

## Short communication

# Laying eggs on the same nest: unusual mixed-species clutch among three colonial shorebirds

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**Short title:** Unusual mixed clutch

**Abstract** - Mixed clutches are relatively uncommon among colonial shorebirds and challenging to document. Here, we report the co-occurrence of three species' eggs, belonging to Pied Avocet *Recurvirostra avosetta*, Little Tern *Sternula albifrons* and Common Tern *Sterna hirundo*, in the same nest within a large colony in the Marano and Grado Lagoon, Friuli Venezia Giulia. To our knowledge, it is the first record with more than two species among Charadriiformes. We discuss the possible explanation concerning the limited suitable nesting space in a human-made breeding habitat. These findings are rarely reported despite their importance for comprehending the phenomenon from an ecological perspective.

**Keywords:** Charadriiformes, Pied Avocet, Little Tern, Common Tern, waders, waterbirds, seabirds.

Colonial breeding is a life history strategy in which bird pairs establish huge and dense aggregations (Danchin & Wagner 1997). Colonial nesting occurs in 13% of bird species (Lack 1968) with varying degrees of philopatry and nest-site fidelity (Pearce 2007, Coulson 2016), leading to the development of intra- or inter-specific social bonds and interactions (Francesiaz et al. 2017). Colonial nesting has several benefits, but also inherent costs. On the one hand, it can determine an increased reproductive success and, therefore, higher fitness, thanks to a more effective predator defence and environmental information exchange (Rolland et al. 1998, Evans et al. 2016). On the other hand, breeding in large colonies could be costly due to lower nesting site availability, increased competition for trophic resources and nest parasitism (Gil et al. 2018). Due to the shortage of suitable nesting sites, colonial breeding may lead to the utilization of communal nests and the occurrence of enlarged or mixed clutches (Amat 1998, Craik 2010). Mixed clutch is defined as the presence of eggs from more than one species in the same nest. Mixed clutches are rare among colonial shorebirds within the order Charadriiformes (Amat 1998, Yom-Tov 2001) and the observations rate is probably skewed by the monitoring and research methods performed (Craik 2010,

Anderson et al. 2016). Limited knowledge exists regarding this phenomenon, underscoring the importance of reporting new data and findings to enhance our comprehension of its ecological implications (Pakanen et al. 2019). Pied Avocet (hereafter Avocet) *Recurvirostra avosetta*, Little Tern *Sternula albifrons* and Common Tern *Sterna hirundo* are colonial shorebird breeders belonging to the order Charadriiformes. Their breeding habitat overlaps, mainly including brackish coastal wetlands such as lagoons, fish-farms and salt pans (Brichetti & Fracasso 2018). Accordingly, breeding pairs can often associate with each other and establish a mixed colony (Brichetti & Fracasso 2018). In this note, we report an unusual mixed clutch among Avocet, Little Tern and Common Tern, which occurred in a mixed breeding colony in the Marano and Grado Lagoon, in North-Eastern Italy.

The Marano and Grado Lagoon is one of the most important brackish ecosystems in the Mediterranean region (Regione FVG 2018, BirdLife International 2024). The Biodiversity Service of the Friuli Venezia Giulia Region coordinates the regional monitoring program of Natura 2000 habitats and species in compliance with Habitat Directive 92/43/CEE and Birds Directive 2009/147/CE. We monitored the most relevant breeding waterbird populations of conservation concern in the Marano and Grado Lagoon, Friuli Venezia Giulia in 2022 (45°40'40" N 13°03'50" E to 45°46'30" N 13°27'20" E; NE Italy) (Sponza & Salvador 2022). The lagoon is characterized by the presence of fish-farms, which are a tidal-free embanked portion of the lagoon and it is a typical artificial habitat of the Northern Adriatic Sea (Fig. 1). Fish-farms are made up of a complex system of freshwater and saltwater enclosures with manually controlled water level. One of the main distinctive morphologies of this artificial habitat is mud banks obtained by the sediment management of fish-farm canals. They are a few tens centimetres high above the water level. The mud banks (Fig. 1) represent the almost exclusive nesting habitat in the Marano and Grado Lagoon for the Common Tern and Avocet and an important habitat for Little Tern (Cosolo et al. 2015). The case of the mixed clutch we document in this note has been recorded inside "Val Noghera", a fish-farm in Marano and Grado Lagoon, which presents multiple linear and circular mud banks, separated by a minimum of 2 m and a maximum of 200 m. It supports one of the two largest breeding colonies of terns in the Marano and Grado Lagoon, playing an essential role in their breeding ecology. Its significance stems from responsible and effective canal dredging management practises, which foster positive ecological outcomes during the breeding season.

Six surveys were carried out in "Val Noghera" fish-farm to estimate the waterbird breeding community from April to July 2022. To minimize the disturbance to the colony, the surveys were conducted approximately every 15 days, considering both the prevailing weather conditions and the availability of the fish-farm manager to facilitate data collection (Fig. 2). Nest census relied on direct visual counting of eggs and hatchlings while walking along the mud banks and a remote visual monitoring was also employed for an accurate estimate of the current population. The reported number of nests refers to the count obtained by each survey. During the breeding season, 35 pairs of Avocet, 30 of Little Tern and 131 of Common Tern have nested inside the whole fish farm, respectively 91%, 26% and 23% of the entire lagoon. In a particular mud bank where the mixed clutch was discovered, up to 15 pairs of Avocet and 17 pairs of Little Tern were observed in May, while 6 pairs of Common Tern in July (Fig. 2). Avocet was the first species to settle in this narrow area, with evidence of broodings by the end of May. On the other hand, the highest abundance of tern nests was recorded at the end of May (Fig. 2). The observed increase of tern nests in July may likely be associated with further nesting attempts following earlier clutch failures. This finding could indicate

suboptimal breeding site conditions for successful brood rearing, possibly due to the scarce availability of suitable nesting sites, thereby intensifying interspecific competition for nesting resources.

During the peak of the occupancy on 30<sup>th</sup> May, an unusual nest was observed. It was a slight depression in the mud with one Avocet egg and two Little Tern eggs (Fig. 3). None of the two species were observed incubating the nest both during this survey and the subsequent one. It is unknown which species laid the eggs initially. However, due to the colony's breeding phenology, we can confidently assume that a Little Tern occupied that nest with the unhatched, presumably abandoned, Avocet egg. Thereafter, on 10<sup>th</sup> June, a Common Tern egg and nest material was found in the mixed nest, and one Common Tern individual was observed to actively incubate it by remote control with a telescope. The overall clutch size was four eggs (Fig. 3). On 27<sup>th</sup> June, Common Tern was still incubating the nest, but the Little Tern eggs were missing. On 14<sup>th</sup> July, only the Avocet egg remained with no evidence of hatchlings. No signs of predation were registered.

To our knowledge, this is the first observation of a mixed clutch of three Charadriiformes species. In the literature, cases involving two species have been described (Amat 1998, Craik 2010, Niemczynowicz et al. 2015, Debata et al. 2018, Haraszthy 2019, Pakanen et al. 2019, Stermin 2021). Two distinct time phases can be discriminated by examining the chronological development of the mixed nest. The first phase involves the Avocet and the Little Tern, which might indicate interspecific brood parasitism. About 400 species of birds are known as nest parasites worldwide (Niemczynowicz et al. 2015), of which 19 species are in the order Charadriiformes (Yom-Tov 2001). Nevertheless, this initial event cannot be deemed as an example of interspecific brood parasitism, because it does not fulfil the assumptions of the interspecific parasitism hypothesis (Yamauchi 1995, Yom-Tov 2001, Soler 2019). Brood parasitism is thought to be a reproductive strategy aiming to shift the burden of reproduction onto individual of other species, in order to focus exclusively on feeding and further breeding (Davies 2000). Therefore, it seems unlikely that the Little Tern parasitized that Avocet nest as the clutch should have been observed to be incubated by an Avocet. The fact that the Little Tern laid two eggs rules out the possibility of brood parasitism. Commonly, brood parasites lay one egg in each parasitized nest to maximize their reproductive success and hence their fitness (Robert & Sorci 2001, Mann 2017). Furthermore, the possibility that the Avocet nest had been abandoned early in the breeding season, and subsequently occupied by the Little Tern, invalidates the interspecific parasitism hypothesis (Mann 2017). The second phase, concerning the settlement of the Common Tern, further disproves the parasitism nesting strategy because the Common Tern was observed to actively incubate after occupying the nest (Amat 1998, Davies 2000). It is worth underlining that the Common Tern only laid one egg, probably due to the presence of three additional eggs in a small suitable nesting space. Shorebirds typically lay three or four eggs per nest, and increased clutch size reduces the feasibility of incubating all eggs successfully (Lengyel et al. 2009). Moreover, it decreases the efficiency of parental care and this might be another reason why mixed clutches of colonial shorebirds are unusual (Amat 1998).

In conclusion, we believe this case may be considered as an unsuccessful breeding attempt where possibly unexperienced or first-attempt individuals of different species used the same nest (Limmer & Becker 2010). Alternatively, as suggested by Niemczynowicz et al. (2015), the mixed clutch might be the result of the increasing number of breeding birds in a small area with limited suitable nesting sites in a human-made habitat. Indeed, the Avocet bred within the fish-farm for the first time ever, possibly due to the artificial development of new semi-

natural habitats (e.g. freshwater basins, mud banks), which are suitable for nesting (Lengyel 2006). The establishment of a new species that breed earlier in the breeding season could have further decreased suitable nesting sites and increased such competition (Amat 1998, Davies 2000). This instance also shows how some shorebird species, under certain environmental conditions, could benefit and respond promptly to human management activities. Nevertheless, the ecological balances of the entire colonial breeding community could be swiftly altered within a human-made habitat, leading to the occurrence of unusual events. Ongoing long-term monitoring could reveal other mixed clutches observations among colonial shorebirds, providing new insights on the ecological mechanisms underlying the phenomenon.

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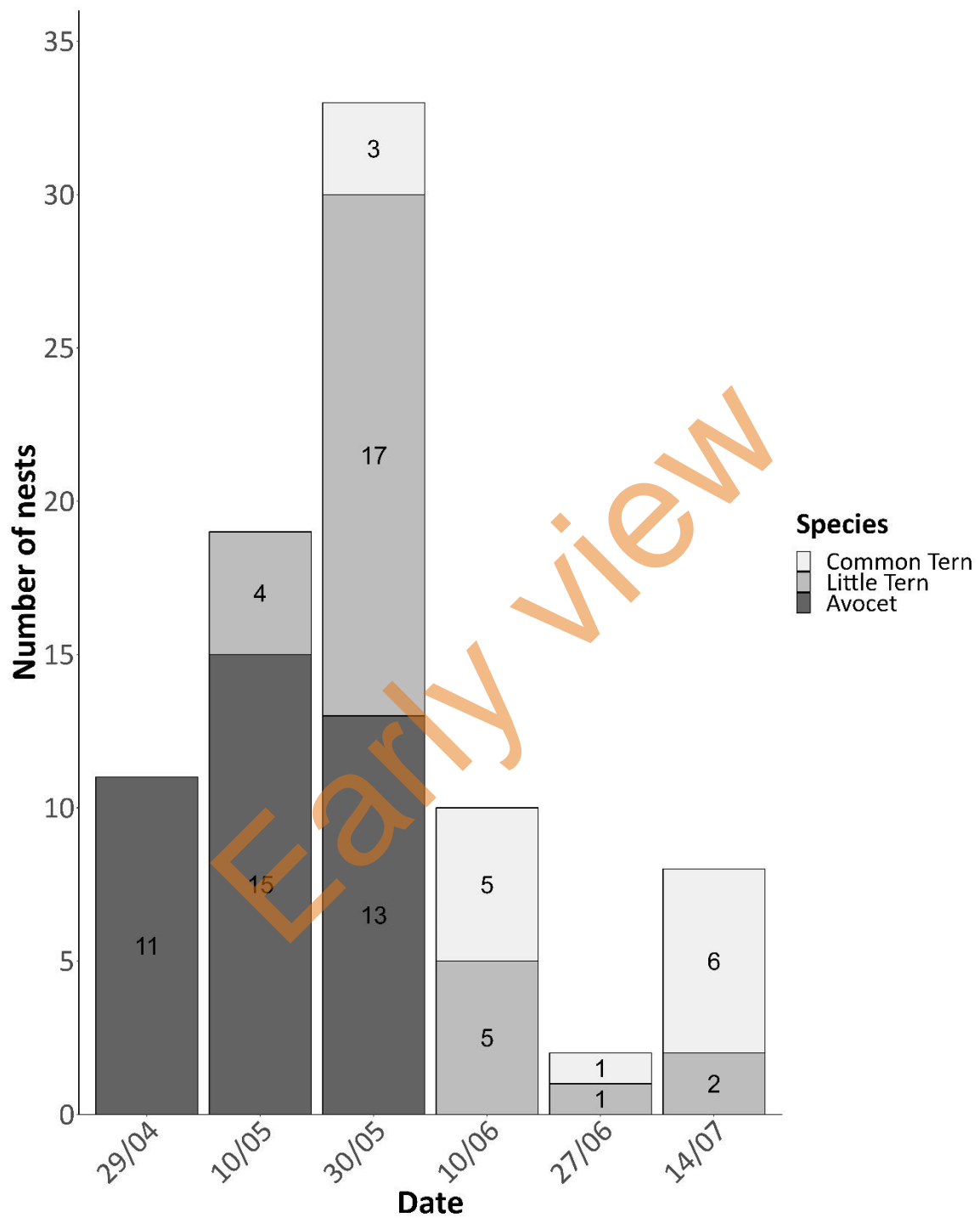
Early view

## Figures captions

**Figure 1.** Mud bank where the nest has been detected. It is a few tens centimetres high above water level and the lack of vegetation is one of the main characteristics. It is located within a fish-farm in the Marano and Grado Lagoon, north-eastern Italy.



**Figure 2.** Number of nests of Avocet, Little Tern and Common Tern recorded on a mud bank within a fish-farm in the Marano and Grado Lagoon, North-Eastern Italy, in 2022. The values inside bar charts show the number of nests for each species throughout the survey period.





**Figure 3.** Unusual mixed-species clutch counting one Avocet *Recurvirostra avosetta* egg, two Little Tern *Sternula albifrons* eggs (indicated by the black arrows) and one Common Tern *Sterna hirundo* egg (indicated by the white arrow).

