Short communications

Sepia cuttlebones pecked by birds along a Mediterranean beach: patterns, frequency and a possible conservation implication

Corrado Battisti[1]*

[1]Torre Flavia' LTER (Long Term Ecological Research) Station, Protected areas - Regional park Service, Città Metropolitana di Roma Capitale, viale G. Ribotta 41, 00144 Rome, Italy
*corresponding author: c.battisti@cittametropolitanaroma.gov.it

iD 0000-0002-2621-3659

Abstract - Birds require calcium carbonate for skeleton and egg development. For this reason, cuttlebones of *Sepia* cuttlefish can be considerate a dietary supplement and often bear signs of pecking by birds (mainly gulls and other seabirds). Due to the different structure of cuttlebones (dorsal vs. ventral regions), I hypothesized that birds peck more intensely the anterior and ventral part these being softer and thicker. On a sample of 89 cuttlebones of *Sepia* cuttlefish, a relatively high percentage (88.8%) showed pecking signs by birds. All cuttlebones pecked showed signs on the ventral part while signs on the dorsal part were observed in significantly lower frequency. In the ventral part, no significant differences were found between the anterior and the posterior region. The longest cuttlebones showed a significant higher frequency of pecking signs, likely because of the longest cuttlebones are also those with greater thickness (and, therefore, with higher availability of calcium aragonite). These are the first evidences from Mediterranean area. Since expanded polystyrene, an increasingly abundant anthropogenic debris in natural environments, was often pecked because confused with cuttlebones, our first data may have conservation implications in the disciplinary arena of marine/beach litter research.

Keywords: *Sepia* cuttlebones, thickness, pecking frequency, calcium aragonite, expanded polystyrene

Cuttlefish bones (i.e. the inner straight shell of cuttlefish: Mollusca Sepiidae genus *Sepia*) often bear signs of pecking by birds (e.g. mainly seabirds as gulls and fulmars; Cadée 2002a, Battisti 2020). This happens because birds need calcium carbonate for their skeleton and during the egg development (Graveland et al. 1994, Perrins 1996). In this regard, cuttlebones are often used for birds in captivity as dietary supplement (e.g. Taylor et al. 1996).

Cuttlefish bones are often found beached in large numbers both because fishermen throw back the shells back into the sea after processing the catch and because these animals die after spawning (Cadée 2002b).

Cuttlebones show a heterogeneous and complex structure, representing a sophisticated buoyancy device combining high porosity with considerable strength since it must withstand the external hydrostatic pressure — with minimum weight (Denton & Gilpin-Brown 1961, Birchall & Thomas 1983, Neige 2006, Cadman et al. 2010). These shells are composed by two main parts: (i) a ventral side (phragmocone) with the visible last-formed chamber complex (lamellar matrix), ending in a layered area composed

of calcium carbonate in its aragonite polymorph (and, secondarily, organic matter: β -chitin, polysaccharide, glycoprotein and protein); and (ii) an upper dorsal side, covered with a dense shield (Checa et al. 2015; North et al. 2017). The dorsal shield, consisting of three hard layers, provides a rigid substrate of calcium carbonate (external periostracum) that encapsulate a middle layer with an aragonite structure, aimed at protecting the ventral ductile matrix of cuttlebone. This matrix is composed of successive calcareous lamellae deposited on each other during growth. The ventral phragmocome shows an anterior smooth area and a posterior striated siphuncular region (Cadman et al. 2012, Le Goff et al. 1998, Le Pabic et al. 2017; Fig. 1).
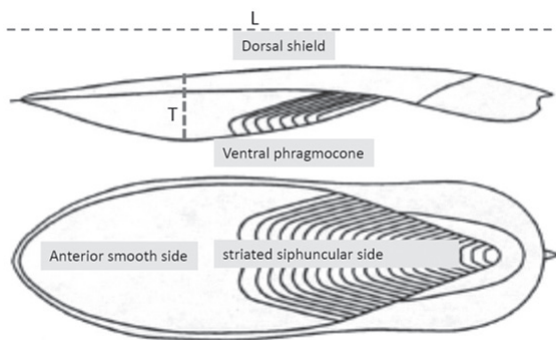

Figure 1. Different parts of a cuttlebone of *Sepia* cuttlefish. L: cuttlebone length; T: thickness.

Due to the different structure between the dorsal part and the ventral part and the higher availability of softer aragonite in the longer cuttlebones, I hypothesized that: (i) birds will obtain more calcium carbonated by peck more intensively on the softer and thicker anterior ventral part, and (ii) birds peck longer cuttlebones more frequently.

I collected *Sepia* cuttlebones (mainly common cuttlefish *Sepia officinalis* Linnaeus, 1758) along a Tyrrhenian sandy beach ('Palude di Torre Flavia' natural monument and Special Conservation Area 79/409/ UE 'Birds' Directive; code IT6030020, Latium, central Italy; 41°57'41.4"N 12°02'43.4"E) following an opportunistic sampling. For three days (May 2020), I recorded all the cuttlebones occurring inside a sec-

tor 1,050 m in length between the shoreline and the foredune (approx. 40 m in breadth; field research effort: about 5 hours). Due to surrounding sandy seafloor, rich in *Posidonia oceanica* (Special Area of Conservation: 'Secche di Torre Flavia, IT6000009), this cephalopod is locally very abundant and actively fished by fishermen (pers. obs.). In this beach, a rich and taxonomically and ecologically heterogeneous bird assemblage occurs including both specialized wetland- and sea-birds (mainly gruiformes and caradriiformes, including three gulls*: Chroicocephalus ridibundus, Larus michahellis* and *Ichthyaetus audouinii*), and vagrant specialized and generalist species (mainly passeriformes, including two corvidae: *Pica pica* and *Corvus cornix*; Battisti and Sorace 2006, Battisti 2014, Battisti et al. 2012).

For each cuttlebone, I calculated length (anterior-posterior distance) and height (thickness), in cm, using a professional calliper. Each cuttlebone was divided in a ventral and in a dorsal side (Fig. 1), each one with an anterior and a posterior part. In each cuttlebone, I recorded the occurrence of linear or triangular signs corresponding to the incision caused by the peck of birds, different from tracks of irregular shape caused by marine invertebrates digging on the cuttlebones (e.g. among the molluscs the Vermetidae and the Pholadomydae, among the sedentary perforator polychaetes *Lysidice ninetta* and *L. collaris;* F. Cerfolli, pers. comm.). When pecking signs were present, I recorded where they occurred on the bone, and their abundance. Since pecking signs on cuttlebones showed different detectability attributable to wear of the calcium layer mainly due to meteo-marine agents and to intra- or inter-specific differences in pecking ability, to minimize errors in measuring signs I chose a threshold approach using only two clear categories of abundance (0-10 and > 10 signs). Then I calculated their relative frequency (i.e. the ratio of numbers of bones with signs/total number of bones, both on the total and subdivided for the two abundance categories). Frequencies of categories of abundance have been calculated also for a set of length classes (< 10 cm, ≥ 10-12, ≥ 12-14,

≥ 14-16, ≥ 16). Since I hypothesized that birds peck longer cuttlebones more frequently, I calculated the frequencies of cuttlebones in the two categories of pecking abundance (0-10 and > 10 signs) for each length class, also obtaining a ratio between two categories of pecking abundance. I would expect a higher pecking abundance ratio in longer cuttlebones.

To test if cuttlebones with pecking had no difference between the ventral vs. posterior and ventral vs. dorsal side, I performed the Chi-Square Test to compare the relative frequencies. Since data distribution of both cuttlebone's length and height is significantly not different from a normal distribution (Kolmogorov-Smirnov test, 2 tail: length: Z = 0.531, p = 0.941; height: Z = 0.855, p < 0.001, n = 89), I performed a Pearson correlation test (2tail) to correlate cuttlebone length vs. thickness (Dytham 2011).

I recorded 89 cuttlebones (length: range 59-197 mm; thickness: 8-25 mm). Among them, 79 showed pecking signs by birds (88.8%), a relatively high percentage when compared to the only other studies quantifying it (e.g. 55-65% in Texel Island, North Sea; Cadée 2002b). All pecked cuttlebones (n = 79) showed signs on the ventral part while signs on the dorsal part were observed with lower frequency (n = 14; 17.7%; χ^2 = 107.06, p < 0.001). On the ventral part, no differences were found between the anterior and the posterior side (anterior: n = 77/89: 86.5%; posterior: 72/89: 80.9%; χ^2 = 1.89, p = 0.17). The longest cuttlebones showed a higher frequency of pecks (> 10 signs), while no cuttlebone < 12 cm in length showed > 10 signs. Ratio between the two categories of pecking abundance is higher in longest cuttlebones, i.e. cuttlebones with high pecking abundance (≥ 10 signs) were longer: about 50% of the longer cuttlebones (> 14 cm) showed a higher number of signs (Tab. 1).

Cuttlebone length was significantly correlated to thickness (r = 0.855, p < 0.001, n = 89, Pearson correlation test). Therefore, the longest cuttlebones were also those with greater thickness (and likely holding higher proportion of calcium aragonite), as well as being more easily detected by animals.

These results suggest that birds preferred to peck the ventral parts (softer), apparently not distinguishing between the anterior and posterior side, and that they pecked more frequently longer cuttlebones, probably because of their higher thickness and detectability.

This behaviour has been studied only in Northern Atlantic (Cadée 2002a, 2002b) and these are the first evidence for Mediterranean basin. However, this research lacks of important details about (i) what are the species that use cuttlebones (are there any possible interspecific differences in the pecking rate? What is the community of birds that depend on this resource? How does it vary its use depending on the season considered?) and (ii) the age of the collected shells (i.e. we can hypothesize that older cuttlefish bone may experience higher peaks than recent ones).

Table 1. Frequencies of cuttlebones (fr) pecked with different intensity (0-10 signs and > 10 signs) by birds subdivided for length classes.

Shell length classes (cm)	n tot	n pecked (0-10 signs)	fr	n pecked (> 10 signs)	fr	ratio 0-10 signs/> 10 signs
< 10	3	3	0.04	0	0	0
≥ 10-12	10	10	0.13	0	0	0
≥ 12-14	30	26	0.33	9	0.31	0.35
≥ 14-16	29	25	0.32	12	0.41	0.48
≥ 16	17	15	0.19	8	0.28	0.53
Tot	89	79		29		

Therefore, further research is necessary in this regard.

In addition to these basic eco-behavioural evidences, these findings may have conservation implications in the disciplinary arena of marine/beach litter research (see Poeta et al. 2016). Indeed, expanded polystyrene, an increasingly abundant anthropogenic debris in natural environments (Gross and Kalra 2002), was often pecked, probably because confused with cuttlebones (Cadée 2002a, Battisti 2020): an initial hypothesis that should be tested is whether this pecking behaviour is repeated with this frequency pattern also on this artificial material, where comparable pecking signs have been observed. Polystyrene tends to fragment and degrade in sea waters: therefore, further experimental research should verify that large fragments of expanded polystyrene, just abandoned and not yet fragmented, were more intensely pecked when compared to old smaller fragments beached for much longer time.

Acknowledgments

REFERENCES

Alonso H., Almeida A., Granadeiro J.P. & Catry P., 2015. Temporal and age-related dietary variations in a large population of yellow-legged gulls *Larus michahellis*: implications for management and conservation. European Journal of Wildlife Research 61: 819-829.

Battisti C., 2014. Bird assemblages on a Mediterranean sandy beach: a yearly study. Rivista Italiana di Ornitologia - Research in Ornithology 84: 5-10.

Battisti C., 2020. Heterogeneous composition of anthropogenic litter recorded in nests of Yellow-legged gull (*Larus michahellis*) from a small Mediterranean island. Marine Pollution Bulletin 150: 10682.

Battisti C. & Sorace A., 2006. Uccelli: check-list aggiornata al 2005. In: Battisti C. (ed.), Biodiversità, gestione, conservazione di un'area umida del litorale tirrenico: la Palude di Torre Flavia. Provincia di Roma, Gangemi editore: 255-269.

Battisti C., Luiselli L. & Vignoli L., 2012. Bird assemblages in a structurally simplified Mediterranean sandy beach: an analysis at spatial and temporal level. Revue d'Ecologie (Terre Vie) 67: 63-70.

Birchall J.D. & Thomas N.L., 1983. On the architecture and function of cuttlefish bone. Journal of Materials Science 18: 2081-2086.

Cadée G.C., 2002a. Seabirds and floating plastic debris. Marine Pollution Bulletin 44: 1294-1295.

Cadée G.C., 2002b. Mass stranding of cuttlebones of *Sepia orbignyana* Férussac, 1826, on Texel, the Netherlands, in July 2002 (Cephalopoda, Decapoda, Sepiidae). Basteria 66: 113-120.

Čadež V., Škapin S.D., Leonardi A., Križaj I., Kazazić S., Salopek-Sondi B. & Sondi I., 2017. Formation and morphogenesis of a cuttlebone's aragonite biomineral structures for the common cuttlefish (*Sepia officinalis*) on the nanoscale: Revisited. Journal of Colloid and Interface Science 508: 95-104.

Cadman J., Zhou S., Chen Y., Li Q., 2012. Cuttlebone: Characterisation, Application and Development of Biomimetic Materials. Journal of Bionic Engineering 9: 367–376.

Cadman J., Zhou S., Chen Y., Li W., Appleyard R. & Li Q., 2010. Characterization of cuttlebone for a biomimetic design of cellular structures. Acta Mechanica Sinica 26: 27-35.

Checa A.G., Cartwright J.H.E., Sánchez-Almazo I., Andrade J.P. & Ruiz-Raya F., 2015. The cuttlefish *Sepia officinalis* (Sepiidae, Cephalopoda) constructs cuttlebone from a liquid-crystal precursor. Scientific Reports 5: 11513.

Coulson R. & Coulson G., 1993. Diets of the Pacific gull *Larus pacificus* and the kelp gull *Larus dominicanus* in Tasmania. Emu 93: 50-53.

Denton E.J. & Gilpin-Brown J.B., 1961. The buoyancy of the cuttlefish, *Sepia officinalis* (L.). Journal of the Marine Biological Association of the United Kingdom 41: 319-342.

Dytham C., 2011. Choosing and using statistics: a biologist's guide. John Wiley & Sons.

Graveland J., Van der Wal R., Van Balen J.H. & Van Noordwick A.J., 1994. Poor reproduction in forest passerines from decline of snail abundance in acïdified soils. Nature 368: 446 448.

Gross R.A. & Kalra B., 2002. Biodegradable polymers for the environment. Science 297: 803–807,

Guillaud E., Morales-Muñiz A., Roselló-Izquierdo E. & Béarez P., 2019. Taphonomy of Yellow-legged Gull (*Larus michahellis*) pellets from the Chafarinas islands (Spain). Canadian Journal of Zoology 97(2): 100-111.

Le Goff R., Gauvrit E., Du Sel G.P. & Daguzan J., 1998. Age group determination by analysis of the cuttlebone of the cuttlefish *Sepia officinalis* L. in reproduction in the Bay of Biscay. Journal of Molluscan Studies 64(2): 183-193.

Le Pabic C., Marie A., Marie B., Percot A., Bonnaud-Ponticelli. L, Jean Lopez P. & Luquet G., 2017. First proteomic analyses of the dorsal and ventral parts of the *Sepia* officinalis cuttlebone. Journal of Proteomics 150: 63–73.

Neige P., 2006. Morphometrics of hard structures in cuttlefish. Vie et Milieu 56(2): 121-128.

North L., Labonte D., Oyen M.L., Coleman M.P., Caliskan H.B. & Johnston R.E., 2017. Interrelated chemical-microstructural-nanomechanical variations in the structural units of the cuttlebone of *Sepia officinalis*. Applied Physics Letters, 5: 116103

Perrins C.M., 1996. Eggs, egg formation and the timing of breeding. Ibis 138(1): 2-15.

Poeta G., Conti L., Malavasi M., Battisti C. & Acosta A.T.R., 2016. Beach litter occurrence in sandy littorals: The potential role of urban areas, rivers and beach users in central Italy. Estuarine, Coastal and Shelf Science 181: 231-237.

Sherrard K.M., 2000. Cuttlebone morphology limits habitat depth in eleven species of *Sepia* (Cephalopoda: Sepiidae). The Biological Bulletin 198(3): 404-414.

Taylor E.J., 1996. An evaluation of the importance of insoluble versus soluble grit in the diet of canaries. Journal of Avian Medicine and Surgery 10: 248-251.