

## Research Article

# The Distribution and Behaviour of Lesser Whistling-Duck (*Dendrocygna javanica*) at Lang Sen Ramsar site in Mekong Delta Vietnam

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### ABSTRACT

This study aimed to investigate the distribution, behaviour, potential food, competitor, and potential predator of Lesser Whistling-Duck (*Dendrocygna javanica*) by direct observations at six sub-zones in the Lang Sen Ramsar site in the south of Vietnam, from September 2021 to August 2022. Two hundred sixty nine (269) individuals of the *D. javanica* were seen on site mainly in pairs and small flocks. The survey showed that Lesser Whistling-Duck often appears in areas with water lettuce and duckweed at sub-zone 9, wild rice and lotus fields at sub-zone 12, and low water level fluctuation at sub-zone 5 in the early morning and late afternoon. The food source of Lesser Whistling-Duck in the reserve Lang Sen are mainly plants (duckweed, water lettuce, young shoots or seeds of lotus, water lily, water hyacinth, and wild rice) and small animals (snail, worm, shrimp, fish, and insect). This bird species is relatively sensitive to environmental influences and their ability to perceive, reflect, and make sound depends on the size of flocks. The carnivores often damage the Lesser Whistling-Duck including black kite, greater coucal, lesser coucal, and python. They are also affected by competition for food and habitat of other waterbirds, activities of tourists, and people around the reserve. The result of observation of Lesser Whistling-Duck is a concern from new area, so it added the information on the distribution of the species, including behaviour, the potential food, competitors, predators, and human threats, which are important in managing the species.

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## INTRODUCTION

Lang Sen Ramsar site, a wetland reserve, was recognized as the 2227th in the world and the 7th Ramsar site in Vietnam and has an area of 4,802 hectares in Tan Hung district, Long An province, which is situated in the Plain of Reeds of the Mekong Delta Vietnam (Hau & Tuyen 2017). Lang Sen is one of the eight important bird zones of Vietnam's freshwater wetlands, which regularly supports more than 20,000 waterbird individuals in the dry season (Ramsar 2016). The reserve has a high biodiversity with 68 zooplankton species, 261 plant species, 11 zoobenthos species, 87 fish species, 17 reptile species, 122 bird species, and 6 mammal species (Cuong 2015; Son et al. 2019). The biodiversity of this reserve has been affected by the hydrological regime of the Vam Co River, climate change (Triet et al. 2019), invasion of alien species, and human activities (Tho et al. 2018).

Lesser Whistling-Duck (*Dendrocygna javanica*) is a species of waterbirds in the genus *Dendrocygna*, order Anseriformes, family Anatidae (del Hoyo et al. 2014; Onwuka et al. 2020). Lesser Whistling-Duck is widely distributed throughout India, Nepal, Sri Lanka, Malaysia, Singapore, Indonesia, Myanmar, and Thailand (Rittiboon & Karntanut 2011; Chukwuemeka 2017; Zakaria et al. 2020). They mainly appear in flocks and live in freshwater wetlands such as ponds, lakes, and swamps with predominant vegetations and aquatic animals (Martins et al. 2017). This waterbird is an indicator organism for wetland ecosystems because they are more responsive to changes in plant composition and fluctuations in water levels than other animal species (Rajpar & Zakaria 2011).

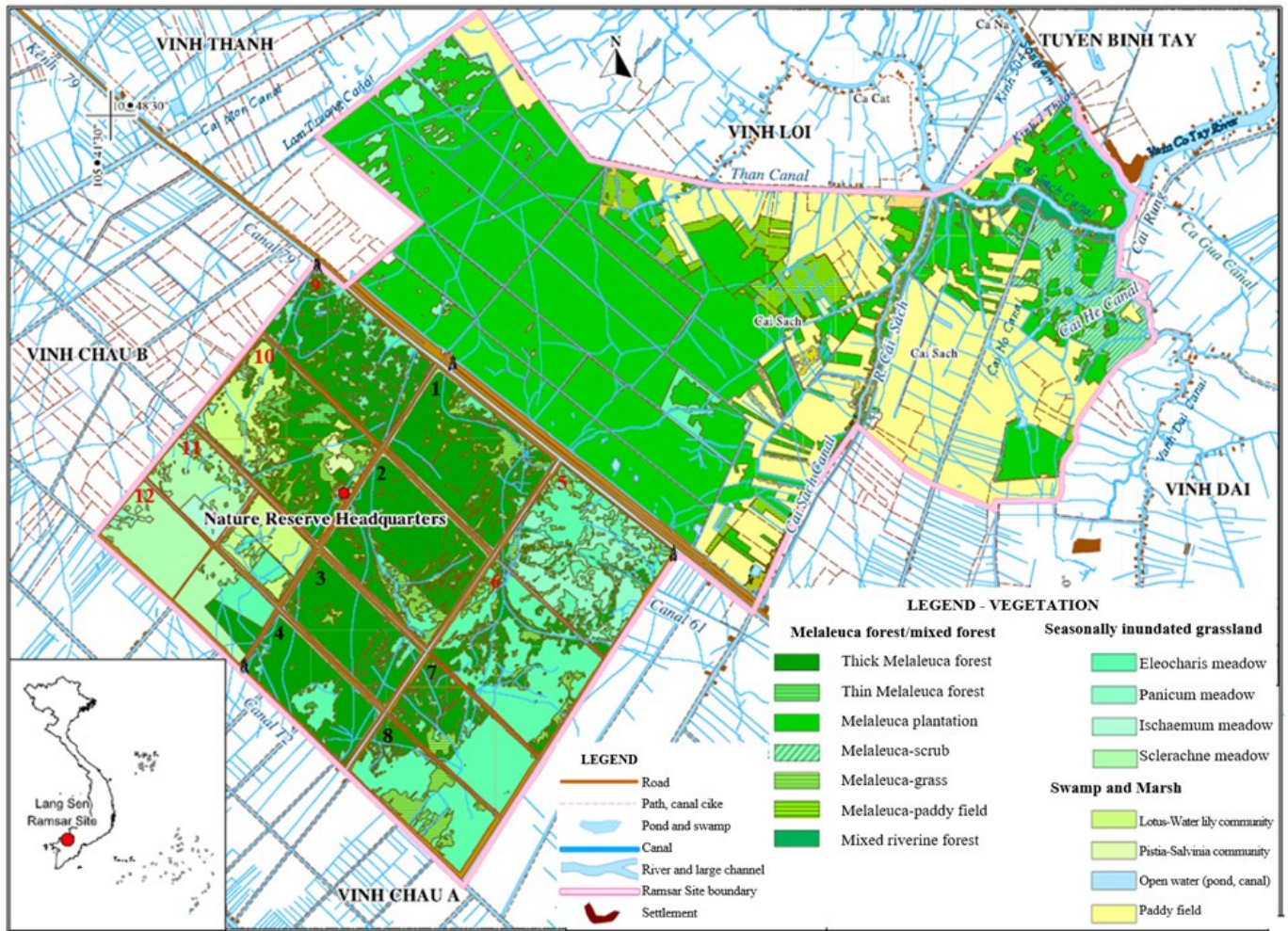
Lesser Whistling-Duck is evaluated as the Least Concern (IUCN Red List 2022), so the monitoring and protection of this bird have not been focused, but the population of this waterbird is decreasing in trend (Bird Life International 2016; Martins et al. 2017). According to the annual bird monitoring results, the number of Lesser Whistling-Duck in Lang Sen Ramsar site decreased from about 4000 individuals in 2015 to 1600 individuals in 2019 and only about 300 individuals in March 2021 (Lang Sen Nature Reserve 2015, 2019, 2022). Although the species is not considered endangered, the information on the use of wetland is important for conservation efforts. Therefore, the study on distribution, behavior, and factors influencing *Dendrocygna javanica* populations in the Lang Sen Ramsar site was carried out to provide data for the management, conservation, and restoration of this waterbird.

## MATERIALS AND METHODS

The survey was carried out from September 2021 to August 2022 at sub-zones 5, 6, 9, 10, 11, and 12, where *D. javanica* has appeared commonly in the last five years in Lang Sen Ramsar Site (Figure 1).

The distribution of Lesser Whistling-Duck in the reserve was surveyed using direct visual observation and point sampling method with binoculars (30 x 80) and photographed with a camera (Nikon-Coolpix P610). The survey was meticulously conducted twice daily, once in the morning (6:00-10:00 am) and once in the evening (4:00-6:30 pm), spanning four days each month. It was a comprehensive sub-zone, cross-sectional survey carried out through direct observation. The behaviours of waterbird were recorded including (1) activity and flock behavior, (2) feeding behaviours and food sources, (3) effect of other birds, and threats from human activities to this bird in the reserve, which were surveyed 4 days per month at the same time surveying of waterbird distribution.

The vegetation structure and water levels were diligently recorded once a month at the same sites where Lesser Whistling-Ducks had been sighted. The water level data was collected for the purpose of comprehending the im-



**Figure 1.** Sample point to survey Lesser Whistling-Duck in Lang Sen Ramsar site.

part of water level fluctuations on the distribution of waterbirds and aquatic vegetation. The monitoring of water quality parameters such as temperature, pH, DO, EC (with AQUACOMBO HM 3070), TDS (with Hanna combo HI 98130); and climatic factors including temperature, air humidity (with ISO-Lab), and light intensity (with WalkLab Digital Lux meter) in the distribution areas of Lesser Whistling-Duck was conducted once a month at the same time surveying of waterbird distribution.

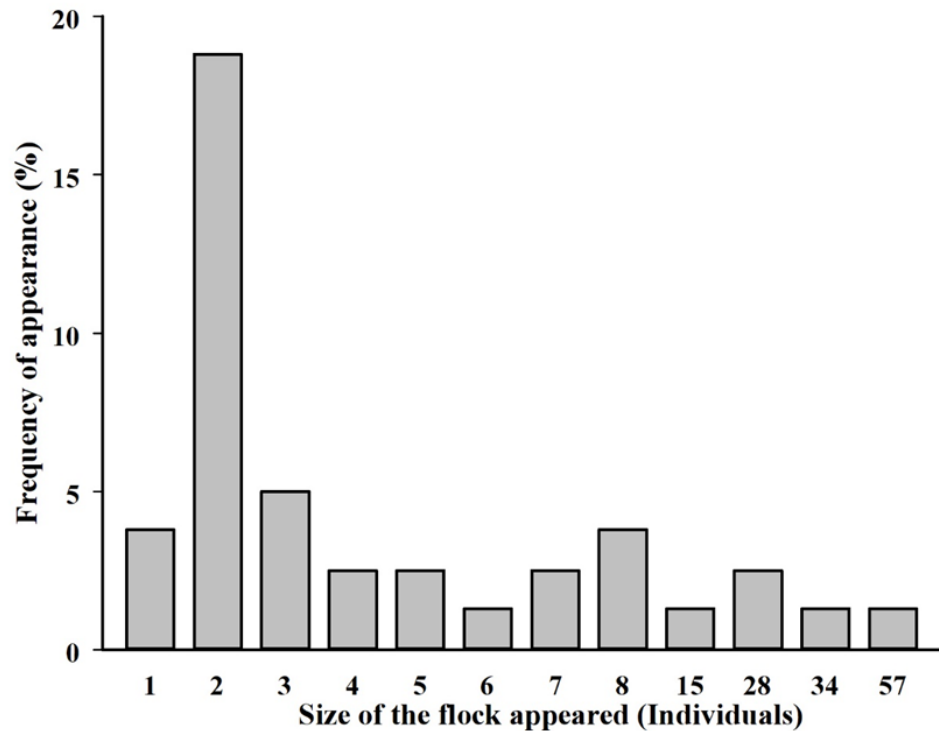
The data were summarized using Excel. The difference in an average number, the occurrence frequency of Lesser Whistling-Duck by time and location were examined using IBM SPSS statistics 22 software. Sigma Plot 12.5 software was used for making graphs.

## RESULTS AND DISCUSSION

### Distribution of Lesser Whistling-Duck in Lang Sen Ramsar site

The survey recorded a total of 269 individuals of Lesser Whistling-Duck from September 2021 to August 2022, at six sub-zones in the Lang Sen Ramsar site. The number of Lesser Whistling-Duck recorded has decreased approximately 15 times compared to 2015 and about 6 times compared to 2019 (Lang Sen Nature Reserve 2015, 2019). Lesser Whistling-Duck is also found in some wetlands in Vietnam such as U Minh Thuong National Park (Kien Giang Province), Tram Chim National Park (Dong Thap Province), Ca Mau Biosphere Reserve, and Cat Tien National Park (Dong Nai Province) (Thang 2011; Uyen et al. 2013; Duc & Dung 2020; Luong et al. 2022). The frequency of appearance Lesser Whistling-Duck during the survey period was about 46.2 %. They mainly appeared in pairs (2 individuals) with 18.8 %, followed by flocks of 3 individuals (5.0 %), and flocks of 15 to 57 individuals only from

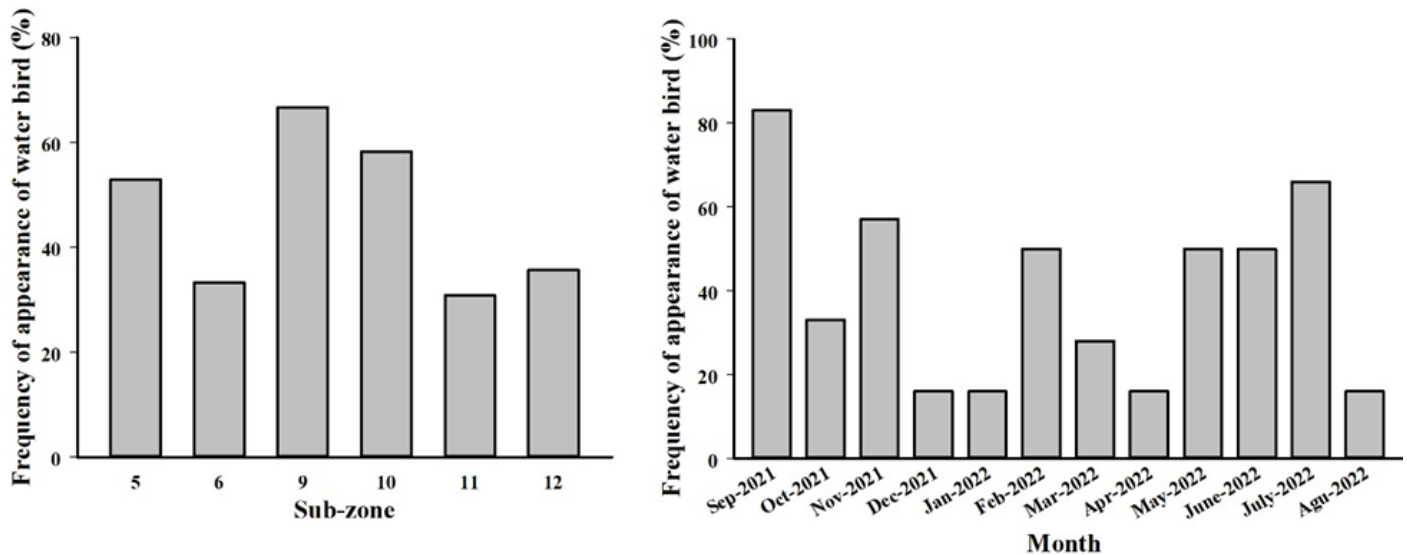
1.3 % to 2.5 %. According to De Silva et al. (2015) and Salari et al. (2016), this bird species is commonly seen in large flocks often comprising several hundred individuals. However, these flocks tend to disperse into smaller groups consisting of mated pairs and families. The size of the flock and frequency of encountering the Lesser Whistling-Duck at Lang Sen Ramsar site are detailed in Figure 2.



**Figure 2.** Size of the flock appeared and frequencies of appearance of Lesser Whistling-Duck at Lang Sen Ramsar Site.

The appearance of the waterbird species among the sub-zones is largely different. The frequencies of appearance of Lesser Whistling-Duck in sub-zones 9, 10, and 5 were 66.7; 58.3; and 52.9 %, respectively, which were higher than those in sub-zones 6, 11, and 12 with 33.3; 30.8; and 35.7 %, respectively. Besides, this spatial distribution also changed in the year, frequency of encountering Lesser Whistling-Duck in September 2021 was 83.3 %, followed by July at 66.7 %, November at 57.1 %, and in January, April, August, December at 16.7 % (Figure 3). In general, the frequencies of encountering and the number of Lesser Whistling-Duck in the sub-zones were correlated. There were 102 individuals in sub-zone 9 followed by sub-zone 12 with 56 individuals, sub-zone 5 with 43 individuals, and sub-zone 11 with 11 individuals (Table 1).

The survey showed that Lesser Whistling-Duck often appeared in areas with water lettuce (*Pistia stratiotes*) and duckweed (*Lemna minima*) at sub-zone 9 as well as wild rice (*Oryza rufipogon*) and lotus (*Nelumbo nucifera*) fields at sub-zone 12, besides water open area and water level fluctuation (sub-zone 5), because these areas will be favorable for them to swim, rest, feed, and hide. The remaining sub-zones are dominated by Melaleuca to obscure the view, the low open water area and high-water level fluctuation limited the distribution of the waterbird population. According to Salari et al. (2016) and Lazuardi et al. (2020), this waterbird preferred freshwater wetlands, which had abundant aquatic vegetation and aquatic invertebrates to feed. The distribution of Lesser Whistling-Duck was inversely proportional to the percentage coverage of the mixed species of spikerush (*Eleocharis dulcis*) and water lily (*Nymphaea lotus*) as well as to the water level fluctuation (Salari et al. 2016). In



**Figure 3.** Frequencies of appearance of Lesser Whistling-Duck in sub-zones (left) and following months of the year (right) in Lang Sen Ramsar site.

cases of elevated water level, such as during heavy rain, the Lesser Whistling-Duck tends to leave (De Silva et al. 2015). Other studies have suggested that water depth is a significant factor influencing the selection of habitat for waterfowl. of habitat for waterfowl, so it directly affects the ability to approach prey (Koli et al. 2014; Sulaiman et al. 2018). Additionally, in polluted water bodies, high nutrient content, low dissolved oxygen content, and low pH contribute to changes in the habitat of waterfowl, leading to a decrease in their population (Kuruvilla 2016).

The distribution of Lesser Whistling-Duck also changed between times of day. Most of them were recorded from 8:30 a.m. to 9:30 a.m., mainly in sub-zone 9, with additional recording from 4:00 p.m. to 5:30 p.m. in sub-zone 6 and 10 (Table 2). Lesser Whistling-Duck foraged from 8-9 a.m, they often found shelter and avoided the hot weather but they were mainly observed in mild morning and afternoon sunshine (De Silva et al. 2015). The results of the study showed that 220 individuals of Lesser Whistling-Duck (accounting for more than 80 % of the total) were distributed and active in sunny conditions in the morning, while Lesser Whistling-Duck less appeared in the cool and cloudy weather in the afternoon. The influence of temperature on the distribution of Lesser Whistling-Duck was reported in the study of Mazumdar and Ghosh (2005) cited by Lazuardi et al. (2020), warm temperature will encourage this bird to fly and mate.

The survey results and correlation analysis of water quality parameters (temperature, pH, DO, EC, TDS); climatic factors (temperature, air humidity, light intensity), and the amount of Lesser Whistling-Duck showed that water quality and climatic factors also contributed to affecting the distribution of this waterbird. The number of Lesser Whistling-Duck appearing positively correlated to the pH of the water (correlation coefficient person of 0.457,  $p < 0.05$ ), TDS (0.572,  $p < 0.05$ ); but negatively correlated to water temperature (-0.739,  $p < 0.01$ ) and water level fluctuation (-0.716,  $p < 0.01$ ). The frequency of occurrence of Lesser Whistling-Duck also positively correlated to air temperature and light intensity with person correlation coefficients of -0.930 ( $p < 0.01$ ) and -0.723 ( $p < 0.01$ ), respectively. Therefore, water quality parameters such as dissolved oxygen, water depth, and salinity fluctuations can affect the presence of aquatic plants and animals in wetland ecosystems and limit the food sources for these birds (Hansson et al. 2010; Rajpar & Zakaria 2011).

**Table 1.** Appearance of Lesser Whistling-Duck in the sub-zones in Lang Sen Ramsar site.

Month	Sub-zone						Total of Lesser Whistling-Duck	
	5	6	9	10	11	12	Individuals	Ratio (%)
Sep-2021	26	2	2	2	2	45	79	29.4
Oct-2021	-	-	-	2	-	3	5	1.9
Nov-2021	4	5	-	-	4	-	13	4.8
Dec-2021	-	-	2	-	-	-	2	0.7
Jan-2022	-	-	2	-	-	-	2	0.7
Feb-2022	-	28	57	8	-	-	93	34.6
Mar-2022	-	-	28	3	-	-	31	11.5
Apr-2022	-	-	6	-	-	-	6	2.2
May-2022	7	-	3	1	-	-	11	4.1
June-2022	4	2	-	2	-	-	8	3.0
July-2022	-	-	2	2	5	8	17	6.3
Agu-2022	2	-	-	-	-	-	2	0.7
Total (Individuals)	43	37	102	20	11	56	269	
Ratio (%)	16.0	13.8	37.9	7.4	4.1	20.8		100

**Table 2.** Distribution of Lesser Whistling-Duck at time of day in sub-zones in Lang Sen Ramsar site.

Time	Sub-zone						Total of Lesser Whistling-Duck (individuals)
	5	6	9	10	11	12	
7-8h30 am	11	-	6	-	-	37	54
8h31-9h30 am	11	5	96	4	5	8	129
9h31-10h30 am	19	-	-	-	-	3	22
4h-5h30 pm	2	32	-	16	6	8	64
Total (individuals)	43	37	102	20	11	56	269

### Characteristics of habitats Lesser Whistling-Duck in Ramsar Lang Sen site

The present study indicated that Lesser Whistling-Duck in Ramsar Lang Sen preferred wetlands habitats with open water surface with lotus (*Nelumbo nucifera*), wild rice (*Oryza rufipogon*), *Eleocharis dulcis*, *Hymenachne acutigluma*, others grass and shrubs, scattered melaleuca (*Melaleuca cajuputi*), and floating aquatic plants such as water lettuce (*Pistia stratiotes*), duckweed (*Lemna minor*). In these habitats, they can easily and quickly observe and detect threats from predators and humans. When Lesser Whistling-Duck detect danger, they often fly to other areas or swim and hide in emergent aquatic plants. Therefore, Lesser Whistling-Duck's activities in grassland and Melaleuca forest habitats were recorded flying and hiding (Table 3). Lesser Whistling-Duck is a gregarious waterbird, widely distributed in marshy swamps, freshwater wetlands with rich vegetation and animals (Zakaria et al. 2020) for their activities, so they are the most dominant species in open water (De Silva et al. 2015), accounting for 42.16 % of total species (Rajpar & Zakaria 2011). They are found resting on the banks, around lakes and water rice fields during the day (Baral et al. 2018). This species often builds nests in freshwater areas with dense vegetation to facilitate access to water and provide concealment and protection from predators for the young birds (Aarif & Babu 2010).

### Lesser Whistling-Duck's behaviors in Ramsar Lang Sen site

#### Activity and flock behavior

Lesser Whistling-Duck in Lang Sen Natural Reserve is relatively sensitive to environmental impacts. During the 12 months of survey, most Lesser Whis-

ling-Duck was recorded flying up and flying to another area, or hiding in the grass, lotus field and shrub, and were recorded the Lesser Whistling-Duck foraging, swimming or resting in open water and grass habitats in the morning with 11 times. Because these birds mainly feed at night (De Silva et al. 2015; Baral et al. 2018); while they mainly preen, swim and flap their wings during the day (De Silva et al. 2015).

Lesser Whistling-Duck's responses to environmental influences depend on the number of individuals in the flocks. When seeing danger, the Lesser Whistling-Duck in large flocks often stops feeding and continuously observes surrounding and then they usually fly away, however in small flocks (1-8 individuals/flock), they often fly up or swim to hide (Table 4). Therefore, Lesser Whistling-Duck preferred to rest, swim, and feed in open water with grass, shrubs, and emergent plants around.

The survey results showed that the ability to detect the environmental impacts of Lesser Whistling-Duck also depends on the number of individuals in the flocks. In the large flocks, Lesser Whistling-Duck can detect humans at a distance of about 300 m. The results of Pearson correlation analysis showed that the number of individuals in the flocks had a very strong positive correlation with the distance of detecting human impact and flying away with a correlation coefficient  $r$  of 0.91 ( $p < 0.01$ ). The reason may be that in large flocks, the members of the flock always take looking around when they are eating or resting, so they can detect threats easily and faster.

The results of the 12-month survey showed that the Lesser Whistling-Duck were mostly catching up from June to August, and their parents were looking for food with young birds in sub-zone 5 and sub-zone 10 from August to September. However, no nests were found except for one old nest (without eggs and juveniles) in the hollow of a melaleuca tree. According to the annual bird monitoring results in the Ramsar Lang Sen reserve, the natural breeding season of the Lesser Whistling-Duck mainly occurs from June to August. The number of their nests surveyed in the reserve has decreased in recent years. This may be due to a significant decrease in the number of Lesser Whistling-Duck in the reserve. The population of the Lesser Whistling-Duck may have been impacted by competitors, predators, and human activity, leading them to nest in more secluded areas far from their food sources.

#### Feeding behavior and food sources

The study also recorded that Lesser Whistling-Duck produces a wheezy sound while flying up, flying or feeding (Table 5), therefore they are called whistling duck. The behaviors of this waterbird in this study are similar to the study of Shaheer Ansari et al. (2017). The majority of Lesser Whistling-Duck makes vocalizations when flying up (54.5 % of times survey) and this behavior mainly occurs when they are active in pairs or small flocks of 1-8 individuals. They also make sound when flying, swimming, and feeding with large flocks (7-28 individuals/flock). In the survey, no instances were found of individuals shouting when feeding in pairs or small groups of 1-6 individuals (Table 5). The reason may be that they want to share information about food sources and danger to other individuals in flocks when operating in large herds, and silent to avoid threats from predators when foraging in small flocks.

Lesser Whistling-Duck is an omnivorous species, in Lang Sen natural reserve they feed in water habitats such as open swamps, water surfaces, floods, grass, and shrublands. Their food is mainly free-floating aquatic plants especially duckweed (*Lemna minor*), water lettuce (*Pistia stratiotes*), *Azolla pinnata*, *Wolffia schleidenii*, giant duckweed (*Spirodela polyrrhiza*), *Salvinia cucullata*; young shoots of water lily (*Nymphaea pubescens*, *Nymphaea indicum*), water hyacinth (*Eichhornia crassipes*), and young shoot and seed of emergent aquatic

**Table 3.** Distribution of Lesser Whistling-Duck in the studied habitats.

Habitat characteristics	Lesser Whistling-Duck's activities	Lesser Whistling-Duck (individuals)	Frequencies of Lesser Whistling-Duck (%)
Melaleuca forest, <i>Eleocharis dulcis</i> field	Fly across the survey area	112	43.3
Open water surface, lotus, <i>Eleocharis dulcis</i> field	Fly up	61	27.0
Open water surface with duckweed, dusty grass, <i>Eleocharis dulcis</i> , scattered melaleuca	Swim, stand, and rest	8	10.8
Open water surface, lotus, wild rice field	Feed	86	16.2
Grassland	Land and hide	2	2.7

**Table 4.** Reaction of Lesser Whistling-Duck when affected by humans and changes in environmental factors.

Lesser Whistling-Duck's activities	Size of flock (individuals)	Total Number of Lesser Whistling-Duck (individuals)	Frequency of total individuals (%)
Fly up	1-8	25	50.0
Swim, hide	2-8	12	21.4
Whistling, swimming, looking for a place to hide	2-34	36	14.3
Silence, constantly observing your surroundings	3-57	60	14.3

**Table 5.** Lesser Whistling-Duck made a wheezy sound.

Activities when made a wheezy sound	Size of flock (Individuals)	Total of Lesser Whistling-Duck (Individuals)	Ratio (%)
Flying up	1-8	19	54.5
Flying	2-28	37	27.3
Swim and feed	34-57	91	18.2

plants consist of lotus (*Nelumbium nucifera*), wild rice (*Oryza rufipogon*), *Hymenachne acutigluma*, *Pseudoraphis brunoniana*, *Eleocharis dulcis*, *Panicum repens*, and *Ischaemum hirtum*. The animal food sources include aquatic invertebrates such as mollusks (snails), worms, crustaceans (small shrimp), and aquatic vertebrates such as small fish and insects (Table 6). The number of Lesser Whistling-Duck had a positive correlation between species and genus diversity of aquatic plants with person correlation coefficients of 0.584 and 0.591, respectively ( $p < 0.05$ ). However, the high density of emergent plants and invasive floating macrophytes (water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*)), affected the free water surface for these waterbird activities (swim, feed), so it also limited the appearance of the waterbird populations. In addition, the rapid decline of the Lesser Whistling-Duck population is also one of the reasons leading to the increase in density and area of two invasive aquatic plant species: water lettuce and water hyacinth in these areas. This expansion is particularly noticeable in the open water surface in sub-zones 9 and 10 of the Ramsar Lang Sen site. This highlights the crucial role of protecting the brown leek population in preserving biodiversity and maintaining the ecological balance of the reserve.



**Table 6.** Food sources of Lesser Whistling-Duck in Lang Sen Ramsar site.

Lesser Whistling-Duck's food at Lang Sen	Size of flock (individuals)	Ratio of Lesser Whistling-Duck /total number of Lesser Whistling-Duck foraging surveyed (%)
Water lettuce	1	0.7
Water lettuce, snail	10	7.2
Water lettuce, wild rice, snail	3	2.2
<i>Lemna minor</i> , water lettuce, <i>Panicum repens</i> , small fish, small shrimp, insect	2	1.4
<i>Salvinia cucullata</i> , seed of lotus, <i>Ischaemum hirtum</i> , water lily, small fish, Small shrimp	34	24.6
<i>Lemna minor</i>	5	3.6
<i>Lemna minor</i> , <i>Wolffia schleidenii</i> , water lettuce, snail	59	42.8
<i>Lemna minor</i> , snail	8	5.8
Small fish	2	1.4
Small fish, small shrimp	7	5.1
Small fish, snail	7	5.1
Total	138	100

### Threats to the Lesser Whistling-Duck in Lang Sen Ramsar Site

In addition, environmental factors such as weather, food sources, and habitat structure, Lesser Whistling-Duck in Lang Sen natural reserve was also affected by carnivores such as black kite (*Milvus migrans*) and python (*Python molurus*), greater coucal (*Centropus sinensis*), lesser coucal (*Centropus bengalensis*), some snakes species, which can hunt and eat young and adult birds and their eggs. Moreover, Lesser Whistling-Duck is also competed for habitat and food with waterbirds such as Indian spot-billed duck (*Anas poecilorhyncha*), little egret (*Egretta garzetta*), little cormorant (*Phalacrocorax niger*), Asian openbill (*Anastomus oscitans*), western swamphen (*Porphyrio porphyrio*), oriental darter (*Anhinga melanogaster*), and pheasant-tailed jacana (*Hydrophasianus chirurgus*). Their populations in Lang Sen are large and active in flocks, making noises and competing for food with the Lesser Whistling-Duck. The Lesser Whistling-Duck often flies away or hides in shrubs and grass. The Lesser Whistling-Duck in Lang Sen Natural Reserve is also very sensitive to human impacts such as noise from travel, tourism, agricultural production (pesticides, equipment) and illegal hunting of people around the reserve. Therefore, it is essential to implement solutions to safeguard the environment and minimize the impact on the Lesser Whistling-Duck population at Ramsar Lang Sen.

There was no evidence found of residents encroaching on the conservation area to hunt *D. javanica*. However, nearby residents are using devices that mimic the calls of *D. javanica* to lure them into the paddy fields and then capture them using nets. This hunting activity typically takes place in the first month of the rice crop. This may be one of the main reasons leading to the rapid decline of the Lesser Whistling-Duck population in the Lang Sen Ramsar site in recent times. Therefore, there is a need for educational solutions to raise awareness among the local community about the conservation of this waterbird species.

### CONCLUSION

In the study, 269 individuals of Lesser Whistling-Duck (*Dendrocygna javanica*) were recorded at Lang Sen Ramsar Site with mainly in pairs and small flocks. Most Lesser Whistling-Duck appeared in sub-zone 9 (102 individuals) and in February (93 individuals) mainly at light sunshine of the day. They often swim, feed and rest in open water surrounding lotus, grass, shrubs or scattered melaleuca where support food source and shelter. Their food source of Lesser Whistling-Duck in the reserve includes plants and animals. This bi-

rd is quite sensitive to environmental influences, their ability to perceive, reflectivity and make sound depends on the size of the flocks. Their carnivores are black kite, greater coucal, lesser coucal, and python. They were also affected by competition for food and habitat of other waterbirds, activities of tourists and people around the reserve. Therefore, protecting the living environment and limiting harmful species and human activities should be paid more attention.

#### AUTHOR CONTRIBUTION

L.D.K. designed the research and supervised all the process, analysed the data and wrote the manuscript; P.Q.N. collected and analysed the data and wrote the manuscript; N.T.L collected the data; N.T.G. collected and analysed the data.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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