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THE DEBATE ABOUT SPLITTING CLUTCHES

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BACKGROUND

One of the topics discussed at a meeting of the Turtle Action Group (TAG) of India in June 2023 was the practice of “splitting clutches”, i.e., dividing a clutch of eggs to create two groups of eggs, before reburial in a hatchery.

The practice of splitting clutches was first described by Balasingam (1967). He noted a higher hatching success (% of eggs that produce a hatchling which leaves the eggshell; Miller, 1999) from naturally smaller clutches of leatherback (*Dermochelys coriacea*) turtle eggs compared to clutches comprising the average number of eggs (85-90) in a hatchery. When the clutch size of eggs moved to a hatchery was manipulated, split

clutches comprising a smaller number of eggs also had a higher hatching success than unmodified clutches (Table 1). Balasingam (1967) hypothesised that smaller clutches generated less metabolic heat, reducing embryo mortality and increasing hatching success, though this was not experimentally tested. After the publication by Balasingam (1967), the practice of splitting clutches was adopted by most hatcheries in Malaysia (Mortimer *et al.*, 1994) as a conservation practice to improve hatching success. Subsequent investigations across species and hatcheries into the outcomes of this practice produced mixed outcomes, which may be due to variables such as nest density and depth which are often not reported.

For example, splitting large natural clutches of olive ridley (*Lepidochelys olivacea*) turtle eggs at hatcheries in India resulted in increased hatching success and may have mitigated the impact of high temperatures and metabolic heating (Abraham *et al.*, 1990 in Shanker, 1994; Mathew *et al.*, 1991) (Table 1). However, statistically significant variation in hatching success with clutch size was not reported in these studies, and no information about nest depth and nest density was provided for the different clutches. Mathew *et al.* (1991) also observed lower mortality of embryos in pipped eggs from split clutches incubated in a hatchery (Table 1) and speculated this was due to greater oxygen availability and/or lower metabolic heat in clutches with fewer eggs. The mortality rate among pipped embryos was later reduced in natural-sized clutches by reducing nest density in the hatchery from 2 nests/m² to 1 nest/m² (Shanker, 1998).

Mortimer *et al.* (1994) compared the emergence success of natural clutches of leatherback and hawksbill (*Eretmochelys imbricata*) eggs with clutches split to contain 40-60 eggs at two hatcheries in Malaysia; nest depth and density in the hatchery were not reported. They found no significant difference ($P > 0.05$) in emergence success (the proportion of eggs that hatch to produce hatchlings that successfully emerge from the nest; Miller, 1999) (Table 1). However, mortality of pipped embryos of both species, and mortality of late-stage embryos and hatchlings, was significantly less ($P < 0.05$) in split clutches. Hence, Mortimer *et al.* (1994) also suggested that splitting clutches could be a conservation strategy to slightly improve hatchling production.

Ibrahim *et al.* (2002) found no significant difference in emergence success between natural clutch sizes and split clutches (Table 1) of green (*Chelonia mydas*) turtle eggs at a hatchery in Malaysia; nest depth and density in the hatchery were not reported. They concluded that there was no advantage for hatchling production to split clutches and also pointed out that splitting clutches would

result in increased space requirements and construction costs for hatcheries (Ibrahim *et al.*, 2002).

Sarahaizad *et al.* (2022) also conducted a study with green turtle eggs in Malaysia. They found a significant difference ($P < 0.05$) between the hatching success of *in situ* whole clutches incubated at Kerachut and clutches collected from Kerachut and Teluk Kampi (Table 1) and split before incubation in a hatchery at different nest depths (Sarahaizad *et al.*, 2022). However, the different incubation locations of eggs in this study and variation in hatching success among study years suggests that environmental factors may have contributed to their findings.

Clarke *et al.* (2021) investigated the potential for splitting clutches to reduce nest incubation temperatures as mitigation for the effects of climate change. They incubated loggerhead (*Caretta caretta*) turtle eggs at a hatchery in Cabo Verde and monitored incubation temperature. Split clutches incubated at natural nest depth for the species had nest temperatures ~1°C lower than control nests and the hatching success was significantly higher, although the difference was not significant in all study years (Table 1). Splitting clutches had no impact on hatchling size or vigour (Clarke *et al.*, 2021).

The variation in findings among these studies indicates that more evidence is required to conclusively demonstrate the advantages of splitting clutches under different conditions. The mixed results suggest that clutch size alone does not explain the variation in success rates, since variables such as nest depth, nest density, nest temperature, substrate moisture, and other incubation conditions can also influence hatching and emergence success. Current guidelines for hatcheries (e.g., Mortimer, 1999; Phillott & Shanker, 2018) recommend that incubation conditions, including nest depth and shape, clutch size etc, should reflect those of the natural nest. The rationale for duplicating natural incubation conditions and clutch size is based on the reasons outlined below, all of which are demonstrated to improve hatchling survival in several ways.

1. Hatchlings in a larger group benefit from the synchronous digging activity, spending less time digging and using less energy to emerge from the nest as (Rusli *et al.*, 2016). Such hatchlings will have larger energy reserves for the crawling and swimming frenzy needed to move hatchlings from the predator-rich beach and in-shore waters (Gyuris, 1994; Pilcher *et al.*, 2000; Wyneken & Salmon, 1992; Wyneken *et al.*, 2008) and the following periods of active swimming and use of currents to reach

Table 1. Comparative studies assessing hatching or emergence success between natural and split clutches of sea turtles in various countries.
 HS: hatching success; ES: emergence success

Species	Country	Incubation Location	Year(s) of Study	# Clutches	Split (# Splits)	Incubation Clutch Size Mean±SD (Range)	Nest Depth (cm)	HS/ES (%) Mean±SD (Range)	Source
Leatherback	Malaysia	Hatchery	1961-1964	32	No	(46-60)	~76.2	63.5 HS	Balasingam, 1967
				137	No	(76-90)	~76.2	52.0 HS	Balasingam, 1967
				216	No	(91-135)	~76.2	33.2 HS	Balasingam, 1967
				8	Yes	(46-60)	~76.2	72.9 HS	Balasingam, 1967
				53	Yes	(76-90)	~76.2	76.0 HS	Balasingam, 1967
				44	Yes	(91-135)	~76.2	70.4 HS	Balasingam, 1967
				28	No	-	-	44.9 ES	Mortimer <i>et al.</i> , 1994
Olive ridley	India	Hatchery	1990	24	Yes (≥2)	(46-60)	-	55.2 ES	Mortimer <i>et al.</i> , 1994
				37	No	144.4 (133-157)	-	48.4 HS	Mathew <i>et al.</i> , 1991
				6	Yes (-)	-	-	73.7 HS	Mathew <i>et al.</i> , 1991
Hawksbill	Malaysia	Hatchery	1991	111	No	-	-	47.0 ES	Mortimer <i>et al.</i> , 1994
				83	Yes (≥2)	(46-60)	-	52.7 ES	Mortimer <i>et al.</i> , 1994
				750	No	-	-	84.8 (79.9-89.0) ES	Ibrahim <i>et al.</i> , 2002
Green	Malaysia	Hatchery	1997-1998	154	No	-	-	47.3 ES	Ibrahim <i>et al.</i> , 2002
				256	Yes (-)	-	-	48.9 ES	Ibrahim <i>et al.</i> , 2002
				10	No	-	-	38.2 HS	Sarhaizad <i>et al.</i> , 2022

Table 1 cont.

Species	Country	Incubation Location	Year(s) of Study	# Clutches	Split (# Splits)	Incubation Clutch Size Mean \pm SD (Range)	Nest Depth (cm)	HS/ES (%) Mean \pm SD (Range)	Source
		Beach	2009/10	10	No	-	-	38.2 HS	Sarahaizad <i>et al.</i> , 2022
		Hatchery	2009/10	10	Yes (3)	38.0 \pm 5.9 (29-49)	45-65	67.6 HS	Sarahaizad <i>et al.</i> , 2022
Loggerhead	Cabo Verde	Hatchery	2012	20	No	92.0 \pm 0.8 (77-117)	45	77.6 \pm 4.9 HS	Clarke <i>et al.</i> , 2021
		Hatchery	2014	23	No	87.6 \pm 4.5 (36-126)	45	61.0 \pm 3.7 HS	Clarke <i>et al.</i> , 2021
		Hatchery, shaded	2012	20	No	88.4 \pm 0.8 (66-111)	45	73.7 \pm 4.91 HS	Clarke <i>et al.</i> , 2021
		Hatchery	2012	20	Yes (2)	45.4 \pm 0.8 (38-57)	45	73.3 \pm 5.4 HS	Clarke <i>et al.</i> , 2021
		Hatchery	2014	23	Yes (2)	48.8 \pm 1.1 (33-63)	45	82.5 \pm 2.3 HS	Clarke <i>et al.</i> , 2021

offshore waters (Briscoe *et al.*, 2016; Gaspar & Lalire, 2017; Gatto & Reina, 2020; Mansfield *et al.*, 2014; Putman & Mansfield, 2015; Salmon *et al.*, 1992) where feeding may commence about a week after emergence from the nest (Kraemer & Bennett, 1981).

2. Emerging as a member of a larger group of hatchlings could also reduce the risk of predation by terrestrial (Erb & Wyneken, 2019; Martins *et al.*, 2021; Santos *et al.*, 2016) and presumably aquatic predators. [Note that the advantages of emerging synchronously in a larger group of hatchlings from an individual nest should not be confused with the higher risk of predation to hatchlings from multiple nests released *en masse* from hatcheries, as described by Pilcher *et al.* (2000)].
3. We do not yet fully understand the biological function (if any) of vocalisation (sound production) during embryo incubation and hatchling emergence from the nest (e.g., Ferrara *et al.*, 2014, 2019; Field *et al.*, 2021; McKenna *et al.*, 2019) for all species of sea turtle. If acoustic communication among embryos and/or hatchlings occurs, then clutch size may be important. Until this information is known, managers should be cautious about changing any incubation parameters that could negatively impact embryo and/or hatchling biology.

RECOMMENDATIONS

Hatchery managers wanting to achieve high hatchling production should ensure they are following best practices for hatcheries (Mortimer, 1999; Phillott & Shanker, 2018) by:

- collecting and moving eggs to the hatchery within 3 (optimal) to 6 (acceptable) hours after oviposition to minimise movement-induced mortality (Limpus *et al.*, 1979; Parmenter, 1980; Williamson *et al.*, 2017), and
- ensuring nest density is no more than 1 nest/m² to minimise the potential for hypoxic (low O₂) and hypercapnic (high CO₂) conditions to develop in the nest (Clusella Trullas & Paladino, 2007; Honarvar *et al.*, 2008) and reduce the likelihood of metabolic heat produced by one clutch of eggs (Deeming, 2004) affecting an adjacent clutch of eggs, and
- digging hatchery nests to the depth of the original nest or the known average for the species so that eggs incubate in thermal conditions for which the species are adapted (Santidrián Tomillo *et al.*, 2017).

If hatchery managers are concerned that conditions in the nest could be reducing hatching and emergence success rates or skewing hatchling sex ratios to female, they can do the following to make an informed decision about managing hatchery nests based on valid data:

- monitor the nest temperature throughout the duration of incubation to calculate the embryos cumulative exposure to high temperatures (Bladow & Milton, 2019) and use the data to determine if temperature mitigation using shading and/or watering) is required (e.g., Esteban *et al.*, 2018; Gatto *et al.*, 2023; Lolavar & Wyneken, 2021; Reboul *et al.*, 2021; Staines *et al.*, 2020; Wood *et al.*, 2014), and/or
- monitor the nest temperature during the thermosensitive period (the time interval of incubation when temperature affects sexual differentiation of the gonads; Mrosovsky & Pieau, 1991) and use the data to predict hatchling sex ratios (Girondot *et al.*, 2018), and/or
- determine the stage of development (Miller, 2017) at embryo mortality to determine if it coincided with specific environmental events (such as high temperatures) during incubation (Mortimer *et al.*, In Press), and/or
- monitor respiratory gases in the nest conditions to assess if hypocapnia and/or hypoxia is being experienced by developing embryos (late-stage embryos are most vulnerable; see Lyons *et al.*, 2022).

In conclusion, there is not yet a substantial body of work that demonstrates splitting clutches increases hatching success without impacting hatchlings. Indeed, splitting clutches might increase the energy for hatchlings to escape the nest and increase the risk of depredation on the beach and while swimming through inshore waters. Until stronger evidence emerges, clutch splitting should be considered experimental, implemented with careful monitoring, and evaluated against natural-sized controls. If the decision is made to split clutches for incubation in a hatchery, then hatchery managers should:

- record data about original and split clutch size, nest depth, incubation period (in days), hatching and emergence success, and indicators of hatchling fitness (e.g., crawl speed, righting ability, swim speed) to compare with the same data for clutches of natural size to assess if splitting clutches is having an impact on hatchlings produced from the hatchery, and
- report such information to help other hatchery

managers understand if the splitting clutches has any benefits or should be avoided.

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