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Breeding Ecology of the Little Egret *Egretta garzetta* in the Anzali Wetland, Northern Iran

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Abstract
The breeding ecology of the Little Egret *Egretta garzetta* was studied during the breeding season of 2016 in two sub-colonies in Anzali wetland. Trees of second sub-colony were younger and the height and diameter of threes were less than half of the first one. Breeding in the second sub-colony done about one month later. The variables tree diameter, the height of nests from the ground and the distance of nests from the top of tree canopy were significantly different. The breeding activities in first sub-colony started from late April and lasted to late July and the second one from 22 May to the middle of August. The mean egg size and volume in the two sub-colonies were significantly different. The average clutch size did not differ between these two sub-colonies. Breeding success in the first sub-colony (82%) was higher than the second sub-colony (74%), however, no significant difference was found at the fledging. Based on generalized linear model, the clutch size and breeding success appeared to be independent of the structural variables of the nesting site in these two sub-colonies. Fish dominated the diet of the nestlings, particularly Eastern Mosquitofish *Gambusia holbrooki,* in terms of the number and *Carassius gibelio,* in terms of the volume percentage.

1. Introduction

The Little Egret *Egretta garzetta* is widely distributed in Asia and southern Europe and winters in Africa. This species often builds its nests in multi-species heronries. In multi-species heronries, the coexistence of hundreds of breeding birds results in dense nesting and partitioning of the available space (Jenni 1969; Maxwell & Kale 1977; Parsons 1995; Pang *et al*. 2019).

Nest site selection varied depending on the height of nesting trees, availability of suitable nesting sites (vegetation structure), and the timing of nest building (Beaver *et al.* 1980; Burger 1985; Arendt & Arendt 1988; Baxter 1994). Little egrets prefer higher trees with less shrubby undergrowth because it decreases the disturbance from the human being or other predators from the bottom of trees (Pang *et al*. 2019).

Little Egrets are widespread across much of Iran on migration and during the winter, but they breed only locally in the south Caspian Sea basin, Khuzestan, Fars and Sistan & Baluchestan Provinces (Mansoori 2008). In Gilan Province (Caspian Sea basin), the species is a common breeder in mixed colonies with other egrets, herons and cormorants with a total population between 1,100−1,600 nests (Ashoori & Abdoos 2013).

There has been many studies on the breeding activities of this species. However the followings

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were used for comparison with our results: a) Uzun *et al.* (2008) in Turkey where they did not find statistically significant difference in annual variations of the mean clutch size, hatching success and fledging success, b) Hilaluddin *et al.* (2003) in Utter Pradesh, India where they suggested that there is a relationship between nest selection and hatching success of the Little Egret, and c) Kazantzidis *et al.* (1997) in the Axios Delta in Greece where they recorded the clutch size and mean number of eggs hatched was smaller in late nesters, but no significant difference was observed in chick survival per nest between early and late nesters. In Iran, a study on the breeding biology and success of the Little Egret has been carried out (Ashoori 2010) that the fledging success was 80.6% and insects were dominant in the diet of nestlings. Additionally, Ashoori & Barati (2013) showed that the height of the nest above the ground affect the breeding success while did not influence the clutch size. Neb & Selmi (2019) showed that the clutch size and hatching success decreased when the breeding season progressed. Pang *et al* (2019) mentioned that nests placed at lower height in trees have produced lower hatching success, due to the nest destruction by the human being. This destruction was marginally contributed to the lowering of the total nesting success and the anthropogenic influences was a greater factor for the reproductive failure.

Our objectives were to compare effects of nest position and the initiation time of nesting on egg sizes, the clutch size and the breeding success in two sub-colonies of the Little Egret in Anzali wetland located in southern Caspian Sea, as well as to determine the nestling diet of this species here.

2. Materials and Methods

2.1. Study area

The Anzali Wetland Complex is situated in Gilan Province at the southwest corner of the Caspian Sea (37°25’ to 37°30’ N, 49°25’ to 49°30’ E, 19,300/ha) and is comprised of four parts; western, eastern and central parts, plus the Siahkeshim wetland. Since 1975, it has been registered as a wetland of international importance by the Ramsar Convention on Wetlands.

The study area is located on Ghalam-Goodeh islet (37°27’ N, 49°27’ E, 81 ha) in the central part of Anzali wetland (Fig. 1). The islet is not inhabited by people. More than one third of this islet is covered by trees, about one third by Common Reed *Phragmites australis*, and the remaining part is composed of a fish pond, grassland, etc. The Common Alder *Alnus glutinosa*, Caucasian Wingnut *Pterocarya fraxinifolia* and Caspian Locust *Gleditsia caspica* are the dominant tree species on this islet. A heronry is situated in the western part of the islet and includes the Black-crowned Night Heron *Nycticorax nycticorax*, Little Egret, Cattle Egret *Bubulcus ibis* and Squacco Heron *Ardeola ralloides*. The total number of individuals of these four heron species in this colony varied during the study period due to yearly changes in habitat conditions. The average number of nests was 840 (range 600–1,100) between 2003 and 2008 with the exception of 2006 (Ashoori & Abdoos 2013). In 2006 and 2015, the colony was abandoned probably due to the human disturbance.

In 2016, we discovered a new heronry with more than 450 nests of the same four heron species (Black-crowned Night Heron (152 pairs), Little Egret (161 pairs), Cattle Egret (33 pairs) and Squacco Heron (106 pairs)). These nests were situated on the Common Alder and Caucasian Wingnut trees at over 15 m above the ground. In this sub-colony, nests of the Little Egret (39 nests) were built at lower height in the middle of April 2016. About one month after the start of nesting in the first sub-colony, another sub-colony was built with 161 nests (Black-crowned Night Herons with 109 pairs, Little Egret with 27 pairs, Squacco Heron with 21 pairs and Cattle Egret with 4 pairs) on a homogeneous mass of the Common Alder (most trees were less than 9 m height). These sub-colonies were located about 80 m from each other, and we could survey all of Little Egret nests in these sub-colonies.

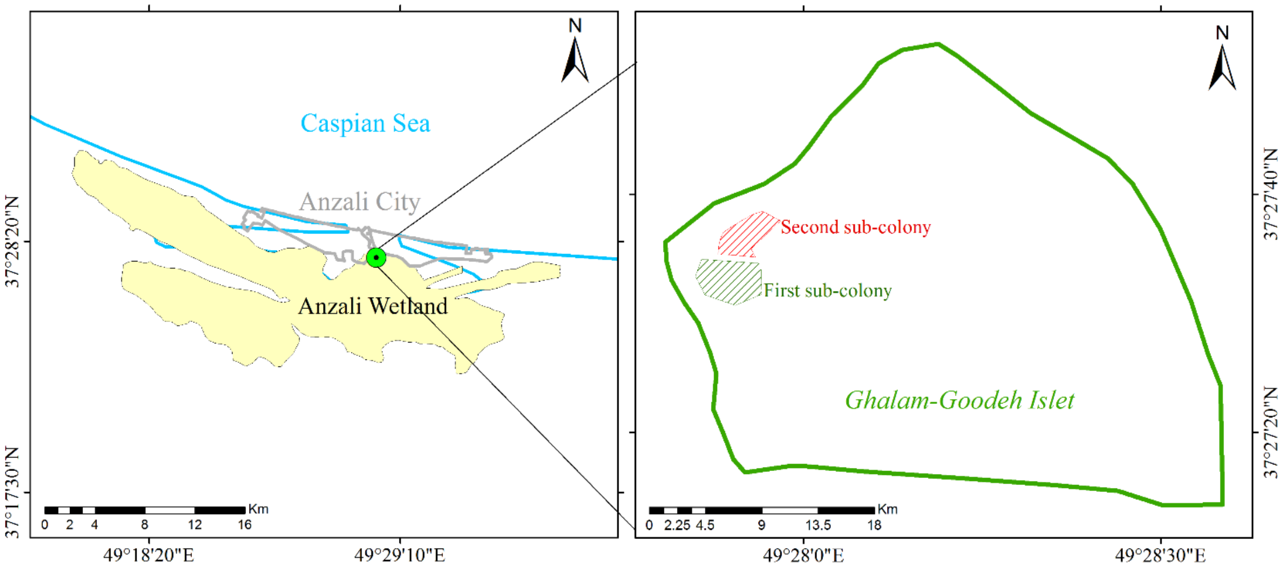


Fig. 1. Map of Anzali wetland and the study area on Ghalam-Goodeh islet.

2.2. Methods

We started monitoring nests from late April 2016 in the first sub-colony. In the second sub-colony we started monitoring nests from late May 2016 until their young fledged around the middle of August. An active nest was defined as one that contained at least either an egg or a nestling (Ashoori 2010, Hilaluddin Shah & Shawl 2006). We used a metal ladder to reach nests in Alder trees. When a nest was found, it was tagged with a numbered plastic plaque. We visited these nests at least twice a week to record the clutch size, number of young fledged and their age. In addition, nest parameters such as the distance of nests above the ground and from the tree canopy were measured using a metal tape. The tree diameter at breast height (DBH) was measured using calipers. The length and width of eggs were measured by an Electronic Vernier Calipers (accuracy of 0.01 mm). To identify the mass and egg shape index, the following formula was used: $V\ (cc) = K \times L\ (cm) \times B^2\ (cm)$, where L is the maximum length, B is the maximum breadth and K as constant (0.51). The egg shape index = B/L × 100 (Hoyt 1979) which means the higher the index value, the more spherical is the shape of eggs. Breeding success was expressed as the ratio of the total number of fledglings to the total number of eggs incubated. Fledging success was also calculated as the proportion of chicks that subsequently fledged successfully. Nest productivity was calculated by dividing the number of young birds at the fledging stage by the number of nests.

2.3. Diet sampling

The diet was studied by analysing nestlings' regurgitations. Regurgitations were collected throughout the nestling period (more than 80% from the second sub-colony). Each sample was placed in a plastic bag, labelled and transferred to the laboratory of Gilan Provincial Office of the Department of the Environment. These individual samples were then stored in formalin (10%), prior to analysis at the end of the field work. The volume of diet items was measured as the volume percentage of each prey item to the whole volume of contents of each stomach using a graduated cylinder. Food items were identified using available guides.

2.4. Statistics

We used a generalized linear model (GLM) for a response with Poisson distribution, to verify effects of nesting site structural attributes on the clutch size and breeding success. In this model, we considered the clutch size and breeding success separately as dependent variables and structural variables as linear predictors (DBH, the height of nests above the ground, the height of nests to the tree canopy, number of nests per tree). After verification of Poisson distribution of the dependent variable, we applied the Kolmogorov-Smirnov test. All pairwise analyses were done with *t*-test. Statistics were performed using R software (R Core Team, 2016) with $P<0.05$ used as the threshold of significance. Means are present ± SD.

3. Results

We measured 66 nests in Ghalam-Goodeh colony (39 nests on 11 trees in the first sub-colony and 27 nests on 24 trees in the second sub-colony). The average diameter at breast height (DBH) of trees in these sub-colonies differed (32.1±6.2 cm in the first sub-colony, range 24.5−44 cm and 13.7±3.8 cm in the second sub-colony, range 6.5−22.45 cm, $t=14.1$ $P<0.001$). The mean height of nests from the ground also differed significantly (10.6±2.58 m in the first sub-colony, range 4.3−15.8 m and 5.8±0.8 m in the second sub-colony range 4.5−7.7 m, $t=-10.1$, $P<0.001$). Also, the height of nests to the canopy was significantly different (3.59±1.9 m in the first sub-colony, range 0.3−8 m and 2.7±0.9 m in the second sub-colony, range 1−4.5 m, $t=2.1$, $P<0.05$). None of the structural variables measured at the nesting sites had any significant effect on the clutch size (Tables 1−2).

In the first sub-colony, the first egg was laid on 23/24 April and the last one on 11/12 May. Hatching began from 19 May and continued until 7 June. Fledglings left the nest between 21 June and 20 July, while in the second sub-colony, the first egg was laid on 22 May and the last one on 21 June. Hatching began on 20 June and continued until 20 July. Fledglings left the nest between 1 to 16 August.

In the first sub-colony, 27 nests were on branches and 12 on the trunk and in the second sub-colony, 11 nests were placed on branches and 16 were on the trunk. In the first sub-colony, the vertical and horizontal distances between nests were 1.03±0.4 (range 0.4−2) and 0.7±0.3 m (range 0.2–1.1), respectively. Vertical and horizontal distances from the other colonial heron species were 1.05±0.7 (range 0.4−2.5) and 0.8±0.4 m (range 0.6–1.5), respectively. In the second sub-colony, there was almost one nest in every tree (84% of all nests) but the vertical and horizontal distances from other species (Black-crowned Night Heron and Squacco Heron) in the colony were 0.7±0.3 (range 0.3−1.2) and 0.6 m, respectively.

Although the average clutch size in the second sub-colony was greater (4.4±0.6, range 3–5) than the first sub-colony (4.12±0.9, range 2–5), this difference was not significant ($t= 1.7$, $P>0.05$). The modal clutch size was five eggs in the first sub-colony, and overall, 41% of the nests had five eggs, 36% four eggs, 18% three eggs and 5% two eggs. In the second sub-colony, the modal clutch size was four and five eggs, and overall, 48% of the nests had four eggs, 48% five eggs, and 4% had three eggs.

The mean egg size and volume in the second sub-colony were greater than the first sub-colony and the differences were significantly different ($P<0.05$). Also, eggs in the first sub-colony were rounder but the difference was not significant (Table 3).

In the first sub-colony, 4 out of 39 nests were destroyed, and the young of 35 active nests reached to the fledging stage. Of 142 total eggs, 132 eggs hatched and 117 chicks reached to their fledging stage, resulting in a breeding success of 82%. In the second sub-colony, 4 out of 27 nests were destroyed, and the young of 23 active nests reached to the fledging stage. Of 103 total eggs, 95 eggs hatched and 76 chicks reached to their fledging stage, resulting in a breeding success of 74%. No difference was found between the fledging stage of these sub-colonies ($t=-0.1$, $P>0.05$). None of the structural variables measured at these nesting sites had a significant effect on the variation in the breeding success (Tables 1−2).

By number, the nestling diet was composed of 15 fish species (45.5%), 12 insect species/families/orders (36.5%), 2 Malacostraca (6%), one Arachnida families (3%), one amphibian (3%), one Clitellata (3%) and one Diplopoda (3%) (Table 3). Fish which were recorded with 229 prey items (45.5%) were the most numerous prey (Table 3, Fig. 2). Among all prey items and fish species identified in the regurgitations, Eastern Mosquitofish *Gambusia holbrooki* was the most numerous species (23.6% and 52%, respectively). Fish comprised about 53.2% of the volume of the nestling diet with the *Carassius gibelio* alone comprising 18.8% and Eastern Mosquitofish as 16.9%, followed by Amphibian (*Rana ridibunda*) with 24.2% and insects with 15.7% items were found in chicks' diet (however, their proportion by volume was low, 5.7%) (Table 4, Fig. 2).

Table 1. Generalized Linear Model (GLM) for a response of Poisson distribution comparing the clutch size and breeding success (dependent variable) with the structural variables of the nesting site (linear predictors) in the first sub-colony of Anzali colony.

Variables	Clutch size			Breeding Success		
	Estimate	Standard Error	*p*	Estimate	Standard Error	*p*
Intercept	1.2	0.5	0.02	0.7	0.6	0.2
Diameter at Breast Height	0.01	0.01	0.3	0.009	0.2	0.6
Nest height above the ground	-0.01	0.03	0.6	0.006	0.4	0.8
Nest height from top of canopy	-0.02	0.04	0.5	-0.02	0.6	0.7
Nest number per tree	0.002	0.03	0. 9	0.03	0.4	0.5

Table 2. Generalized Linear Model (GLM) for a response of Poisson distribution comparing clutch size and breeding success (dependent variable) with the structural variables of the nesting site (linear predictors) in the second sub-colony of Anzali colony.

Variables	Clutch size			Breeding Success		
	Estimate	Standard Error	*P*	Estimate	Standard Error	*p*
Intercept	1.7	0.9	0.07	0.6	1.2	0.6
Diameter at Breast Height	-0.007	0.02	0.8	-0.04	0.04	0.3
Nest height above the ground	-0.05	0.1	0.6	-0.01	0.15	0.9
Nest height from top of canopy	0.01	0.1	0.8	0.2	0.16	0.1
Nest number per tree	0.09	0.3	0.7	0.5	0.4	0.2

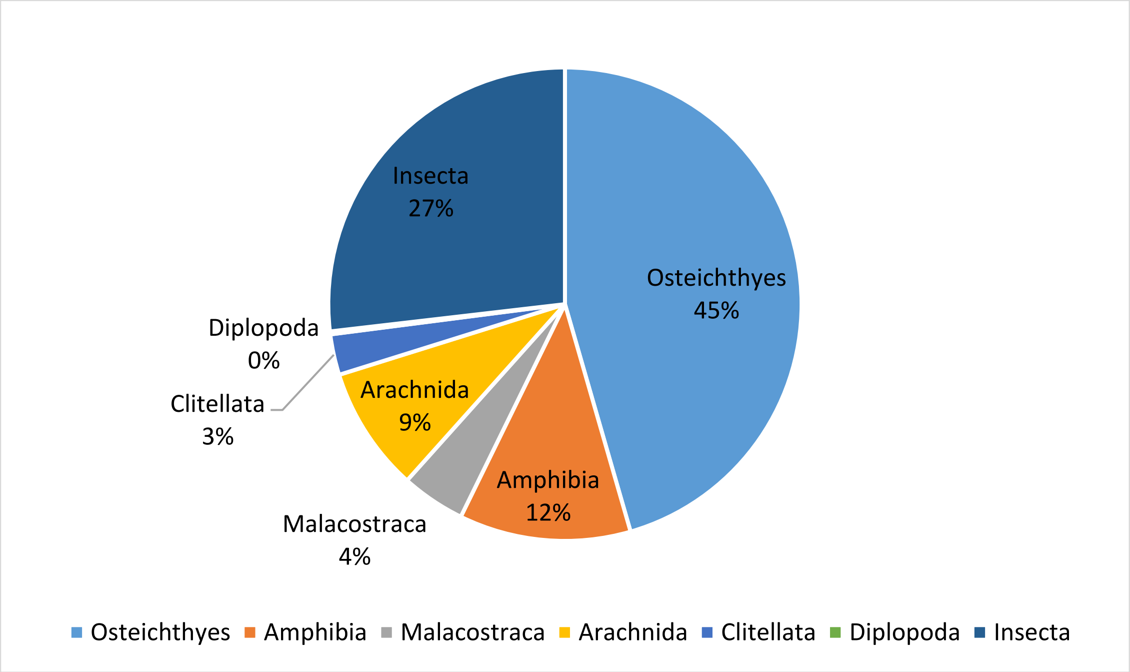
Table 3. The mean ± SD of egg traits of Little Egret in the two sub-colonies in the Anzali colony in 2016 (N_1= 100, N_2= 105).

Anzali colony	Length (mm)	Width (mm)	Egg volume (cm^3)	Egg Shape Index
First Sub-Colony	45.1 ± 1.9	32.6 ± 1.1	24.5 ± 1.9	72.3 ± 3.5
Second Sub-Colony	45.8 ± 1.5	32.9 ± 1.25	25.24 ± 2.17	71.9 ± 3.5
***t*–test**	2.9	2.2	3.3	- 0.7
P	0.001	0.01	0.001	0.4

Table 4. The number of prey items (N), their relative abundance (RA) and the percentage of volume frequency (PVF) identified in the nestling regurgitations of the Little Egret in Anzali wetland, Iran.

Class	Order/ Family	Species	N	RA (%)	PVF (%)	RA (%)
Osteichthyes	Cyprinidae	*Carassius gibelio*	52	10.3	616.5	18.8
Osteichthyes	Cyprinidae	*Hemiculter leucisculus*	9	1.8	189.6	5.8
Osteichthyes	Cyprinidae	*Blicca bjoerkna*	4	0.8	21.2	0.6
Osteichthyes	Cyprinidae	*Rutilus rutilus*	2	0.4	18	0.5
Osteichthyes	Cyprinidae	*Rutilus frisii kutum*	9	1.8	19	0.6
Osteichthyes	Cyprinidae	*Pseudorasbora parva*	2	0.4	15.3	0.5
Osteichthyes	Cyprinidae	*Alburnus hohenackeri*	5	1	45	1.4
Osteichthyes	Cyprinidae	unidentified	3	0.6	105	3.2
Osteichthyes	Poeciliidae	*Gambusia holbrooki*	119	23.6	554.9	16.9
Osteichthyes	Esocidae	*Esox Lucius*	1	0.2	30	0.9
Osteichthyes	Mugilidae	*Liza Saliens*	1	0.2	15	0.4
Osteichthyes	Cobitidae	*Sabanejewia caspia*	15	3	75	2.3
Osteichthyes	Gobiidae	*Neogobius fluviatilis*	2	0.4	18	0.5
Osteichthyes	Gobiidae	*Ponticola gorlap*	3	0.6	12	0.4
Osteichthyes	Gobiidae	Rinigobius spp	1	0.2	4.1	0.1
Osteichthyes	Atherinidae	*Atherina boyeri*	1	0.2	10	0.3
Amphibia	Ranidae	*Rana ridibunda*	59	11.7	793	24.2
Malacostraca	Palaemonidae	*Macrobrachium nipponense*	13	2.6	140	4.2
Malacostraca	Gammaridae	unidentified	9	1.8	8	0.2

Class	Order/ Family	Species	N	RA (%)	PVF (%)	RA (%)
Arachnida	Araneidae	unidentified	43	8.5	22.5	0.7
Clitellata	Piscicolidae	unidentified	14	2.8	57	1.7
Diplopoda	unidentified	unidentified	1	0.2	1	0.03
Insecta	Gryllotalpidae	*Gryllotalpa gryllotalpa*	2	0.4	130	4
Insecta	Agrionidae	unidentified	8	1.6	78	2.4
Insecta	Acrididae	unidentified	31	6.2	119.3	3.6
Insecta	Tettigoniidae	Tettigonia spp	39	7.7	58.85	1.8
Insecta	Tetrigidae	unidentified	1	0.2	1.3	0.04
Insecta	Gryllidae	Gryllus spp	21	4.2	12.25	0.4
Insecta	Tabanidae	unidentified	2	0.4	70	2.1
Insecta	Carabidae	unidentified	7	1.4	8	0.2
Insecta	Dytiscidae	unidentified	9	1.8	14.9	0.4
Insecta	Mantidae	unidentified	9	1.8	16.6	0.5
Insecta	Nepidae	unidentified	1	0.2	2	0.06
Insecta	Mecoptera	unidentified	1	0.2	2.5	0.07
Insecta	unidentified	unidentified	4	0.8	8.5	0.2
Total			**503**	**100**	**3292.3**	**100**



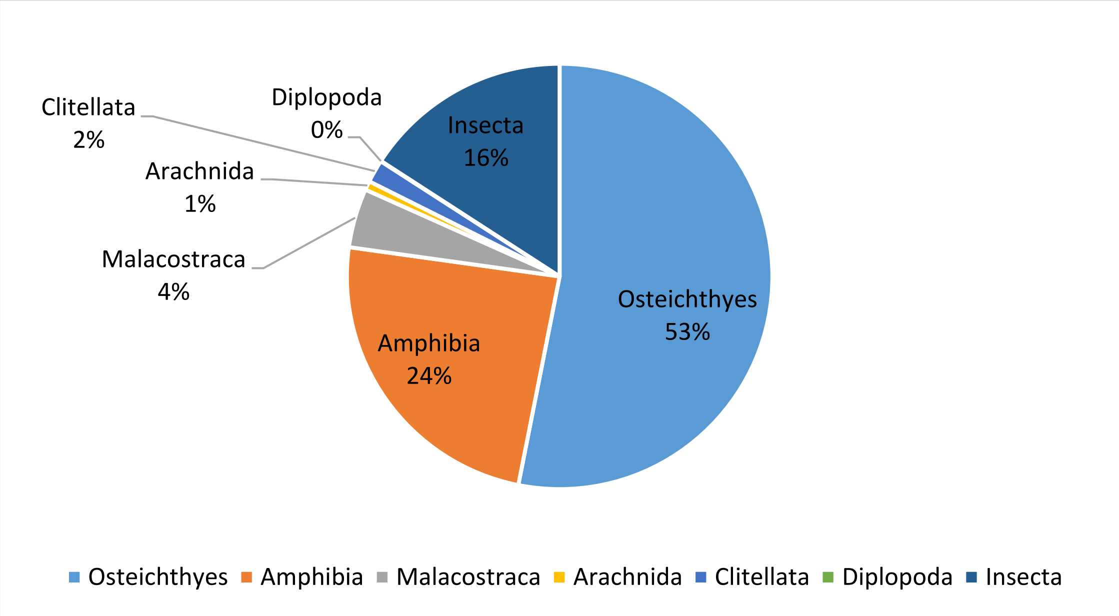


Fig. 2. Diet of the Little Egret nestlings by the number (above) and the percentage of volume frequency of prey categories (below).

4. Discussion

Little Egrets, like other herons are quite adapted to exploiting available local nesting resources such as vegetation type and height (Voisin 1991; Perennou *et al.* 1996). The two sub-colonies that were occupied by the Little Egret in Anzali wetland in the 2016 breeding period were different because of the variables tree size, the nest height from the ground, and the height to the top of the canopy and the differences in the date of nesting beginning.

In both sub-colonies, nests of Little Egrets were located at the lowest point of the trees, in comparison with the other three heron species in these two sub-colonies (Black-crowned Night Heron, Cattle Egret and Squacco Heron), and it seems that the nest siting is determined through interspecific interactions, which had also been mentioned by Kazantzidis *et al.* (1997) and Ashoori & Barati (2013).

Breeding of the Little Egret in Anzali wetland in 2016 started later than that of the Axios Delta, Greece (mid-April) (Kazantzidis *et al.* 1997), Lake Poyrazlar, Turkey (mid-March to early April), (Uzun *et al.* 2008), and Karfestan Ab-bandan (late March to early April) (Ashoori & Barati 2013). However, the Amroha'colony in India (Hilaluddin Shah & Shawl 2003) began earlier than the first sub-colony.

The mean clutch size in Anzali wetland in these two sub-colonies (4.1 and 4.4 eggs, respectively) were similar to the clutch size recorded in Camargue (4.1 eggs) (Kazantzidis *et al.* 1996), in the Axios Delta (4.3 eggs) (Kazantzidis *et al.* 1997) and in Karfestan Ab-bandan (4.2 eggs) (Ashoori 2010), but were larger than the Amroha (3.2 eggs) (Hilaluddin Shah & Shawl 2003) and the Lake Poyrazlar (3.2 eggs) (Uzun *et al.* 2008). Although, there is no clear tendency, but it seems that the latitude alone is not an important factor for the clutch size and probably other factors (e.g. quality of the diet) have more important role in the clutch size, as mentioned for the Little Egret by Hafner *et al.* (1994). Late breeders of the Little Egret have previously showed decrease in the breeding performance with poorer quality compared to early ones (Neb & Selmi 2019). Although, it has been reported that the clutch size in birds is often dependent on the age of parents, with younger parents laying fewer eggs (Klomp 1970; Coulson & Porter 1985), but it seems that the age and body condition in Little Egrets in the two sub-colonies were not substantially different ($P<0.05$), because there was no significant difference between the clutch size between these two sub-colonies in the colony. Probably, when food is available, groups of mature birds avoid competition for nest building by starting breeding later. The clutch size of Little Egrets has been found to be dependent on the quality of the diet and body condition of female birds (Hafner *et al.* 1994).

Although it is known that the laying date is an effective factor on the egg size (Murphy 1994; Sandercock *et al.* 1999), despite the greater egg size in the second sub-colony, we did not find any significant differences between egg sizes between these two sub-colonies.

The breeding success of the Little Egret in Karfestan Ab-bandan (Ashoori & Barati 2013) was within the range observed in our study but the breeding success of this species between these two sub-colonies was higher than the Amroha (40.8%) (Hilaluddin Shah & Shawl 2003) and Lake Poyrazlar (71.2%) (Uzun *et al*. 2008). Additionally, although breeding success in the first sub-colony was higher than the second sub-colony, we did not find any significant differences of the breeding success between the two sub-colonies in Anzali colony.

Different studies mentioned that the diet analyses based on regurgitated items are potentially biased (Marquiss & Leitch, 1990; Delord *et al.* 2004; Ashoori *et al.* 2012; Ashoori & Rakhshbhar 2013). In France and Greece, the most important prey category was fish in the nestling diet of the Little Egret (Kazantzidis *et al*. 1996; Kazantzidis & Goutner 2005), similar to results of the present study, while in three other studies in Italy, Greece and Iran, the contribution of fish in the diet was lower (Fasola *et al*. 1981; Kazantzidis *et al*.1996; Ashoori 2010). Although Little Egrets are typically opportunistic foragers, they respond flexibly to changes in food resources in time and space (Hafner & Britton 1983; Dugan *et al.* 1986; Kersten *et al.* 1992; Hafner *et al.* 1993), and they also adapt well to different habitat types (Kazantzidis *et al*. 1996), but fish seems to be the most important prey for the Little Egret (Voisin 1991). *Gambusia holbrooki* by number and *Carassius gibelio* by volume were the dominant species in the diet of young in our study, and *Carassius gibelio* was the dominant species in the diet of young of the Grey Heron and Purple Heron in two other previous studies in Anzali wetland (Ashoori *et al.* 2012; Ashoori & Rakshbahar 2013). These two fish species are invasive alien species in the Anzali wetland ecosystem.

Our results suggest that the height of nests from the ground and the distance of nests from the top of canopy do not play any important role in the clutch size and the breeding success of the Little Egret in the Anzali colony. Similarly, it was concluded by Parejo *et al.* (2000) and Ashoori & Barati (2013). Furthermore, we did not find any relationship between the size of trees and date of nesting. It seems that because of the limited number of nesting positions available for the Little Egret in the colony and the limited availability of food resources, a number of mature pairs avoid intraspecific and interspecific competition by starting to breed later, without any adverse effect on their breeding success.

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