Diptera assemblages in avian nest-boxes

PhD Thesis

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„Aquila non captat muscas.”

1. Introduction

It is well-known, that many insects live in the nests of birds and mammals - but it is less well-known, that the vast majority of these insects are actually dipterans. These populations are most diverse as regards their lifestyle. The majority of them both as imagoes and larvae are saprophagus, and these insects spend the longer period of their imago life outside the nests.

The larvae of some species are predators of the other larvae in the nest (*Muscina*), and some species instead of egg immediately lay third phase larvae, which can pupate within a short time (Hippoboscidae), and hatching imagoes suck the blood of the birds. Few are aware that the larvae of some fly species are bloodsucking parasites of nestlings birds (*Protocalliphora*). There are also fly species that have saprophagous larvae, but their imagoes are bloodsuckers (*Carnus*). Populations of another important group of species live outside the nests as larva, but their as imagoes are bloodsuckers of birds. These latter species represent serious threat by the transmission of viruses and other microbes (protozoan parasites).

The bird nests provide very diverse habitat due to their location, size, plant or other composition. The term "bird nest", although trivial, cannot therefore be regarded as a uniform habitat in an ecological sense. Probably the varied lifestyle of dipterans explains that our knowledge of those dipterans that live in bird nests is insufficient, despite the relatively extensive research. Because of the deficiencies of such knowledge, complex and thorough researches are required in this field with not little significance from practical point of view.

In order to create a somewhat comprehensive picture of the dipterans which live in bird nests, three comprehensive publications of HICKS (1959, 1962, 1971) were used, summarizing the findings of arthropods related to birds up to 1970. To search for publications since 1971, we used the online database of Zoological Record to find relevant articles. Keywords were: (Diptera or Ceratopogonidae or Culicidae or Muscidae or Fanniidae or Carnidae or Mosquitoe*) and (bird* or avian* or aves). We found Forty-nine Diptera families in the literature (383 species and 108 non-species-identified taxa) that live in bird nests.

In Hungary, the following bird pathogens were spread with dipterans: West Nile Virus, Usutu virus, *Pox* virus, *Papilloma* virus, *Plasmodium*, *Haemoproteus* and

Leucocytozoon. From these pathogens, the West Nile Virus is the most significant for a human health aspect.

2. Aims

The purpose of our studies is the qualitative and quantitative examination of dipterans which live in bird nests. The flies of birds were difficult to study due to the birds' flying lifestyles. The only exception is the time of the breeding season when these animals are partially fixed. In our studies, flies and mosquitoes were sampled during the breeding season and from the nesting material remaining after the birds leave the nest.

2.1. Development and testing of a sampling method optimal for dipterological sampling during breeding season of birds

For sampling during the breeding season, there was a need to find a method that does not interfere with the birds, is easy to handle, and does not affect the identification of fragile dipterans. In the literature we found an idea of (TOMÁS *et al.* 2008) to apply Johnson & Johnson Baby oil on plastic sheets which looks promising to use as a sticky plate.

2.2. Investigating dipterans in the nest during the breeding season

For singing birds and Red-footed Falcons we wanted to know

- what kind of Diptera species visited the birds during the breeding season,
- what was the abundance of the parasites,
- whether there was a pattern according to the stage of the breeding and the number of chicks.

2.3. Examination of Diptera in artificial nest boxes

We were looking for the answer to the following questions regarding the Red-footed Falcon's nest box:

- What kind of Diptera species develop in artificial nest boxes?
- Are there any parasitic flies?
- Is there any synchronization between the emergence of the flies and the hatching of the nestling, in a colony where 4 bird species use the nest-boxes?
- What parameters influence the abundance of the Diptera species which develop in the nest boxes?

2.4. Study on West Nile Virus

We were looking for the answer to the following questions regarding the West Nile Virus:

- What kind of mosquitoes carry the virus in Hungary?
- Is the virus found in the Red-footed Falcon - mosquito system examined by us?

3. Materials & methods

3.1. Rearing imagoes of Diptera from avian nest material

The nest materials were collected for the Red-footed Falcon's colony in Borsodi-Mezőség, and stored in 10 × 30 cm linen sacks. The sacks were placed in crates and the positions of the sacks within the box were changed every two weeks (to eliminate the effects of microclimate deviations). We overwintered the nest substances in a cellar (to ensure stable temperature and similar overwintering probability), and sprayed with water to avoid their drying out. For the simple picking of the hatching flies, a fish pot made of a translucent PET bottle was inserted into the mouth of the sack. The bottle was cut in half and top turned inwards (towards the bottom of the bottle with the screwed end, forming a kind of trap). The hatching dipterans move in the direction of light, - in the direction of the transparent plastic bottle - so they are trapped in the bottle. The hatching insects were collected every 14–15 days. The overslept with chloroform and preserved with paradichlorobenzene until identification. To protect the birds, we collected the nest material only after the nestlings were already flown out in September. We collected the nest material in 2009 from 57, and in 2010 from 17 nest boxes. The nesting material collected in 2009 had accumulated for 3 years while in 2010 only in the course of 1 year.

3.2. "Gel trap"

In dipterological studies, the fundamental problem is the identifiability of the collected individuals. A method had to be found that samples dipteran parasites of hatching birds with minumum disturbance to the birds. The idea of using

Johnson & Johnson Baby Oil Gel is based on TOMÁS *et al.* (2008). The material is suitable for dipterological studies because it is relatively fast and well soluble in benzine and the dipterans remain intact. We had modified the method of TOMÁS. They either put the gel in a Petri dish or in another examination on a piece of water-resistant paper which was reinforced with a wire mesh and fixed to the top of the nest-box of the singing birds. Because of the wire mesh it was not possible for the birds to reach the gel.

Initially, we applied 5 ml gel adhesive (Johnson's Baby Oil Gel with Chamomille; Johnson & Johnson, Dusseldorf, Germany) on one side of 10×15 cm transparent (0.2 mm) plastic sheets. We then secured these sheets (with the gel facing upwards) on the inner side of the nest-boxes' roofs for 24 hours.

The baby oil gel traps were tested in two ways. On one hand, they were used to test whether hematophagous vectors are attracted to either the nest-box or the adhesive gel alone (56 samples collected) by comparing the results obtained here with that in active nests. On the other hand in 2010, we tested the capturing efficiency of the method near Kardoskút and in the Pilis Mts. We tested the gel in 9 Red-footed Falcons' and 5 Collared Flycatchers' nest boxes: 10 × 15 cm plastic sheet was placed with the baby oil gel on one side and a non-drying glue on the other (latter is commonly used in agricultural tests). We compared the number of caught individuals in two different materials in pairs. The gel traps - in order to disturb the birds as little as possible - were pre-prepared and carried in a closed box to the nests. After trapping the sticky plates were transported and stored in the same closed box until processing. After the gel was dissolved in benzine, all collected animals were stored in 70% ethanol.

In the Pilis in 2011, we sampled the Great tit (28 nests, 18 days) and the Collared Flycatcher (31 nests, 18 days) nest boxes during the breeding. In Kardoskút, sampling was carried out in three consecutive breeding seasons (2010: 37; 2011: 107; 2012: 165).

3.3.Virological studies

In 2011–2012 we participated in a country-wide West Nile Virus (WNV) monitoring program, we tested the mosquitoes for West Nile virus. During the analysis, we tested 23227 individuals of 30 mosquito species from 24 area of six counties.

WNV prevalence has been shown to cumulate in vertebrate hosts late in the summer (HAMER *et al.* 2008), therefore in order to minimize laboratory costs and

to maximize the probability of detecting WNV presence we only used mosquitoes (from the nest boxes) trapped in the late breeding stage in 2011. A total of 779 mosquitoes of three species (*Culex pipiens*, *Culex modestus* and *Coquillettidia richiardii*) were investigated.

We used 42 broods that were also selected for mosquito abundance sampling to assess WNV sero-prevalence in the studied population. Blood samples of 0.8–1 ml were taken by basilic venipuncture (*vena cutanea ulnaris*) from fledglings (n=139) reaching the second half of the breeding stage (17–24 days) in 2011. Blood samples were also tested for Western Nile virus and its' antibody.

The countrywide mosquito sample were tested for Western Nile Virus with RT-RT-PCR (Reverse Transcription Real-Time PCR) as described by BAKONYI *et al.* (2013), mosquitoes from nest of Red-footed Falcon and the falcons' nestling were tested with RT-PCR (Reverse Transcription PCR) as described by ERDÉLYI *et al.* (2007). Serum samples were tested for the presence of anti WNV antibodies using the ID Screen® West Nile Competition ELISA kit (ID VET, Montpellier, France) according to the manufacturers' instructions.

4. Results

- Methodological
  - We found a feasible and efficient method for sampling dipterans in nest boxes of Red-footed Falcon, furthermore, the collected material is suitable for virological examination.
- Ecological
  - The nests of Red-footed Falcon were unknown hereto in terms of dipterological aspect. We found 6 species of Diptera that are new for bird nests and 13 species new from Red-footed Falcon. The most abundant species was *Carnus hemapterus.*
  - We investigated a nest box colony, which is a breeding place for four bird species, where the nest boxes have not been cleaned for three years, and we found that only the last breeding bird species had a significant effect on the number of *C. hemapterus.* The number of *C. hemapterus* was significantly higher in nests where the last breeding bird species was Common Kestrel and not Red-footed Falcon.

- We also observed a supposed intra-nest synchronization. In those nests where two different bird species bred in the former year, a part of the *C. hemapterus* specimens were synchronized to the Jackdaws while the others were synchronised to the Common Kestrels/Red-footed Falcons, which bred remarkably later.
- Brood size had a positive effect on the number of attracted *Culex pipiens* and *Culex modestus* individuals
- Age of nestlings had a positive effect on the number of attracted *Culex pipiens* individuals. Blood feeding success rate of both dominant *Culex* species (*Culex pipiens* and *Culex modestus*) was largest of the first 14 days of the age of nestlings.

- Virological
  - We examined the presence of the West Nile virus in mosquitoes, during a national sampling scheme. We have detected the pathogen in tree mosquito species (*Culex pipiens, Coquillettidia richiardii, Ochlerotatus annulipes*).
  - We have detected the RNA of the WNV (Lineage 2) in *Cx. pipiens* that were caught in the nestbox of Red-footed Falcon.
  - We found significant negative correlation between the age of the nestlings and the WNV antibody level, which refers to maternal origin. The antibody level is higher in younger nestlings, when due to less developed plumage, the mosquitoes are more successful.
- Faunistical
  - In the Pilis we found a mosquito (Culicidae) which was new to the Hungarian fauna: *Aedes geminus* Peus, 1970.
  - In the Borsodi-Mezőség from the nest boxes of Red-footed Falcon we found a Fanniidae species which was new to the Hungarian fauna: *Fannia lineata* (Stein, 1895)
  - We found 5 Ceratopogonidae species that were new to the Hungarian fauna: from the nest boxes of Red-footed Falcon: *Leptoconops bidentatus* Gutsevich, 1960, *Culicoides alazanicus* Dzhafarov, 1961, *Culicoides manchuriensis* (Tokunaga, 1941), *Culicoides duddingstoni* Kettle & Lawson, 1955; from the nest boxes of Collared Flycatcher: *Culicoides truncorum* Edwards, 1939.

5. Summary

In our study two sampling methods were used to investigate the dipterans of bird nests. Altogether we identified 58427 dipteran individuals to species level collected in the artificial nest boxes, and we based our conclusions on this collection.

In one of these methods, we collected the residual nest material, after the fledglings left the nest, from nest boxes of Red-footed falcons, and the emerging imagoes were identified to species. The nests of these bird species were hitherto unknown from dipterological point of view. We found 6 species of Diptera that are new for bird nests and 13 species new to the Red-footed Falcon. The most abundant species was *Carnus hemapterus*, the larvae of this species are saprophagous, but the imagoes suck the blood of fledglings. We investigated a nest box colony, which is a breeding place for four bird species, where the nest boxes have not been cleaned for three years, and we found that only the last breeding bird species had a significant effect on the number of *C. hemapterus.* The number of *C. hemapterus* was significantly higher in nests where the last breeding bird species was Common Kestrel and not Red-footed Falcon. We also observed a supposed intra-nest synchronization. In those nests where two different bird species bred in the former year, a part of the *C. hemapterus* specimens were synchronized to the Jackdaws while the others were synchronized to the Common Kestrels/Red-footed Falcons, which bred remarkably later. Although our study covered only two years, we captured one order of magnitude lower number of *C. hemapterus* specimens in freshly cleaned nest boxes suggesting the necessity of cleaning the boxes after breeding.

The other sampling method we used was the sticky gel trap, which is a modified version of the trap described by Tomás *et al.* (2008). We tested this trap and we found it suitable for the sampling of fragile dipterans, furthermore, the collected material is also suitable for virological examination. We used this gel trap in the artificial nest boxes of song-birds and Red-footed Falcon. However, due to the small number of Diptera individuals (from the nests of the songbirds) we could not statistically evaluate the results but with this special collection method we found a new species for the Hungarian fauna. During the sampling in the nest of Red-footed Falcons, we found surprisingly high numbers of mosquitoes. Brood size and the age of nestlings had a positive effect on the number of attracted

Culex pipiens individuals, while blood feeding success rate of both dominant *Culex* species (*Culex pipiens* and *Culex modestus*) markedly decreased after the nestlings reached 14 days of age. Based on the results, it seems that at the younger Red-footed Falcon nestlings (with less-developed plumage) the probability of infection by vector borne pathogens is higher.

We examined West Nile virus (WNV) in Hungary cooperating with virologists. We examined the presence of the virus in mosquitoes, during a national sampling scheme. We have detected the pathogen in three mosquito species (*Culex pipiens, Coquillettidia richiardii, Ochlerotatus annulipes*). We also have tested the Red-footed Falcon's fledglings and their mosquito vectors for WNV. Within this study we have comprehensively investigated vector attraction patterns, blood feeding success rate, WNV prevalence and host serum sero-prevalence under natural conditions in a colonial raptor. We have detected the RNA of the WNV in *Cx. pipiens* (that were caught in the nest), and the antibody in the serum of the fledglings. The origin of the antibodies could be the sign of a previous infection. However, virus RNA was not present in the fledglings. Thus, possibly the nestlings were infected soon after hatching and/or the duration of detectable viremia was remarkably short. This scenario is not very likely because the length of the viremia in related falcons lasts over several weeks (ZIEGLER 2013). More probable is that the majority, if not all, seropositive nestlings had maternally derived antibodies against WNV. The literature results show that the time-scale of maternally derived antibody decay can be measured in days; however, antibody decrease in free ranging birds with active immune response is detectable over months. Based on the ELISA OD ratios, it is concluded that younger fledglings have more antibodies, while less in the older ones, and the Ig level negatively correlates with the hatching order in a nest. Therefore, according to our analysis, the antibodies in Red-footed Falcon fledglings are likely to be maternal.

With the relatively new gel trap method, we found six Diptera species which were new to the Hungarian fauna, while in the nest material an additional species was recorded firstly from Hungary.

6. Publications in connection with the thesis

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