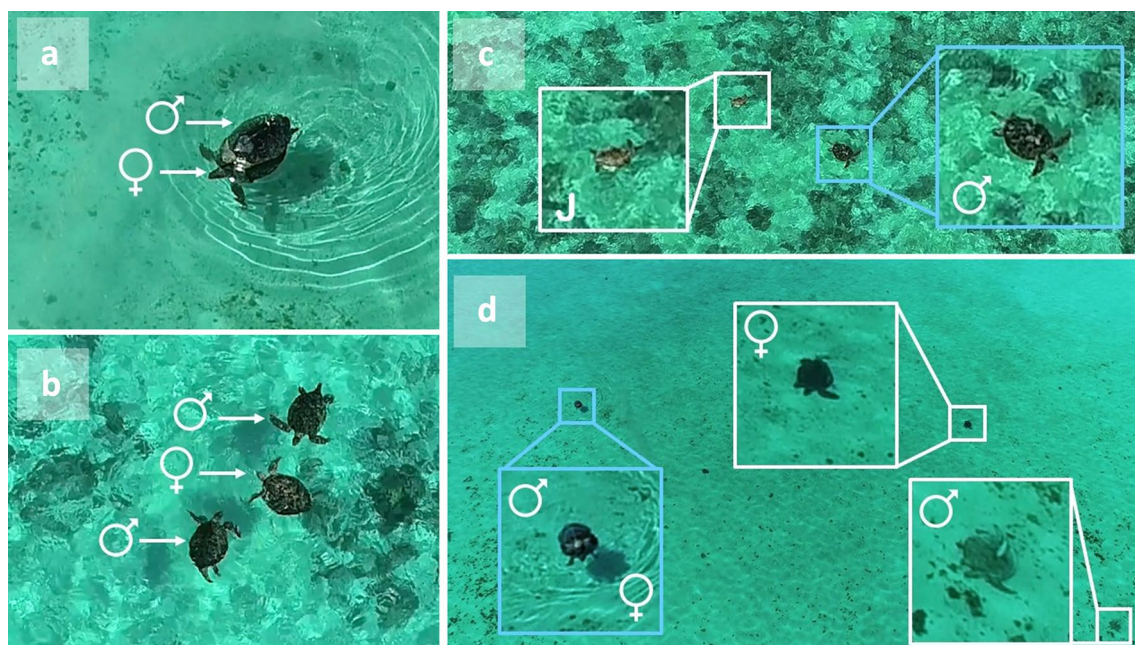

Fig. 2 Still images of green turtles captured from drone footage on Heron Reef in July and October 2021. **a** A breeding pair consisting of an adult male (tail not visible) mounted ontop of an adult female green turtle. **b** A courtship group consisting of a single adult female and two rival adult males (tails visible). **c** An adult breeding male (blue square) swimming away from a juvenile green turtle (white square) after attempting to mount. **d** A breeding pair (blue square) in close vicinity to two foraging turtles (white squares) in the 'mid-lagoon' area. Images have been cropped and adapted for this figure

Information videos) (Schofield et al. 2017; Dickson et al. 2022). Observable foraging behaviours include individuals on the seafloor with the head down, presumably feeding on algae (e.g., *Enteromorpha* sp.) or patchy seagrass (*Halophilia ovalis*) (Forbes 1996), and/or crawling along the seafloor searching for food (Fig. 2d; Supplementary Information videos). Seafloor foraging may also be interspersed by the turtle surfacing for a breath before swimming straight back down to the seafloor. Both breeding and foraging turtles could be seen basking on the water's surface, so this behaviour was excluded in our behavioural classification system. Tallies for adult male and female green turtle observations during the July and October periods were also converted into turtle density estimates based on turtles observed per km and km^2 across the 'nearshore' survey area and all three survey areas; 'nearshore', 'mid-Lagoon' and 'east-Lagoon', combined.

Both adult green and loggerhead turtles (*Caretta caretta*) are year-round residents to the lagoon areas of Heron Reef, whereas resident hawksbill turtles (*Eretmochelys imbricata*) primarily remain on the reef crest and slope. Because green and loggerhead turtles occur in the same areas of the lagoons, they had to be distinguished from each other in the drone footage. Loggerhead turtles were identified by their 'heart-shaped' carapace, orange–brown scales, and a proportionally large head and smaller front and hind flippers in comparison to body size. Adult green turtles were identified by their oval-shaped carapace with a grey–green and black speckled pattern, and a proportionally small head and larger front and hind flippers in comparison to body size. To further distinguish green and loggerhead turtles, a protocol for identifying the species in the drone-imagery was developed. Firstly, a subsample of the still images of clearly identifiable green and loggerhead were imported into ImageJ Ops Software. If the head was clearly visible in the drone footage, then the head dimensions (length and width) were measured, to calculate the head L:W ratio of both species. Using this index, any ambiguous turtle images where the head was visible could be more confidently assigned.

Results

Shore-based surveys

No adult-sized turtles or courting pairs were observed in the shore-based surveys throughout August when the surveys started. The first pair of courting turtles was observed on 14 September 2021 (Fig. 3). The number of mating turtle observations then increased to a peak on 4 October and then decreased to 1–2 sightings per survey after 16 October (Fig. 3).

Size-class and species identification

The width and length of the carapace was measured for four juveniles and a subsample of 12 adult-sized green turtles (initial identification was based on carapace colouration), where the mean carapace L:W ratio was 1.09 ($\pm$0.04 SE, range = 0.96–1.16) and 1.32 ($\pm$0.03 SE, range = 1.14–1.47), respectively. Therefore, adult-sized turtles were identified as having a L:W carapace ratio > 1.15.

The L:W head ratio was calculated from measurements of 17 adult-sized turtles that could be confidently identified by the observer as a loggerhead or green turtle by their visible features (e.g. carapace shape, colouration, head, and limb proportions). The head L:W averaged 0.94 ($\pm$0.06 SE, range = 0.83–1.09, $n = 5$) for loggerhead turtles and averaged 1.51 ($\pm$0.06 SE, range = 1.2–1.82, $n = 12$) for green turtles (Fig. 4). Therefore, any individuals with a L:W ratio for head proportions (where the head is visible) > 1.2 were classified as green turtles.

Drone surveys

A total of 23 and 18 drone flights were conducted in July and October respectively, culminating in a combined survey distance of 94 km. Adult-sized green turtles were observed

Fig. 3 The number of adult-sized green turtles (light diamonds) and breeding pairs (dark circles) observed in the nearshore area from shore-based visual surveys on Heron Island conducted from 1 Aug 2021 to 17 January 2022. Adults are rarely observed in the nearshore areas outside of the breeding and nesting months (September–March) due to the lack of suitable food available close to the island
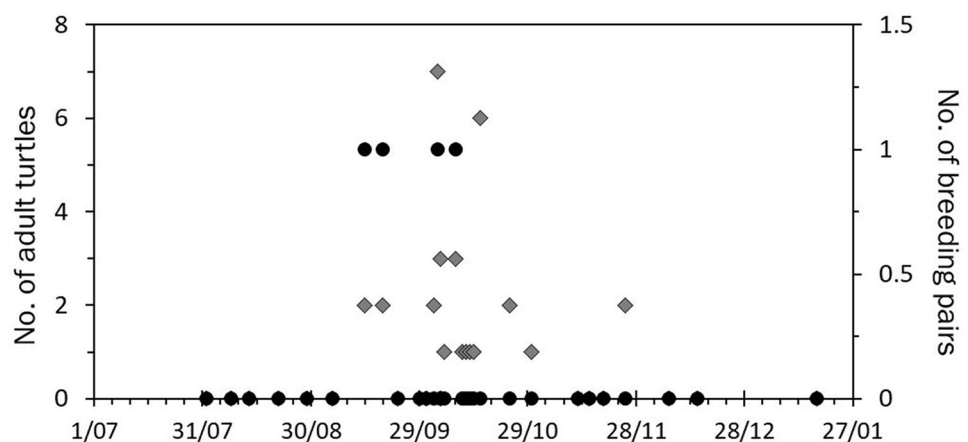
Fig. 4 Frequency plot of head length to width (L:W) ratio of adult-sized green (green bars) and loggerhead (purple bars) turtles measured in ImageJ Software from a subsample of the turtles observed in footage from drone surveys on Heron Reef. This figure includes images of *Caretta caretta* (loggerhead) and *Chelonia mydas* (green turtle) as a reference to show key morphological differences seen in the two species at the adult life stage
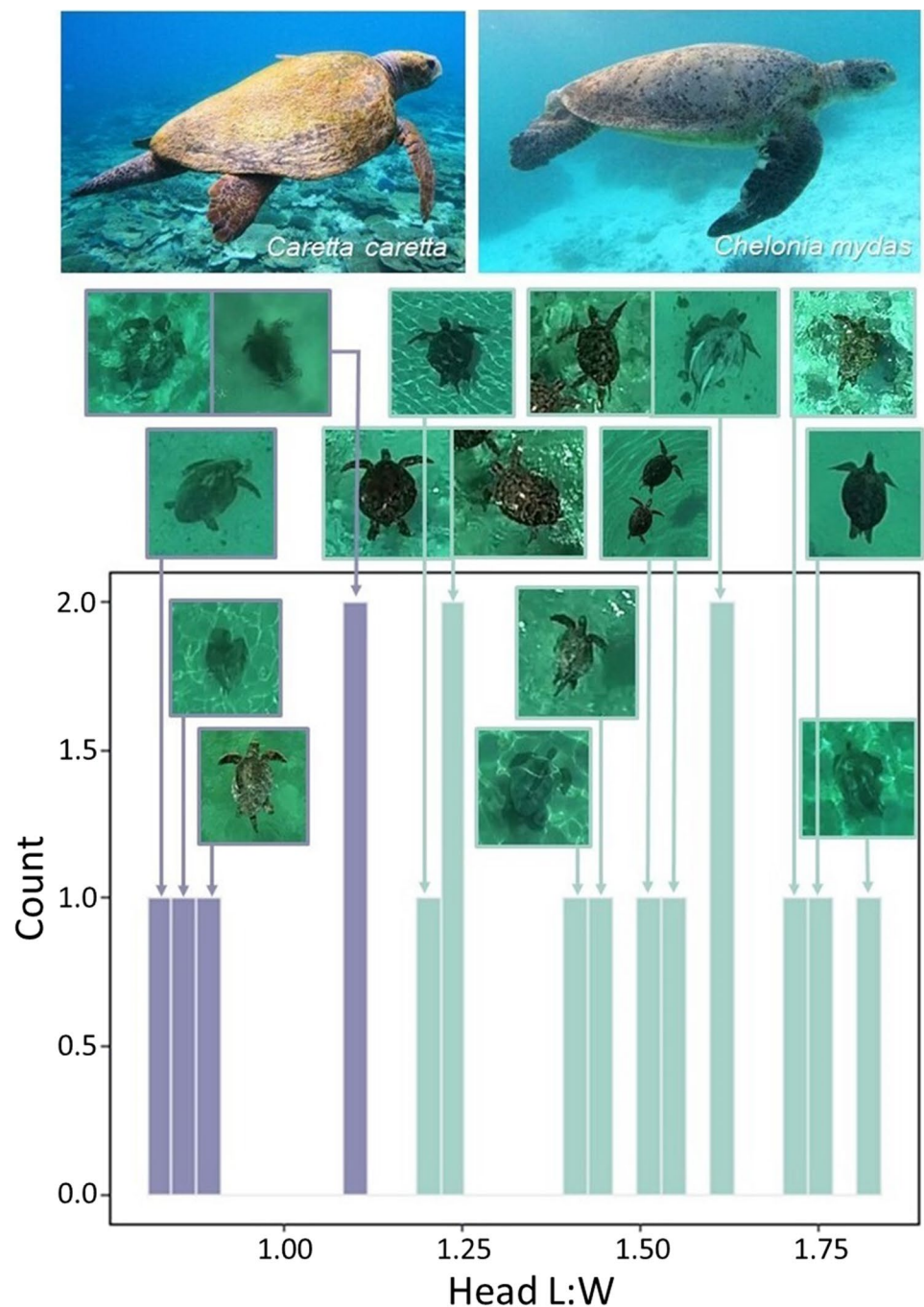
in July on 23 occasions (4 males and 19 females) and had an overall density of 3.8 turtles per km² (Table 1). None of these individuals were observed courting, mounting, or chasing each other. Adult-sized turtles in July exhibited typically foraging behaviours on the seafloor of the lagoons or rising to the surface to breathe.

In the October survey period, 45 male and 68 female turtles were observed ($n = 113$). Overall density increased five-fold for adult-sized green turtles in October (21.2 turtles per km²) compared to July and resulted in density estimates of

8.4 males and 12.7 females per km², respectively (Table 1). Whilst breeding behaviours were observed across all areas, foraging behaviours were only observed in the 'mid-lagoon' and 'east lagoon' areas. During the July surveys, no foraging behaviours or breeding aggregations of adult-sized turtles were observed in the 'nearshore' area. It is unlikely that an adult turtle would forage in the nearshore site because their preferred foods (seagrass or algal turf) are likely scarce immediately adjacent to the island. Therefore, to estimate OSR and exclude non-breeding individuals and subadults,

Table 1 Results from drone surveys of adult-sized green turtles in the three survey areas of Heron Reef prior to and during the mating season (July and October, respectively)

Survey period	Parameter	Males	Females	Both sexes	Survey Distance (km)	Survey area (km^2)
July (residents)	Total	4	19*	23	51.4	6.1
	Turtles/km	0.1	0.4*	0.4		
	Turtles/km^2	0.7	3.1*	3.8		
October (breeders + residents)	Total	45	68*	113	42.6	5.3
	Turtles/km	1.1	1.6*	2.7		
	Turtles/km^2	8.4	12.7*	21.2		

The total represents the total number of male, female and all turtle observations during each survey period. Turtle density (turtles/km and turtles/km^2) was calculated from the number of observations divided by the total distance and area covered during by the drone during each survey period

*Adult-sized turtles without a long tail (may include subadult males and females)

we examined the sex ratio of adult-sized green turtles in the 'nearshore area' only. This resulted in an OSR of 1.0 M: 1.1 F, or 0.51 female (± 0.17 CI limit) (Table 2). Of the 113 observations of adult-sized turtles, 75 (66.4%) were engaged in courtship or other breeding behaviours. Of those 75 turtle observations, 38 were male and 36 were female. Breeding behaviours were seen in all survey areas during October, and the lowest observations of breeding activity occurred in the 'east-lagoon' area. On average, two breeding pairs were sighted per day (total = 10, SE ± 0.4) and there were two occasions where attendant males were sighted with a breeding pair. However, a female being courted by two or more males (not mounted) was only seen once during the survey period. The other 33.6% of turtle observations were of adult-sized green turtles that were either on the seafloor feeding, resting, or surfacing to breathe.

Discussion

Drone surveys of Heron Reef yielded an OSR estimate for the sGBR green turtle population of 1 M: 1.1 F during the peak of the courtship aggregation in October 2021. This corresponds with previous estimates of OSR for green turtles on Heron Reef made by Yaney-Keller et al. (2021) during drone surveys from 17 to 21 October 2016 (1.05 M: 1 F). Our estimate also corresponds to the balanced estimates from the 1993–1996 'mark and recapture' surveys by Fitzsimmons (1997) (1.2 M: 1 F), and similarly found proportionally

greater numbers of breeding males to non-breeding males present in the nearshore areas around the island (e.g. 'shark bay') during the courtship period. The consistency in the OSR across 25 + years suggests that the OSR may show little inter-annual variability in contrast to nesting numbers which can vary greatly between years (Limpus 2008). This may indicate there has been a stable recruitment of males into the adult cohort, which is also supported by sex ratios reported for juvenile, subadult and adult cohorts of the sGBR green turtle population at other GBR foraging sites from mark-recapture surveys (e.g. Jensen et al. 2018). This is likely due to the low sand temperatures within the CBG where this population primarily nests (Booth and Freeman 2006), and has likely buffered against climate change induced feminization. However, in recent years, there has been some evidence to suggest that rising sand temperatures on Heron Island may be causing reduced male production (SI Figs. 1, 2). Therefore, increased feminisation of the adult population may only become apparent in over 30 years-time, as those turtles reach maturity (Limpus 2008).

A balanced OSR despite a female-biased hatchling sex ratio

To estimate the OSR of sea turtle populations, researchers have typically used mark-recapture surveys (Fitzsimmons 1997; Hays et al. 2010), multiple paternity tests for clutches (Wright et al. 2012a; Stewart and Dutton 2014; Prakash et al. 2022) and more recently, drone surveys (Schofield

Table 2 Observations of adult female and male green turtles recorded in drone surveys during the October survey period in the 'nearshore area' of Heron Reef

Survey	Males	Females	Both sexes	Sex ratio (M:F)	Proportion female	95% CI limits
07-10-2021	10	9	19	1.1:1.0	0.47	± 0.22
10-10-2021	7	9	16	1.0:1.3	0.56	± 0.24
Total	17	18	35	1.0:1.1	0.51	± 0.17
Mean	8.5	9	17.5	1.0:1.1	0.52	± 0.23

et al. 2017; Yaney-Keller et al. 2021). Regardless of the method used or species surveyed, most estimates of OSR reported for sea turtle populations around the world to date do not reflect the female-skewed ratio typically seen in hatchling sex ratio estimates (Phillips et al. 2013; Wright et al. 2012a; Stewart and Dutton 2014; Schofield et al. 2017; Yaney-Keller et al. 2021; Prakash et al. 2022). Theoretical considerations of the energetics of breeding suggest that the interval between breeding seasons (termed the remigration interval) will generally be much shorter in males than females across sea turtle populations (Hays et al. 2017). This suggestion is also supported by empirical observations (Fitzsimmons 1997; Limpus 2008; Wright et al. 2012b; Hays et al. 2017; Papafitsoros et al. 2022). It is likely that the mating system of sea turtles also allows populations to persist even with female-biased hatchling sex ratios because of the male's ability to breed with multiple females (Limpus 1993; Fitzsimmons 1997; Lee and Hays 2004; Papafitsoros et al. 2022). The differential survival between males and females is another mechanism by which OSRs could differ from hatchling sex ratios (Hays et al. 2022). Additionally, some freshwater turtle species are known to have high intra-population variation for pivotal temperature and the transitional range in temperatures, so males can be produced at warmer temperatures (Carter et al. 2019). However, such phenomena (including inter-clutch variation) are yet to be confirmed for most sea turtle populations (Mrosovsky 1988; Lockley et al. 2020; Porter et al. 2021). Regardless, our findings show optimistically that female-skewed hatchling sex ratios may still translate into more balanced OSRs.

The OSR can be estimated using multiple paternity tests of clutches (Wright et al. 2012a; Stewart and Dutton 2014; Prakash et al. 2022). However, there is evidence for relatively low rates of multiple paternity in clutches ($n = 13$) of the sGBR green turtle population when compared to other nesting populations around the world (Lee et al. 2018). The incidence of multiple paternity in clutches is likely driven by adult male–female encounter rates and so it is important to investigate the links between the OSR and the incidence of multiple paternity at nesting sites (Lee et al. 2018). It may be, for example, that a very high incidence of multiple paternity is linked to sites where the OSR is more heavily male-biased than that reported at Heron Island.

Breeding phenology

Shore-based observational surveys were used in this study to corroborate that mating activity peaked in early to mid-October, a finding consistent with previous estimates for the peak of mating activity for Heron Reef green turtles (Limpus 1993; Fitzsimmons 1997). Shore-based surveys of the incidence of mating pairs have been used elsewhere (Godley et al. 2002) and provide a simple, cheap and effective method of assessing mating seasonality and so complement drone-only surveys. The nesting season on Heron Island peaks from December to early January (Limpus 2008), approximately 8–10 weeks after the peak in mating. Our findings are also consistent with observations from around the world. For example, in green turtles nesting on Ascension Island and loggerhead turtles nesting in Greece, the mating season also peaks 8–10 weeks before the peak of the nesting season (Godley et al. 2002; Schofield et al. 2013). Throughout the mating season, the sex ratio of breeding sea turtles is also highly dynamic, with males arriving earlier to the breeding grounds and departing earlier than females (Schofield et al. 2013). Several studies have also noted that over the course of several weeks, the adult sex ratio shifts from predominately male, to balanced and then to predominately female (Schofield et al. 2017; Yaney-Keller et al. 2021). Our results suggest that 8–10 weeks before the peak in nesting activity is the optimal period during which to survey mating interactions and assess the relative abundance of breeding males.

Accounting for residents and other species in drone surveys

Measuring sex ratios of the adult cohort in a sea turtle population is made difficult because unlike females, males do not come ashore and so at-sea census surveys are often needed (Hays et al. 2010; Schofield et al. 2017). Surveying Heron Reef to determine the OSR is further complicated by the presence of (i) non-breeding, resident adults and (ii) subadult turtles (e.g. Limpus 1993, 2008; Forbes 1996; Fitzsimmons 1997), so it is not as straightforward to identify adult breeding turtles in drone footage. For example, the July drone surveys from our study do not allow an accurate estimate of the sex ratio of the foraging population because subadult individuals cannot be reliably distinguished from small adult female turtles in drone videos. We also acknowledge that discriminating visiting breeding turtles from resident foraging turtles by only their observed behaviours in the drone videos is not as rigorous as other methods. Potentially some of the adult turtles seen "foraging" on the sea floor may in fact be breeding adults resting or opportunistically feeding between bouts of courtship activity (Schofield et al. 2017). A study by Tucker and Read (2001) conducted on Raine Island (nGBR) during the nesting season, found that 50% of the female green turtles sampled ($n = 48$) had traces of vegetation in the upper digestive tract. However, the prevalence of opportunistic foraging by breeding turtles from the sGBR stock remains understudied (Forbes 1996). The short duration of observations in the present study and the occurrence of breeding activities in known foraging areas (e.g. 'mid' and 'east-lagoon') might make assignments of breeding status erroneous. Therefore, we identified breeding adults by both their behaviours and occurrence in the 'nearshore'

area where no observations of adult-sized turtles foraging were ever documented. However, improved methods to distinguish breeding turtles from both non-breeding adults and subadults will certainly help improve OSR estimates at Heron Reef. In courtship areas where both non-breeding adults and subadults are rare, then it is easier to reliably estimate the OSR from drone surveys, such as for loggerhead turtles in Greece (Schofield et al. 2017). A further potential complication in some areas, may be that some females do not nest close to courtship and mating sites. In such cases, potentially high-resolution satellite tracking (e.g., Esteban et al. 2017; Shimada et al. 2019) may help identify the links between where females mate and where they nest, thereby revealing further key sites for drone surveys to assess OSRs.

In some locations, identifying the turtle species in drone footage is very simple, as only a single species occurs in the area (Schofield et al. 2017). However, around the world multiple turtle species are often found coexisting in the same general area on both foraging and breeding grounds. In such cases, objective criteria are needed to distinguish species. Sea turtles differ in several external characteristics, most notably their scute patterns and carapace colour and shape. Species also differ in the general shape of the head, for example, loggerheads having a relatively large and wide head consistent with powerful jaws needed to eat their hard prey (Tomas et al. 2001). We demonstrated how the L:W ratio of the head could be used to distinguish loggerhead from green turtles in drone surveys. This approach for distinguishing species could be extended to other species of sea turtles as drone surveys become more widely used with this group.

Conclusion

Assessing OSR has been identified as a key factor to advance our knowledge and to assist with the conservation effort of sea turtle populations (Rees et al. 2016). There may be many high-risk populations; however, the current literature demonstrates that balanced OSRs can persist even with more female-biased primary sex ratios (Wright et al. 2012b). The findings from our drone surveys further emphasise this point, and it is likely that the more frequent breeding by male turtles helps to mitigate female-biased hatchling sex ratio skews (Hays et al. 2014, 2022). However, climate warming has raised concerns for the viability of populations with extreme female-bias in hatchling sex ratios (Poloczanska et al. 2009; Hays et al. 2017). Hence, estimating OSR should be prioritised for populations such as in the nGBR green turtle genetic stock, where the primary sex ratio is already 99% female (Jensen et al. 2018; Booth et al. 2020). It is hoped that the techniques we have described can be used to estimate OSR for more populations and to better understand turtle mating systems under a changing climate. More broadly, OSR estimates may help to inform wildlife managers whether direct intervention by nest cooling (e.g., Esteban et al. 2018; Smith et al. 2021) is required to increase male hatchling production (Santidrián Tomillo et al. 2021).

Declarations

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