#### SHORT REPORT

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# Blowfly *Trypocalliphora* braueri larvae infestation in Willow Warbler *Phylloscopus* trochilus nestlings on the Kola Peninsula, Russia

Larver av spyflugan Trypocalliphora braueri angriper lövsångarungar Phylloscopus trochilus på Kolahalvön, Ryssland

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**THE BLOWFLY** *Trypocalliphora braueri* is a subcutaneous parasite infesting nestlings. We investigated the occurrence of blowfly larvae in Willow Warbler *Phylloscopus trochilus* nestlings on the Kola Peninsula, Russia, in 2023–2024. The infestation rate was 46.9% of nests and 37.9% of chicks. The highest infestation rates were observed during a season with a late start of the breeding season and an unusually warm summer. In the infested nests, 28.6–100% of nestlings (average 86.2%) contained 1–11 larvae (average 3.4). Chicks with many larvae or with vital organ damage died. Nestling survival in the infested nests decreased by 15.4% compared to that in uninfested nests, but these differences were not statistically significant. We also found no significant differences in infestation rates between areas with varying levels of anthropogenic pressure.

Keywords: birds | breeding | subcutaneous parasite | nestling survival | wildlife disease

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## Introduction

Parasites that infect nestlings can cause weakness, injuries, or even mortality, which can affect survival at both individual and population levels (Warner 1968, Åkesson et al. 2002). In this paper, we report on infestations of the blowfly Trypocalliphora braueri (Diptera: Calliphoridae) in Willow Warbler Phylloscopus trochilus nestlings. The blowfly T. braueri is a parasite that primarily infests nestlings of ground-breeding passerines. This species is unique to the Palaearctic. Its larvae are subcutaneous parasites that penetrate the birds' skin and burrow into the flesh. The larvae develop inside encapsulations that can measure up to 12 mm deep and 4.1 mm wide on various parts of the nestlings' bodies. Mature larvae leave the host at death or fledging stage through a warble-like opening under the nestling's skin and pupate in or near the nest on the ground (Rognes 1991, Gaponov & Truphanova 1995, Whitworth 2012).

The Willow Warbler is a small passerine bird weighing approximately 9–10 g (in the study region, on average 9.79 g, n = 48). Over two years, we found 39 nests, 32 of which were not predated before the nest-lings hatched. In the study region, Willow Warbler breeding began in late May to mid-June (first egg laid: 28 May–15 June) in 2023 and only in June (predominantly 5–12 June, with the latest nest dated 27 June) in 2024, a season characterized by a cold, late spring but an unusually warm and dry summer. The nests of Willow Warblers are dome-shaped structures with a side entrance, built by females primarily on the ground. Their average clutch size was 6.3 eggs (4–7 eggs) and females incubated the eggs for 13 days.

Although *T. braueri* is considered a common parasite in some populations of Willow Warblers, the rate of infestation is usually quite low (Rognes 1991, Åkesson *et al.* 2002). However, during our study of the reproductive biology of Willow Warblers on the southern Kola Peninsula, north-western Russia, we observed an unexpectedly high infestation rate and a decreased nestling survival in 2024.

#### **STUDY AREAS**

In 2023 and 2024, during a study on the reproductive biology of Willow Warblers, we located their nests along the shore of the Gulf of Kandalaksha, White Sea  $(67^{\circ}10' \text{ N}, 32^{\circ}69' \text{ E}, \text{ southern Kola Peninsula, north-western})$ 

Russia). Half of the study plots were situated near the Luvenga outpost of the Kandalaksha Nature Reserve in humid mixed deciduous-coniferous forests with European Blueberry Vaccinium myrtillus undergrowth, with minimal human impact. The remaining plots were located in fragmented forest habitats near Luvenga village, including abandoned sites with destroyed buildings and sites containing domestic waste. These areas had a higher level of anthropogenic impact. They are frequented by people more often than those within the reserve. Some of these sites are located along the seashore, where people engage in recreational activities such as camping. Others are situated near the village, where residents sometimes walk their dogs or collect berries and mushrooms. We inspected 16 nests in plots with low anthropogenic impact and 16 nests in plots with high anthropogenic impact. All study plots were within a 3-km<sup>2</sup> area.

## Material and methods

We visually inspected all chicks for encapsulated larvae, which remain in them for only 3–5 days before pupating (Eastman *et al.* 1989, Howe 1992). These larvae were observed in 5–9-day-old nestlings. In our research, we have found and described the location of 208 *T. braueri* larvae over two years.

We counted the number of infested nestlings per nest and the number of larvae on each nestling. We also noted the larvae's locations on the nestlings' bodies and collected some larvae for identification in the laboratory. Some of the larvae had already exited the nestlings, leaving dark crater-like openings in the skin. Later, we checked the nests for dead nestlings. We assessed nestling survival by calculating the proportion of fledglings to hatchlings and compared this rate in infested and uninfested nests where the nestlings reached eight days of age and were not predated before fledging, using the Wilcoxon rank sum test with continuity correction.

It is worth noting that the weather conditions varied slightly between the study seasons. In 2023, the Luvenga region experienced a relatively warm season. During the second half of May, nighttime temperatures remained above freezing, occasionally reaching 12°C, while daytime temperatures fluctuated between 10 and 19°C. However, a cold spell occurred in late May and early June, coinciding with the initial breeding period of Willow Warblers. During this time, nighttime temperatures dropped to  $1-2^{\circ}$ C, and daytime temperatures ranged from 7–8°C. The first ten days of June remained cool, with daily temperatures varying between 3 and 11°C. By mid-June, the weather warmed significantly, with daytime temperatures rising to  $12-17^{\circ}$ C and occasionally peaking at 18–21°C. Early July, which marked the conclusion of the breeding period for most Willow Warblers, saw stable daytime temperatures of  $11-15^{\circ}$ C. Throughout the season, the region experienced predominantly overcast conditions with intermittent rainfall. The mean daily temperature (and day temperature) for May was 6.1°C (8.9°C), for June 10.4°C (13.7°C), and for July 13.0°C (16.4°C).

Spring 2024 was unusually cold, with nighttime frosts persisting until the final week of May. Only by the end of the month did temperatures consistently rise above 3°C. As a result, Willow Warblers postponed their nesting activities until early June, coinciding with a sharp increase in temperatures. Morning temperatures ranged between 9-11°C, rising to 18-20°C during the day and occasionally reaching 24-26°C - remarkably high for the region. This unusually warm and dry weather persisted until the end of June. Overcast days were rare, but during the latter part of June, daytime temperatures occasionally decreased to 11-17°C, accompanied by light rainfall. July continued to experience atypically warm conditions for the region, with nighttime temperatures remaining above 9°C and daytime temperatures fluctuating between 14-23°C. Considering significant daily temperature fluctuations, the mean daily temperature (and day temperature) for May 2024 was 4.0°C (7.3°C), for June 12.7°C (16.3°C), and for July 14.8°C (18.5°C). Overall, the 2024 season was characterized by a prolonged cold spring, followed by an exceptionally warm and dry summer.

## **Results and discussion**

In total, we recorded *T. braueri* larvae in six out of 15 nests (25 out of 93 nestlings) in 2023 and in nine of 17 nests (47 out of 97 nestlings) in 2024 (Table 1). In infested nests, on average 86.2% of nestlings were infested (range: 28.6–100%), and the average number of larvae per nest was 15.9 (range: 1–37). The overall infestation rate for the two years was 46.9% of nests and 37.9% of nestlings (Table 1). These infestation rates

rABLE 1.   - Detaljer	<sup>&gt;</sup> arameters of blowfly <i>Try</i> , kring angreppen av spyflu	oocalliphora brauer. Igelarver av arten Ti	r larvae infestation in Will rypocalliphora braueri på	low Warblers Phylloscopu Jövsångarungar Phyllosc	<i>us trochilus</i> nestlings on the copus trochilus <i>på södra Kol</i> s	southern Kola Peninsul ahalvön i nordvästra Ry.	la, north-western Russi ss <i>land.</i>	ġ.
			% nests with c % bon med d	dead nestlings <i>löda boungar</i>			% dödu	l nestlings a boungar
	-	% infested	-	of all inspected	N inspected	% infested	of infested	of all inspected
Voor år	N inspected nests	nests %	of infested nests	nests av alla	nestings N	nestlings %	nestlings av	nestlings av alla
Year Ar	N UNDELSOKTA DON	angripna pon	av angripna pon	undersokta pon	undersokta boungar	angripna poungar	angripna poungar	unaersokta poungar
2023	15	40.0	0	0	93	26.9	0	0
2024	17	52.9	44.4	23.5	97	48.5	27.7	13.4
Total	32	46.9	26.7	12.5	190	37.9	18.1	6.8

were unexpectedly high. The average rate of Willow Warbler infestation revealed during previous studies on the south of the Kola Peninsula was only 17% of nests (Shutova 1997). During 15 years of studying the breeding biology of Willow Warblers in the neighbouring region of Karelia, north-western Russia, we observed only a few cases of infestation by *T. braueri* (our unpublished data). In Sweden, Åkesson *et al.* (2002) reported that 14% of Willow Warbler nests and 9.6% of nestlings were infested, and earlier research in that country found only a few rare cases of such infestation.

In 2024, infestation was especially high (Table 1). This year was characterized by a late, cold spring, which delayed and synchronized the beginning of breeding. However, the following summer was unusually hot and dry, with almost no rainfall in June and the first half of July. Previous studies (Pavel *et al.* 2008, Timoshkin 2019) revealed that infestation rates tend to be higher in warmer summers. Some researchers also concluded that later broods were more often infested with these parasites (Prokofieva 2000, Timoshkin 2019). Our results also support the finding of Åkesson *et al.* (2002) that the infestation rate of Willow Warblers is geographically restricted, but outbreaks may occur during specific seasons.

We did not find significant differences in infestation rates between nests in plots with low (50%, 8 infested nests of 16) and high anthropogenic impact (44%, 7 infested nests of 16). For other bird species, Timoshkin (2019) showed higher infestation rates in anthropogenic landscapes. In our case, the study plots of different types may have been too close to each other to provide more accurate estimates.

We observed between one and eleven larvae per infested nestling, with an average of 3.4 larvae per nestling (n=63). These data correspond to Shutova (1997) from this region, which reported an average of 4.4 larvae per nestling (range: 1–14). Most larvae (65.4%, 136 larvae from 208 observed) were found in the wings of infested nestlings. Larvae also encapsulated in the nestlings' legs, heads, and backs, with fewer larvae found in the throat, belly, neck, and sides of the body (Appendix 1). Tissue surrounding the encapsulated larvae was swollen (Figure 1), and in some cases, there were deformations of wings, legs, and bills, as well as damages to eyes, ears, and even brains.

In 2023, no dead nestlings were found in the infested nests. However, in 2024, 27.7% of infested chicks (commonly, with several larvae or with vital organ damage) died before fledging (Table 1, Appendix 2). Overall, nestling survival in the infested nests decreased by 15.4% compared to uninfested nests, but the difference was not statistically significant (Wilcoxon rank sum test with continuity correction:  $W_{12,12}=65$ , p=0.079). Nevertheless, the figure aligns with the decrease in survival (14–18%) of ground-breeding birds on the



**FIGURE 1.** Nestlings of the Willow Warbler *Phylloscopus trochilus* at four days of age (left) and seven days of age (right), infested with larvae of the blowfly *Trypocalliphora braueri* on their wings and legs. Southern Kola Peninsula, north-western Russia, 2024. Photo: Sergey Simonov. — *Boungar av lövsångare* Phylloscopus trochilus vid fyra (vänster) och sju dagars ålder (höger) angripna av spyflugan Trypocalliphora braueri på vingar och ben. Södra Kolahalvön, nordvästra Ryssland, 2024. Foto: Sergey Simonov.

southern Kola Peninsula in seasons with high infestation rates, which exceeds the regional average of 4% (Shutova 1997).

Infestations by blowfly larvae can cause blood loss, leading to anaemia, and damage to muscle tissue and organs, as observed in our study. Damages of muscle tissue and organs like we observed are also common. Severe infestations may result in death or leave nestlings weak and vulnerable to predators (Åkesson et al. 2002, Pavel et al. 2008, Whitworth 2012, Gaponov & Tewelde 2019). In some cases, larvae cause bacterial subcutaneous infections (Warren 1994, Matsuoka et al. 1997). Nestlings infested early after hatching are particularly susceptible (Eastman et al. 1989, Prokofieva 2000). In a previous study on the Kola Peninsula, it was noted that the presence of eight or more larvae in a single Willow Warbler nestling often led to its death (Elena Shutova, pers. comm.). However, in our study, we also observed cases where nestlings died from fewer larvae but suffered severe injuries, especially in the head and belly areas.

In the study region, T. braueri also parasitizes other ground-breeding birds, such as pipits Anthus spp. and wagtails Motacilla spp. However, its larvae are occasionally observed in other species, including thrushes Turdus spp., sparrows Passer spp., Common Redstart Phoenicurus phoenicurus, Northern Wheatear Oenanthe oenanthe, Spotted Flycatcher Muscicapa striata, European Pied Flycatcher Ficedula hypoleuca, Great Tit Parus major, and Grey-Headed Chickadees Poecile cinctus (reviewed by Shutova 1986, 1997). Even at a low average level of infestation, about one third of nestlings infested with T. braueri larvae die, while surviving nestlings may suffer severe damage that could reduce their future survival prospects (Shutova 1986, 1997). Streby et al. (2009) further revealed that while T. braueri may have no significant impact on nestling health and survival during the nestling stage, it negatively affects fledgling survival after they leave the nest. Therefore, although we observed a limited effect on nestling survival, particularly in 2023, this does not necessarily indicate a minor impact on juvenile survival post-fledging. It is reasonable to conclude that even when the immediate effects on nestlings appear minimal, blowflies can significantly influence bird population dynamics by reducing recruitment rates.

Thus, during our study on the southern Kola Peninsula, we identified an unexpectedly high infestation rate of Willow Warbler nestlings by T. braueri larvae, particularly in the season characterized by delayed breeding and warmer, drier summer conditions. At present, no significant differences in infestation rates between areas with varying degrees of anthropogenic pressure have been observed. The number of larvae per nestling varied substantially. A high number of larvae or infestation of vital organs led to the mortality of some nestlings. Additionally, some nestlings that did not die in the nests appeared weakened or exhibited injuries that could potentially reduce their future survival. Although the current findings did not reveal a significant impact of the studied parasites on host survival, the high infestation levels of Willow Warbler nestlings on the Kola Peninsula raise serious concerns and warrant further investigation.

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### Svensk sammanfattning

I den här studien undersökte vi angrepp av larver av spyflugan *Trypocalliphora braueri* på lövsångare *Phylloscopus trochilus* som häckar på Kolahalvön i nordvästra Ryssland, under häckningssäsongerna 2023 och 2024. Larverna hos denna spyfluga är subkutana parasiter, vars angrepp på fågelungar potentiellt leder till en rad hälsoproblem, inklusive skador, svaghet och till och med döden. Vår forskning syftade till att utvärdera omfattningen av angrepp, dess effekter på ungöverlevnad och hur antropogena faktorer potentiellt inverkar på angreppsfrekvensen.

Under studien fann vi att spyflugelarver angrep 46,9% av bon och 37,9% av ungarna under de två åren (tabell 1). De högsta angreppsfrekvenserna observerades 2024, som kännetecknades av en ovanligt kall vår följt av en varm och torr sommar. Totalt hade 86,2% av ungarna i angripna bon mellan en och elva larver, med ett genomsnitt på 3,4 larver per unge. I fall av allvarliga angrepp dog några ungar, särskilt de med betydande organskador.

Larver hittades mestadels i vingarna på angripna ungar (65,4% av larverna), även om de även observerades i andra kroppsdelar, inklusive ben, huvud och rygg (appendix 1). Förekomsten av larver orsakade svullnad och deformationer i den omgivande vävnaden (figur 1), med vissa ungar som uppvisade skador på vingar, ben och till och med ögon och hjärna. Vissa boungar, särskilt de med stora angrepp eller allvarliga skador, dog innan de blev flygga (appendix 2), vilket resulterade i 15,4% minskning av häckningsöverlevnaden i angripna bon jämfört med icke angripna. Dessa skillnader var dock inte statistiskt signifikanta, vilket tyder på att även om det fanns en nedgång i överlevnad, kanske den inte är tillräckligt stor för att ha en stor omedelbar effekt på populationen.

Det är värt att notera att även om den direkta inverkan av spyflugelarver på ungöverlevnad var begränsad, kan det finnas bredare konsekvenser för populationsdynamiken. Angripna ungar som överlever de tidiga utvecklingsstadierna kan drabbas av långvariga konsekvenser, vilket kan påverka deras förmåga att överleva som flygga. Tidigare studier har visat att effekterna av sådana parasitangrepp kan sträcka sig bortom tiden i boet, vilket påverkar överlevnadsgraden hos flygga och potentiellt den övergripande rekryteringen av nya individer till populationen. Vi hittade inga signifikanta skillnader i mängden angrepps mellan områden med låga och höga nivåer av mänsklig påverkan, även om tidigare studier tyder på att antropogena landskap kan bidra till högre nivåer av flugangrepp hos vissa fågelarter. Angreppsfrekvensen som observerades i vår studie var oväntat hög, särskilt i jämförelse med tidigare rapporter i regionen, där angrepp var mycket sällsynta.

Sammanfattningsvis belyser den här studien den oväntat höga angreppsfrekvensen på lövsångare av spyflugelarver, särskilt under häckningssäsongen 2024, som kännetecknades av ovanliga väderförhållanden. Även om de omedelbara effekterna av spyflugeangrepp på boungeöverlevnad inte var statistiskt signifikanta är de potentiella långtidseffekterna på flygga ungars överlevnad och den totala populationsdynamiken fortfarande ett bekymmer. Ytterligare forskning behövs för att fullt ut förstå konsekvenserna av spyflugeangrepp för fågelpopulationer, särskilt i regioner med fluktuerande miljöförhållanden.



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## Appendices

APPENDIX 1. Number of Trypocalliphora braueri larvae in infested Willow Warbler Phylloscopus trochilus nestlings on the southern Kola Peninsula, north-western Russia, in 2023 and 2024.

— Antal larver av spyflugan Trypocalliphora braueri i angripna lövsångarungar Phylloscopus trochilus på södra Kolahalvön, nordvästra Ryssland, 2003 och 2024.

Body part Kroppsdel	Parameter	2023	2024	Total Totalt
	Median	1	1	1
Head Huvud	Range Variationsbredd	1–2	1–3	1–3
	Ν	4	12	16
	Median	2	1.5	2
Throat <i>Strupe</i>	Range Variationsbredd	1–6	1–2	1–6
	Ν	4	6	10
	Median	0	1	1
Belly Mage	Range Variationsbredd	0-0	1–3	1–3
	Ν	0	8	8
	Median	3	2	2
Back <i>Rygg</i>	Range Variationsbredd	2-4	1–2	1–4
	Ν	9	5	14
	Median	0	2	2
Flanks <i>Sidor</i>	Range Variationsbredd	0-0	2-2	2-2
	Ν	0	2	2
	Median	1	3	2
Wings <i>Vingar</i>	Range Variationsbredd	1–3	1–7	1–7
	Ν	32	104	136
	Median	1	1	1
Legs Ben	Range Variationsbredd	1–2	1-3	1–3
	Ν	5	17	22

APPENDIX 2. Characteristics of injuries documented in dead Willow Warbler *Phylloscopus trochilus* nestlings caused by *Trypocalliphora braueri* larvae on the southern Kola Peninsula, north-western Russia, in 2024.

<ul> <li>Skildring av</li> </ul>	v skador hos c	döda lövsångarur	<i>ngar</i> Phylloscopus	trochilus c	orsakade av	larver från	spyflugan	Trypocalliphora	braueri <i>på</i>	södra k	Colahalvön,
nordvästra Ry	ssland, 2024.										

Nest no. Bo #	Nestling no. <i>Unge #</i>	N larvae in nestling N larver i unge	Approx. age at infestation (days) Ungefärlig ålder (dagar) vid angrepp	Approx. age at death (days) Ungefärlig ålder (dagar) vid död	Description of injuries Beskrivning av skador (endast på engelska)
1	1	2	4	13	A large hole in the upper back caused by larvae penetrating the body cavity near each other and exiting from it. The nestling likely died after larval emergence due to the non-healing wound and/or infection.
	2	3	1	4	Two larvae in one wing and one in the other. The nestling was likely too small to survive the infestation.
2	3	3	1	3	Larvae penetrated deep into the head of the small nestling (two near the ears and one in the occipital region).
	4	2	5	10	In 7-day-old nestling, two larvae were observed in the wings; the nestling was found dead at 10 days. The exact cause of death is unknown, but all nestlings in this nest died. It is possible that the female abandoned the nest after the death of more heavily infested nestlings, which were too large to be removed from the nest.
	5	3	5	10	In 7-day-old nestling, two larvae were observed in the right wing and one on the left chest. The nestling was found dead at 10 days, likely due to internal organ damage caused by larval penetration into the body cavity. The cause suggested for Nestling no. 4 also cannot be ruled out.
3	6	4	5	10	At 7 days old, the nestling showed severe infestations: two larvae are in one wing, one is in the other wing, and one is in the center of the back. At 10 days old, it was found dead. The back injuries appeared critical.
	7	3	4	9	At 6 days old, two larvae were observed in one wing and one in the other. The nestling was found dead at 9 days. The presumed cause of death is similar to that of Nestling no. 4.
	8	1	5	10	In 7-day-old nestling, one larva was observed in the wing; the nestling was found dead at 10 days. The presumed cause of death is similar to that of Nestling no. 4.
	9	4	5	10	In 7-day-old nestling, three larvae were observed in the wing and one in the abdomen. The nestling was found dead at 10 days. Abdominal injuries appeared critical
	10	1	5	10	In 7-day-old nestling, one larva was observed in the wing; the nestling was found dead at 10 days. The presumed cause of death is similar to that of Nestling no. 4.
	11	3	8	10	
4	12	3	8	10	Nestlings were infested in the wings at 8 days old and likely weakened, as they were dead by 10 days old.
	13	4	8	10	