Stress response in pied flycatchers (*Ficedula hypoleuca*) in relation to monoterritorial and polyterritorial behavior

Anders Schanche

CEES

Centre for Ecological and Evolutionary Synthesis

Master of Science Thesis (30.sp)

University of Oslo

Department of Biology

2015

2015

Stress response in pied flycatchers (*Ficedula hypoleuca*) in relation to monoterritorial and polyterritorial behavior

https://www.duo.uio.no

Trykk: Reprosentralen, Universitetet i Oslo

Abstract

When facing life-threatening perturbation the following stress reaction will usually turn current behavior towards survival behaviors. However, this can have a potential fitness trade-off if it turns behavior away from reproduction towards survival. Animals will therefore try to modify their sensitivity to stressors so that they will turn current behavior towards survival when this gives the highest fitness. Animals that, for varies reasons, are more reactive to stressors should therefore avoid behavior that can more quickly resemble an emergency stress response. In a pied flycatcher (*Ficedula hypoleuca*) population, only a portion of the males becomes polyterritorial while the rest stays monoterritorial. Polyterritoriality is an energetically demanding strategy, and the behavior could trigger emergency stress responses more quickly than monoterritorial behavior. The hypothesis in this study is therefore that pied flycatcher males that are more sensitive towards stressors should avoid polyterritorial behavior, and males with this behavior should also show a suppressed stress response in order to continue to be polyterritorial. To examine this male pied flycatcher were captured twice, once in the territorial establishment period and once in the nestling feeding period. Breathing rate and corticosterone levels were used as stress response measurements. Three predictions were tested: 1. Pre-breeding males that later become polyterritorial will show a lower acute stress response than males who stay monoterritorial. 2. Males that have become polyterritorial will show a lower acute stress response than males who stay monoterritorial. 3. Males that are polyterritorial will have higher baseline corticosterone levels than monoterritorial, because polyterritoriality is an energetically demanding strategy.

None of the predictions regarding stress response tested in this thesis showed any significant difference between mono- and polyterritorial males. This suggests that responsiveness to stressors does not affect the likelihood of becoming polyterritorial. Other proximate explanations for this behavior are necessary, or a combination of many factors together could explain why some males become polyterritorial. Further, the tendency for higher baseline corticosterone levels in polyterritorial males during the nestling feeding period supports previous studies that polyterritorial behavior is in fact an energetically demanding strategy.

Acknowledgements

This master thesis was carried out at the University of Oslo, and supervised by Professor Helene M. Lampe. The thesis was approved by CEES and the Animal Research Authority in Norway (FOTS ID 6421). There would have been no thesis if it were not for the help I have received from others so many thanks to:

- My co supervisor Meta Landys, for guidance and advice.
- Wolfgang Goyman for analyzing all the blood samples.
- Anders Herland, for help in the field work and collecting data.
- CEES department for having provided a nice environment to work in.
- Thomas B. Martinsen for some long weekends helping me with formats, graphs, and statistics.
- Julie Johnsen for proofreading the thesis and giving suggestions for improvements.

- Finally, a special thanks to my supervisor Helene M. Lampe for giving me the opportunity to work with this thesis, and for all the help, assistance, knowledge and fun I got to experience during the field work, as well as all suggestions, guidance and advices throughout the master thesis.

Index

1.0 Introduction

Stress is a common term in everyday speech, where we like to talk about a pattern of physiological, behavioral, emotional or cognitive responses to some environmental conditions or happenings perceived as threatening to our intentions or goals. The term stress is often defined as a threat to homeostasis, and is commonly in reference to both predictable (e.g "migration stress") and unpredictable (e.g "capture and handling stress") events (Mc Ewen and Wingfield, 2003). However, the use of the term "stress" for predictable events, such as migration, reproduction etc, is rather misleading because natural selection has evolved traits to perform such events advantageously. Therefore events that are predictable for the individual (though still energetically demanding) should be viewed in the context of allostasis. The concept of allostasis means that animals can achieve stability in alternative homeostatic states, such that predictable events (e.g migration, territorial establishment, hibernation) will allow for an internal change that is not devastating for the individual, but rather a necessity to meet the new demands in a favorable way (Landys et al., 2006). In contrast, unpredictable events that are a threat to homeostasis should be referred to as a stressor and the following reaction a stress response (Mc Ewen and Wingfield, 2003).

In the face of an acute stressful situation (a stressor) the autonomic nervous system is activated through the Hypothalamic-Pituitary-Adrenal (HPA) axis. The incoming stimuli from a stress response increase the production of corticotropin in the hypothalamus. This hormone will stimulate the anterior pituitary gland and synthesize adrenocorticotropin. Adrencocorticotropin moves through the blood to the adrenal cortical tissue cells, which are followed by the release of corticosterone (Siegel, 1980). Corticosterone secretion will increase both when birds are exposed to a stressor and if the birds are entering/preparing for a new life stage that, for instance, needs higher energetic demands (e.g Siegel, 1980, Silverin, 1998b, Wingfield and Silverin, 2009). However, stressors will increase corticosterone many times more than such predictable events, and the high levels of corticosterone resulting from stressors will usually turn performed behavior away from the current life-history stage towards survival. Prolonged increase in corticosterone levels, resulting from chronic stress or repeated stressors, is believed to be devastating for the individuals, while the smaller

corticosterone increase resulting from predictable events is not, but is rather a requirement to meet energetic demands (Landys et al., 2006, McEwen and Wingfield, 2003).

The evolutionary explanation behind a stress response is to redirect the current behavior to an emergency state with survival behaviors. To have a well-functioning stress response is therefore an evolutionary advantage (Landys et al., 2006, Silverin 1998b). However, to be sensitive towards stressors may also have fitness disadvantages, e.g turning reproductive behavior towards survival behavior when the actual stressors probably would not kill the individual, but the redirection of behavior will mean the death of the offsprings (Silverin, 1986). It is therefore important for individuals to have accurate sensitivity towards stressors, so that they will redirect behavior towards survival when this gives the best opportunity for higher fitness. One example of this is from Schmid et al. (2013) that have shown that the Eurasian hoopoe (*Upupa epops)* modulate its adrenocortical response in a way so that the hormonal stress response is higher when having fewer nestlings compared to more nestlings. They also found that those birds that bred later in the season (and therefore were not able to have a second brood that year) would show a lower acute stress response. These results indicate that birds try to optimize their stress response in a way that is less likely to turn their behavior towards survival when there is a high fitness trade off in form of reproduction.

By exposing the birds to stressors, e.g. in the form of capture and handling, it is possible to observe individual stress responses and thereby compare the differences in a stress response (Wingfield, 1994, Wingfield et al., 1994). This method has been used in several studies as a way to find how sensitive birds are towards stressful events (e.g Astheimer et al., 1995, Schmid et al., 2013). Even though exposure to capture and handling is not a natural stressor which the birds might be exposed to in the field, this method for determining the strength of stress response is assumed applicable. This is because individuals have been shown to differ non-randomly in the way they respond to a given stressor (Laiolo et al., 2009, Sol et al., 2013). Although passerines have been shown to be able to modulate their hormonal stress response during the breeding season (Wada and Shimizu, 2004), the stress response has been shown to have distinctive individual variation (Cockrem and Silverin, 2002, Class et al., 2014). The handling stress protocol has been used in several studies to compare stress responses across species, between population, and between individuals within a population (Silverin and Wingfield, 1998, Torne-Noguera et al., 2014, Wingfield, 1994,). The rate of increase and the corticosterone peak is assumed to give a good indication of the sensitivity of

an individual to a stressor (Silverin, 1998a, Silverin, 1998b). The breathing rate of the bird has also been shown to be reliable indicator of the acute stress response (Carere and van Oers, 2004) and can also be measured by counting chest movements over a fixed time interval (Torne-Noguera et al., 2014, van Oers and Carere, 2007,). Although not directly linked to corticosterone increase, both Torne-Noguera et al (2014) and Carere and van Oers (2004) suggest breathing rate as an indicator of acute stress. This is because their results from studies on breathing rate shows the same tendencies as corticosterone increase in previous studies on the same kind of individuals (e.g shy individuals show higher breathing rate than bold individuals (Carere and van Oers, 2004), which is in line with studies showing higher stress induced corticosterone levels for shy individuals (Carere et al., 2003). Also, catecholamines (epinephrine and norepinephrine) that goes up within seconds of a stressors has been shown to trigger a rapid increase in breathing rate (Li and Nattie, 2006).

Whether a bird has one (monoterritorial), or more than one (polyterritorial) territory may have large effects on its fitness in form of reproduction (Slagsvold and Lifjeld, 1988). The pied flycatcher (*Ficedula hypoleuca*) is well known to have monoterritorial males as well as males that will try to occupy multiple territories to attract a second mate (Slagsvold et al., 1992). The ratio between mono- and polyterritorial males in a population has been shown to be dependent on the density of the pied flycatcher population (Alatalo and Lundberg, 1984, Alatalo et al., 1987), with more males employing the polyterritorial strategy when densities are low. Still, this variation in pied flycatchers strategies, to be monoterritorial or polyterritorial, may be explained by a trade-off of benefits and costs. While polyterritoriality may lead to increased reproductive success, if a male succeeds in attracting a second mate, it may also have its disadvantages (von Haartman, 1951). The disadvantages include: 1) Increased energy demands associated with the defense of two territories (Lifjeld and Slagsvold, 1986), and 2) Increased risk of suffering zero reproductive success for the season, as polyterritorial males may more easily lose both their primary and secondary territories to an intruder (Krebs, 1982). In comparison to monoterritorial males, polyterritorial males display increased baseline levels of corticosterone (Silverin and Wingfield, 1982). Since the increased demands associated with polyterritoriality are predictable, the increased baseline corticosterone is probably due to allostasis and assist the individual in meeting these new demands. For example, corticosterone has been shown to act in the mobilization of energy stores, to affect locomotion activity, and to be permissive for feeding behavior (Landys et al., 2006). These are probably all necessary adjustments, linked to polyterritorial behavior, that

corticosterone will help individuals to modify. Nevertheless, very high levels of corticosterone may induce pied flycatchers to abandon their nest (Silverin, 1986), increase the risk of diseases (Siegel 1980, Silverin, 1998b), and delay molting (Wingfield and Silverin, 2009). It is therefore intuitive to believe that individuals that are more sensitive to stressors will try to avoid behavior that provides higher levels of corticosterone (Silverin and Wingfield, 1982), and therefore are closer to the levels that will trigger emergency behavior. Even if the new behavior (polyterritoriality) seemingly is a new stable state, with non-harmful corticosterone levels, further challenges may trigger the emergency state more quickly in easily stressed individuals. In other words, they could be more susceptible to stressful events if they occur in addition to the normal environmental condition (Wingfield et al., 2010). This can, as mentioned, ruin reproductive success; it can therefore be an advantage to avoid polyterritorial behavior when the individual is sensitive towards stressors. I will therefore in the thesis investigate this as a third cost of polyterritorial behavior. It has also been shown that birds that are more sensitive to stressors may have more trouble keeping competitors away (Garamszegi et al., 2012). Hence, birds that are more sensitive to stressors might find it challenging enough to defend their primary territory, and will therefore not be polyterritorial. Therefore, the ability a given pied flycatcher has to cope with environmental stressors may have a large effect on its optimal strategy.

How sensitive an individual is towards stressors may e.g. be affected by the birds body condition, if the bird is suffering from diseases or if it has previous experience with the stressor (e.g. Marko et al., 2013, Walker et al., 2006, Siegel, 1980, Silverin, 1998b). For instance a bird in poor condition might be more sensitive to stressors, so that in the advent of an energetic challenge a strong stress response can help to immediately redirect behaviors toward survival. Such a bird may not choose to employ a polyterritorial reproductive strategy, because the corticosterone elevations associated with this strategy compounded with a higher responsiveness to unpredictable stressors would cause frequent entry into an emergency life history state (Wingfield et al., 2010). Because birds with low body condition, and/or low experience are more sensitive to stressors (Marko et al., 2013, Silverin et al., 1997, Walker et al., 2006), these males may stay monoterritorial. Also, since there is need for more energy to defend two territories (Lifjeld and Slagsvold, 1986), and it involves the risk of losing both its primary and secondary territory to an intruder (Krebs, 1982), only males in good body condition and with more experience might be able to perform the polyterritorial strategy.

Polyterritorial behavior will be a situation where the baseline corticosterone level will increase for those males that have this strategy, however only while they are focusing on protecting both territories (Silverin and Wingfield, 1982). The baseline corticosterone levels return to same levels as monoterritorial males later in the breeding season when males are no longer polyterritorial (Silverin and Wingfield, 1982). According to Silverin and Wingfield (1982), pied flycatchers that established a secondary territory retained elevated levels of corticosterone throughout all breeding stages from early nest-building until the first half of the nestling-feeding period (1-7 day old nestlings). During the second half of nestling-feeding period (8-14 days old nestlings), the difference in baseline corticosterone levels between males occupying secondary territories and those that remained at their home territory were shown to be no longer significant (Silverin and Wingfield, 1982). The polyterritorial male will gradually pay less, if any, attention to a secondary territory when the first clutch hatches, so that the secondary female receives very little or no help from the male (Lifjeld and Slagsvold, 1989, von Haartman, 1956). Because the male now pay less (if any) attention to another territory or female, baseline corticosterone levels in this male will gradually decline so that they resemble those of monoterritorial males (Silverin and Wingfield, 1982). This is because at this stage most males that used to be polyterritorial now in essence have become similar to the monoterritorial males.

To determinate whether individual differences in sensitivity to stressors may explain variations in a chosen territorial strategy, I investigated the connection between the stress response (measured as breathing rate and the capture-induced corticosterone increase) and the territorial strategy (mono- or polyterritorial) in a wild population of pied flycatchers. I hypothesized that sensitivity to stressors in individual male flycatchers affects their choice of territorial strategy. Results of this project will thus provide further understanding of one of the evolutionary aspects that may limit an individual in a population from becoming polyterritorial.

To examine my hypothesis, I measured breathing rate and corticosterone levels in response to capture both in pre-breeding males and in males during the nestling stage. My predictions were the following:

1. Pre-breeding males that later become polyterritorial will show a relatively lower acute stress response (i.e., breathing rate) than future monoterritorial males.
2. During the nestling stage males that are polyterritorial will show a relatively lower acute stress response (i.e., breathing rate and rate of corticosterone increase) than monoterritorial males.

To confirm corticosterone patterns found in past studies (Silverin and Wingfield 1982), I also measured baseline corticosterone levels in polyterritorial and monoterritorial males during the nestling stage, I predicted that:

3. Baseline corticosterone levels will be relatively higher in polyterritorial males.

Information on the correlation between baseline corticosterone levels and territorial strategy may explain why some individuals will try to avoid polyterritorial behavior, e.g., because of the associated fitness cost from an elevated baseline.

2.0 Materials and methods

2.1 Study area

The study area was at Sinober in Sørkedalen close to Oslo (figure 1, 59°58'N 10°37"E). Sinober is an approximately 1 km^2 deciduous–coniferous woodland area (Slagsvold and Lifjeld, 1988). The area contains 270 nest boxes that are available to birds for breeding. Each year between 40 and 50 pairs of pied flycatchers breed in these nest boxes along with other small passerine birds, such as blue tits (*Parus caeruleus*), great tits (*Parus major*), coal tits (*Periparus ater*), and sometimes one or two nuthatches (*Sitta europaea)*. Nest boxes were placed approximately 1.5m above ground level. The boxes were spread evenly across the entire area, and placed 30-50 meters apart. From the end of April to the end of June all nest boxes were checked regularly. Nest boxes occupied by other birds than pied flycatchers were checked roughly once a week, and unoccupied boxes or those occupied by pied flycatchers were checked at least every other day. The entire area was examined for pied flycatcher activity by 2-3 people on a daily basis (except from a few days with heavy rain). Territorial activity was examined by observing male pied flycatchers singing fairly constant around a nest box, and individual birds were identified by their unique color bands.

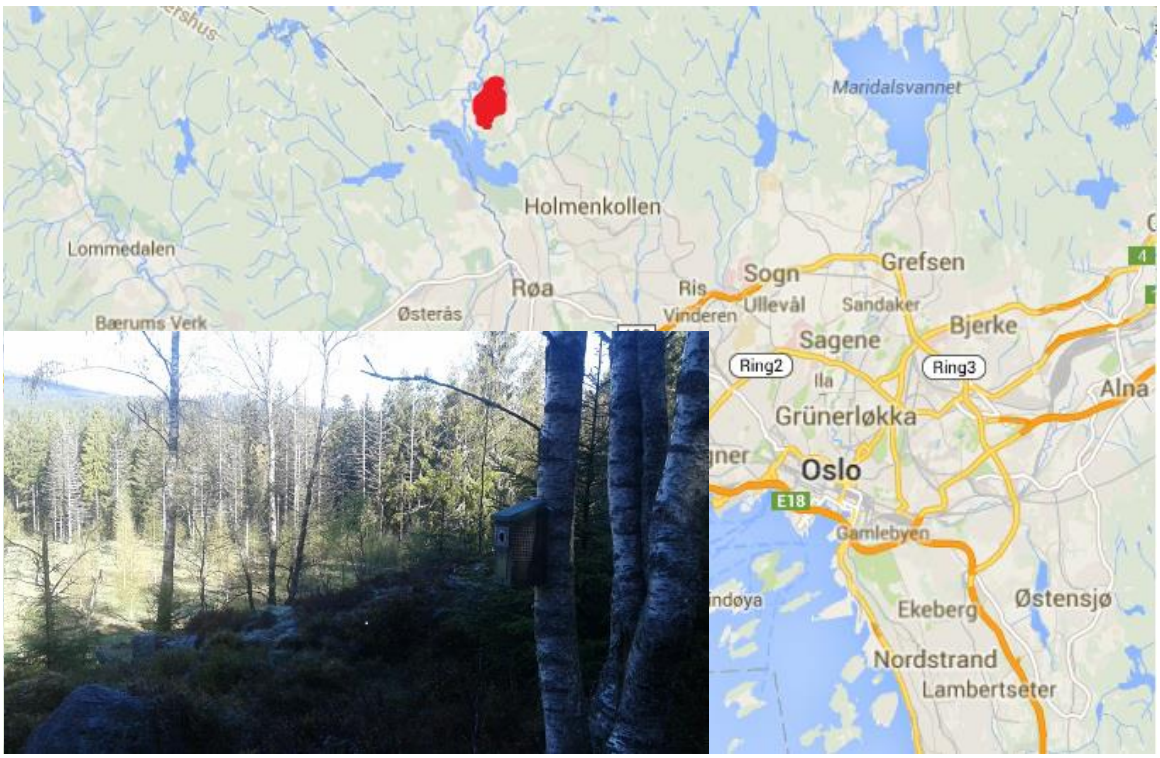


Figure 1: The red area indicates the study area Sinober (google maps). Inserted photo is a picture from the area (© Anders Schanche).

2.2 Study Species

Flycatchers are a migratory passerine that breed in Norway from late April to August. Male pied flycatchers are generally a bit larger than their female counterparts and have an average weight around 12-13g. Flycatchers are a well-known model organism because they easily nests in artificial nest-boxes (Lundberg and Alatalo, 1992). The male pied flycatcher will establish its territory immediately after arriving to Norway from its overwintering sites in western Africa. The territory is generally small (ca. 10-15m radius), and concentrated around a nest hole in a tree or a nest box (von Haartman, 1956). The function of this small territory is mainly to secure a nest site (Dale et al., 1990).

The male will immediately start to sing once a territory is established in order to attract a female partner (von Haartman, 1956). Once the male has successfully attracted a female, it stops the intensive singing and only sings to call for its mate. However, some males will establish a second territory and will start to sing in this territory to attract a second mate (Lundberg and Alatalo, 1992)**.** Such polyterritorial males fly between their two territories to ensure control over both (Alatalo and Lundberg, 1984, Alatalo and Lundberg 1990, Stenmark et al., 1988, von Haartman, 1956). In males that become polyterritorial, a second territory is usually obtained during the egg-laying period. However, once eggs hatch, males will mostly focus only on helping their primary partner to feed nestlings (Lifjeld and Slagsvold, 1989). In spite of possible fitness gains from polyterritoriality, many pied flycatcher males remain monoterritorial throughout the breeding season (Alatalo and Lundberg, 1984, Alatalo et al., 1987)**.** See Appendix (figure A1, table A1) for details of all the males in the Sinober area spring/summer 2014.

2.3 Territory establishment period (First time capture)

During the period April 25 – June 25 2014 we were able to capture 22 male pied flycatchers as soon as they arrived to the Sinober area, i.e., before they were able to attract a mate. Traps were placed inside the nest boxes in order to capture the males while they established their territory. The trap consisted of a lattice with duct tape attached to the nest box and held open by a stick (Figure 2). When male entered rigged nest boxes, they dislodged the stick, causing the lattice to block the exit. In order to trick the male into the nest box, I broadcasted the song of a male pied flycatcher using an audio player placed behind the nest, so that the male was tricked into thinking that the sound originated from within the given nest box. The male would therefore in most cases fly inside the nest box to chase away what he believed to be a competitor.

Nest boxes were continuously monitored during song broadcast, so that I could determine the exact time of capture. Once captured, birds were immediately taken out of the nest box. No males stayed inside a nest box for longer than 60 seconds. Breathing rate (over a 30 second interval) was measured within one minute after a bird was taken out of a nest box, using a stopwatch and a tally count (Figure 3). To decrease inter-observer differences in data collection, breathing rate measurements were performed by two persons. Body mass, wing length, and tarsus length were measured. Age (juvenile/adult) and plumage color was estimated according to Svensson (1992) and Drost (1936). Birds that were not already color banded from previous years were given a unique combination of color rings.

Figure 2: Spring trap. (© Anders Schanche).

Figure 3: Breathing rate measurement. (© Anders Schanche).

To determinate whether there are any differences in this decline between mono- and polyterritorial birds, we repeated the measurements of the breathing rate after 10 minutes (from the time taken out of the nest box), measuring chest movements during another 30 seconds.

2.4 Nestling feeding period (Second time capture)

Three to seven days after hatching, male pied flycatcher were captured a second time. We were able to capture 25 males during this stage of breeding.

During the nestling period, birds were captured with a trap consisting of a wooden block placed outside the nest box (Figure 4). This was connected to a fishing line and as the male flew inside to feed the nestlings, the fishing line was pulled so that the exit was blocked. In connection with this capture event a blood sample (50 – 75 µL volume) was taken from the brachial vein 1-2 min after capture to determine the baseline level of corticosterone. When blood samples are taken 1-2 minutes of disturbance (i.e., capture) plasma consentration relects baseline corticosterone levels, as corticosterone in birds is shown not to increase significantly in blood until 2-3 minutes after a stressful event (Romero and Romero, 2002). Such baseline corticosterone level will therefore reflect the corticosterone levels in a free-living bird prior to capture. An additional blood sample was taken 15 min following capture to determine the rate of corticosterone increase for each bird (calculated as the difference between capture induced and baseline levels).

Figure 4: Block trap (© Anders Schanche).

To collect blood samples, the brachial vein was punctured with a sterile 25-cauge needle (Figure 5). Blood droplets were drawn into heparinized microhematocrit capillary tubes and transferred into small test tubes (Figure 6). The tubes with the blood samples were stored in a thermos filled with ice. The tubes were surrounded by a plastic bag and pieces of paper to avoid direct contact with the ice, so that blood would not freeze and cause rupture of erythocytes. Immediately after blood sample were taken, breathing rate was also measured following the same procedure as described during the first capture event. All breathing rate measurements were obtained within 3 minutes of birds being taken out of the nest box.

Breathing rate measurement were repeated after 10 min. Blood samples were collected only during the morning (between 8:00 and 13:00), as baseline levels of corticosterone have been shown to be fairly constant over this period (Silverin, 1998b, Silverin and Wingfield, 1998).

Figur 5: The brachial vein punctured with a sterile 25-cauge needle (© Anders Schanche).

Figur 6: Transferring blood from heparinized microhematocrit capillary tubes into small test tubes (© Anders Schanche).

2.5 Blood sample analysis

Blood samples were centrifuged (3800 rpm) for 10 min within 8h of collection (following Landys et al., 2007). After the samples were centrifuged, approximately 20 µL of the blood plasma was removed to an ependorph tube. Samples were than immediately frozen and kept at -80°C at the University of Oslo. The samples were later transported on dry ice to the Max Planck Institute for Ornithology, where corticosterone levels were measured via RIA by Wolfgang Goymann (following Goymann et al. 2006).

2.6 Ethical consideration

This study was performed under licenses from the Animal Research Authority in Norway (FOTS ID 6421), and the Directorate of nature conservation for catching and ringing the birds. Stangel (1986) argued that blood sampling will not affect future baseline stress level, or mortality rate for small birds. Handling, however, may affect the immediate stress level in pied flycatchers, but is not believed to affect their survival (Silverin, 1998c).

2.7 Statistical methods

I used Chi-square test, standard T- test (unpaired and paired), Mann Whitney U tests and Pearson correlation tests to analyze collected data. According to Hill and Lewicki (2007), a Mann Whitney U test should be conducted instead of a T- test when both sample size is small (less than <50) and there is a large departure from normal distribution. In the data sets involving corticosterone there was large expands in the data points, therefore Mann Whitney U test was conducted for all corticosterone analyzes.

All tests were two tailed, and the level of significance used in this study for rejecting the null hypothesis was $p = 0.05$.

3.0 Results

Breathing rate has been shown to decline with time since capture (van Oers and Carere, 2007). However, I found no significant intra-individual differences between first and second breathing rate measurements, neither for the males captured during territorial establishment period (Paired t- test, n = 22, t = 1.50, p = 0.15), or the males captured during feeding nestling period (Paired t- test, n = 25, t = 1.62, p = 0.12). Mean breathing rate was therefore used for statistical analyzes.

3.1 Territorial establishment period (TEP)

Mean breathing rate measurements (combining 1 and 10 min) for mono- and polyterritorial males during the territorial establishment period were not significantly different (figure 7, Unpaired T-test, t = 1.38, p = 0.18). Thus there was no support for the first prediction.

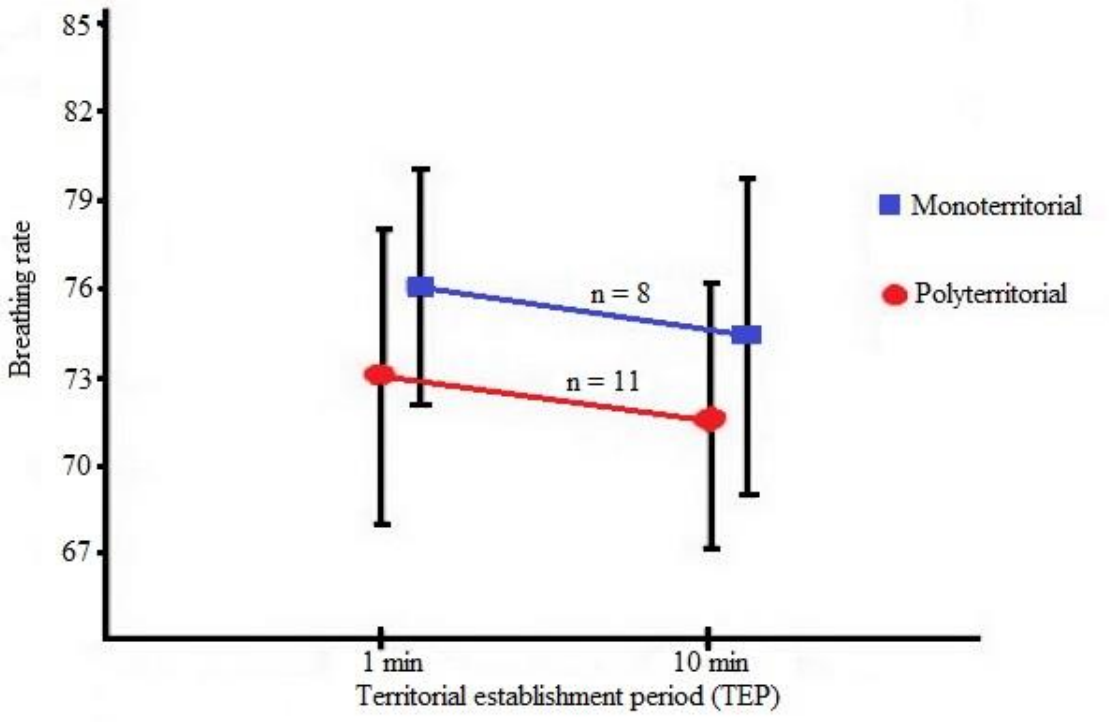


Figure 7: The mean breathing rate during 30s (± SD) measured for mono- and polyterritorial males during the territorial establishment period, one and ten minutes after capture.

3.2 Nestling feeding period (NFP)

3.2.1 Breathing rate

Mean breathing rate measurements (combining 3 and 10 min) for mono- and polyterritorial males during the nestling feeding period were not significantly different (figure 8, Unpaired T-test, t = 0.52, p = 0.60). Thus there was no support for the first part of the second prediction.

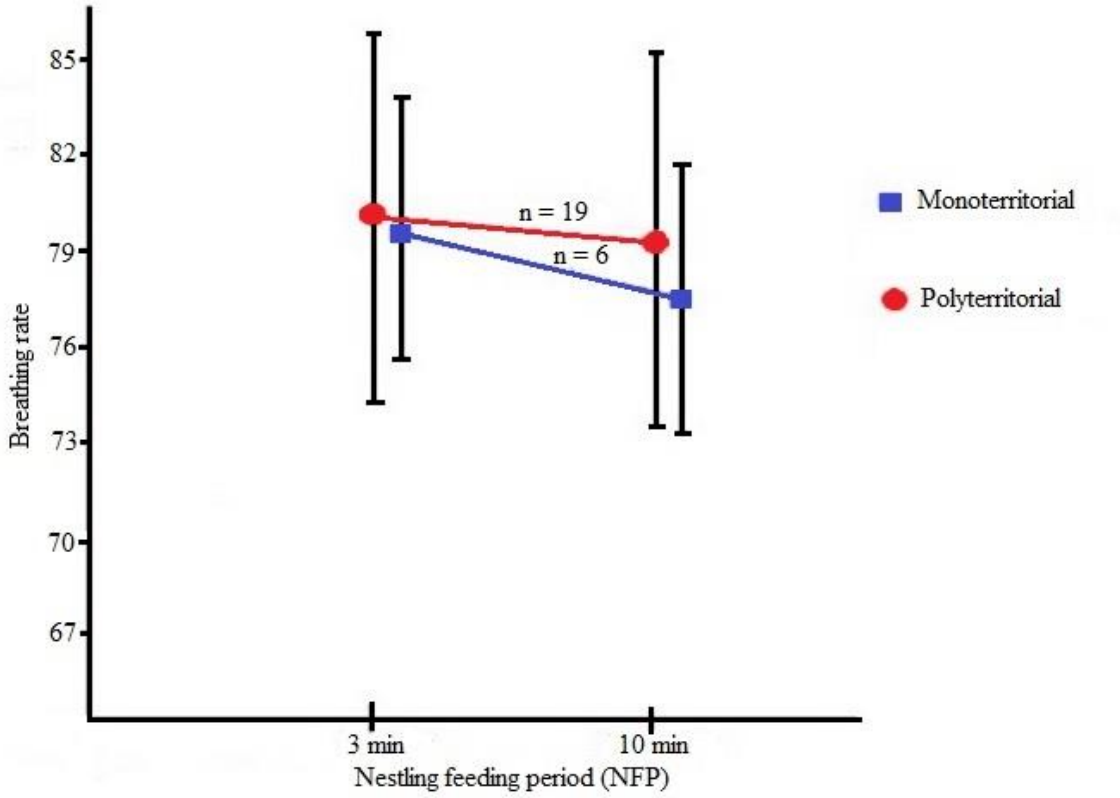


Figure 8: The mean breathing rate during 30s (± SD) measured for mono- and polyterritorial males during nestling feeding period, three and ten minutes after capture.

3.2.2 Corticosterone rate of increase

The corticosterone rate of increase measurement for mono- and polyterritorial males during the nestling feeding period were not significantly different (figure 9, Mann-Whitney U-test, U = 12, p = 0.65). Thus there was no support for the second part of the second prediction either.

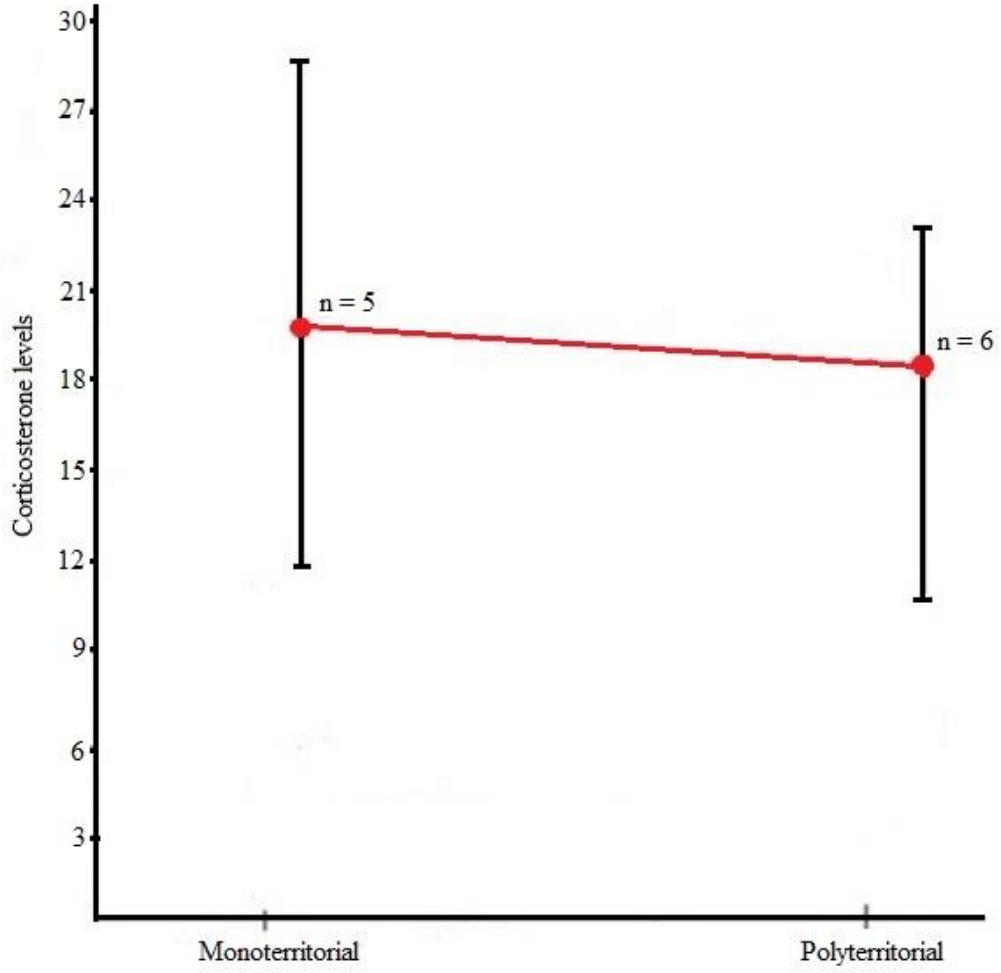
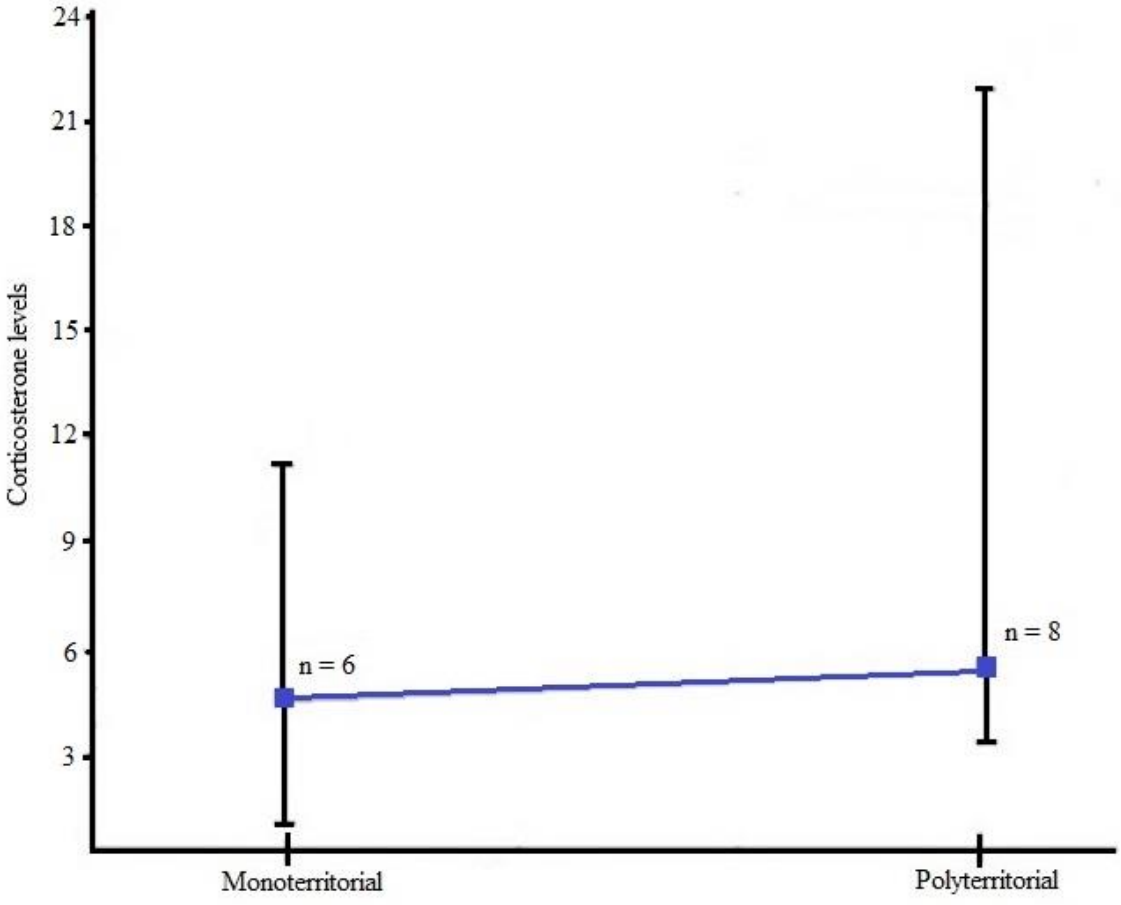


Figure 9: The median and the ranges of the corticosterone increase levels (measured as ng/ml blood) for mono- and polyterritorial males, measured during the nestling feeding period.

3.3 Baseline corticosterone measurement

The baseline corticosterone measurements for mono- and polyterritorial males during the nestling feeding period were not significantly different (figure 10, Mann Whitney U test, U = 14, p = 0.22). However, a small tendency was revealed considering the fact that from the small sample size the three lowest values (1.32ng/ml, 2.55ng/ml, 3.12ng/ml) were from the monoterritorial group while the three highest corticosterone levels (21.61ng/ml, 15.99ng/ml, 12.25ng/ml) were from the polyterritorial group (appendix, table A1), which is in the predicted direction.



Figur 10: The median and the ranges of the baseline corticosterone levels (measured as ng/ml blood) for mono- and polyterritorial males, measured during the nestling feeding period.

3.4 Mean breathing rate versus corticosterone increase

There was not a strong correlation between the mean breathing rate values and corticosterone rate of increase measured during the nestling feeding period (figure 11, Pearson correlation test: n = 11, r = - 0.51 and p = 0.11). The correlation was negative, but not significant, which is not in line with the expected positive correlation. Thus, the predicted distribution of monoterritorial males having lower breathing rate and corticosterone increase levels than polyterritorial males was not supported.

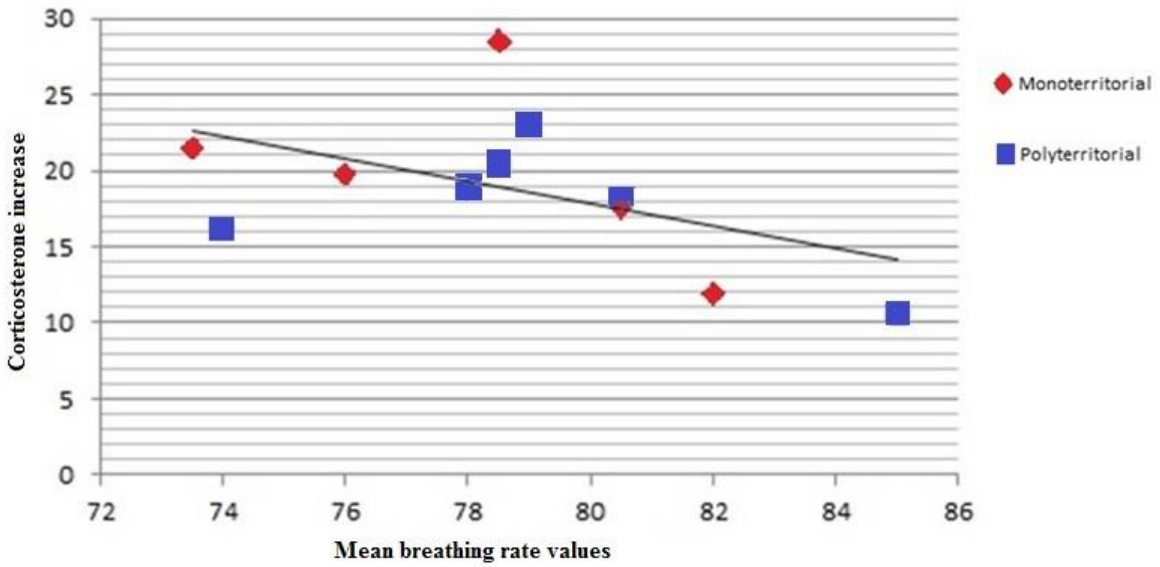


Figure 11: The correlation between corticosterone increase and mean breathing rate during the period of feeding nestling. The mean breathing rate is the mean of the measurements three and ten minutes after capture.

3.5 Breathing rate analysis including monoterritorial and polyterritorial males

There was a highly significant difference in breathing rate for male pied flycatcher between territorial establishment period and nestling feeding period (figure 12, unpaired t-test, t = 4.44, p = 0.0001).

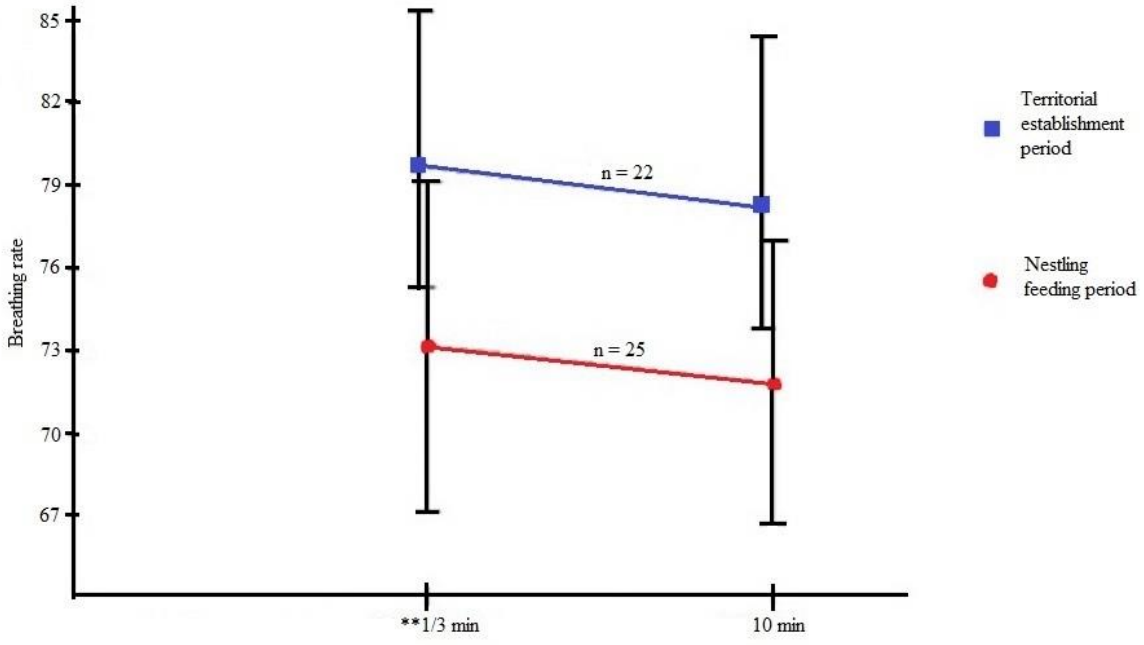


Figure 12: The mean breathing during 30s (± SD) of first and second measurements from the territorial establishment period, and the nestling feeding period.

3.6 Experience and body condition in relation to mono- and polyterritorial behavior.

There was not any significant difference in age between monoterritorial and polyterritorial males during the territorial establishment period. Although, twice as many adult males were polyterritorial than juvenile males (Table 1, Chi-square test, $x^2 = 2.23$, $p = 0.13$).

Likewise, there was no significant difference in previous knowledge to the area (Sinober) between monoterritorial and polyterritorial males during the territorial establishment period (Table 1, Chi-square test, $x^2 = 2.44$, $p = 0.12$)

Table 1: The number of mono- and polyterritorial males in two different experience categories (previous knowledge to the area, and age).

	Been to Sinober previous years (Experienced)	First time in Sinober (Unexperienced)	Adult	Juvenile
Monoterritorial	2	6	3	5
Polyterritorial	11	8	13	6

The difference in body condition (body mass / tarsus length) between mono- and polyterritorial males during the territorial establishment period were not significant (Unpaired t-test, mean, ± SD, Monoterritorial: 0.61, 0.017, n = 8. Polyterritorial: 0.63, 0.017, n = 11, t = 1.58, p = 0.13.).

3.7 Confounding factors on breathing rate

The correlation between breathing rate and wing length, plumage color and age (Unpaired T-test, $n_1 = n_2 = 11$, mean, ± SD, Juvenile: 72.40, 5.93, Adult: 72.73, 4.97, $t = 0.12$, $p = 0.9$) were not significant (table 2). Breathing rate with body mass and tarsus length were significantly correlated. However, body condition was not (table 2).

Table 2: Different variables measured compared to breathing rate. Significant results in bold.

		Pearson correlation x breathing rate	
Variables	**n**	**r**	**p**
Body mass	22	-0.48	**0.02**
Wing length	22	0.01	0.96
Tarsus length	22	-0.45	**0.04**
Plumage color	22	0.27	0.22
Body condition	22	-0.07	0.74

4.0 Discussion

This study investigated pied flycatcher males to determine: (1) whether the acute stress response (measured as breathing rate) has an impact on the decision to become mono- or polyterritorial, as well as the converse, namely (2) whether a chosen territorial strategy has an impact on the acute stress response (measured as breathing rate and corticosterone response to capture and handling). The study also determined (3) whether baseline corticosterone levels differs between mono- and polyterritorial males. Results suggest that there is no significant difference in acute stress response in birds prior to expression of a distinct territorial strategy, suggesting that the acute stress response does not influence the decision to become polyterritorial. Second, results suggest there is no difference in the acute stress response of birds already expressing a distinct territorial strategy suggesting that the demands associated with a particular territorial strategy in the pied flycatcher do not influence sensitivity to stressors. Finally, as found in previous studies (Silverin and Wingfield, 1982) polyterritorial males showed a trend for relatively higher baseline corticosterone, suggesting that polyterritoriality may be associated with relatively higher energetic demands.

4.1 Stress response as proximate explanation for mono- or polyterritorial behavior.

In the present study there were no significant differences between mono- and polyterritorial males in breathing rate or corticosterone rate of increase. Other studies have suggested that there are differences between the immediate stress responses for individuals within a population (Silverin, 1998b), but to my knowledge there have been no studies on this as a proximate explanation for mono/polyterritorial behavior. However, Silverin and Wingfield (1982) have been studying the differences between polyterritorial and monoterritorial males regarding baseline corticosterone levels. Their study has shown that the effects of being polyterritorial is higher baseline corticosterone levels, but the reason why some males become polyterritorial in the first place, while others do not, is not known.

Since there was no difference in the stress response measurements between future mono- and polyterritorial males from the Sinober area, other explanation for why males have different territorial strategies are therefore relevant. Perhaps the best proximate explanation shown for why some pied flycatchers establishes a secondary territory while others do not, is that high levels of testosterone are shown to be necessary to establish a secondary territory (Silverin

and Wingfield, 1982), and that pied flycatchers male given testosterone during the egg laying period will establish a secondary territory (Silverin, 1980). However, the reason for why some males remain at low levels of testosterone while others do not is unknown. It is therefore possible that those males more sensitive to stress also avoid the stimulus that might increase testosterone secretion, or that sensitivity towards stress might be one of the factors explaining territorial behavior in birds. Ketterson et al. (1991) have shown evidence for a reciprocal relationship between testosterone and corticosterone. High levels of testosterone might on the other hand be a high cost for the males, since high levels of testosterone may suppress the activation of the immune system according to the immunocompetence handicap hypothesis (Folstad and Karter, 1992, Poiani et al., 2000).

Slagsvold and Lifjeld (1988) propose three reasons for why pied flycatcher males become polyterritorial: When the territory has a small spatial extent and is easy to defend, male competition is low, and the duration of male resource holding is short. This might explain why pied flycatchers are able to become polyterritorial. However, those terms are ultimate causes and more or less explain why the species is preforming this type of behavior. The proximate causes that trigger the behavior for males to become polyterritorial while others stay monoterritorial in one population are more unknown.

It has been shown that the ratio between polyterritorial and monoterritorial males in one area is density dependent (Alatalo and Lundberg, 1984, Alatalo et al., 1987). However, why are some males establishing a secondary (sometimes even tertiary) territory, while others keep staying monoterritorial when the male density is low? Would it be possible to point out which males of a population that is more likely to become mono- or polyterritorial? This depends on what is triggering this kind of behavior.

The explanation for why pied flycatcher males become polyterritorial might be found in other traits than in the sensitivity to acute stress, which were proposed as a factor in determining territorial behavior in this study. Other factors could be that they have chosen a nest box with no other nest boxes available at short distance (Slagsvold et al., 1992). It may be lots of males close to their primary nest box and they are afraid of being cuckolded, which might prevent them from getting additional territories (Alatalo and Lundberg, 1984). Slagsvold et al. (1992) has shown that polyterritorial males establishing secondary territories farther away from their primary territory are more successful in attracting a second mate, and that this may be explained by female aggression from the primary female who is trying to deter the male from

attracting a second partner. It might therefore also be possible that polyterritorial behavior is prevented by the female, if some females are better to intimidate their males from being polyterritorial than others. The explanation for why some bird become polyterritorial could also be explained by their body condition and experience (Marko et al., 2013, Silverin et al., 1997, Walker et al., 2006). However, experience and body condition was not significantly different between mono- and polyterritorial males in the present study.

Nevertheless, there is the possibility that covariation between more than one of these factors may explain the territorial strategy of pied flycatcher males. In this thesis both body condition and age, although not significant, were slightly higher in polyterritorial males, also the polyterritorial males did have (also not significant) a slightly lower breathing rate, all in accordance with what would be expected for polyterritorial males. If more than one factor is affecting which males will become mono- or polyterritorial it is still possible that individual differences in stress response can contribute to who will establish more than one territory.

4.2 Baseline corticosterone measurements

In this study I found no significant difference in baseline corticosterone levels between mono- and polyterritorial males during the nestling feeding period. However, there was a large variability in the baseline corticosterone levels within the two groups (mono- and polyterritorial) that may be explained by random stressful events experienced by the flycatchers before capture (Silverin, 1998b), making the corticosterone level measured differ from the initial baseline corticosterone level. Events triggering corticosterone increase could be the experience of an attack from a woodpecker or another predator not long before capture or that they have been fighting with another male. Polyterritorial males defending two or more territories most likely would run into more of such encounters and thereby get higher corticosterone levels than monoterritorial males.

The sample size we investigated was on the other hand small, and it is interesting that even though there was no significant difference between mono- and polyterritorial males, the three lowest measured baseline corticosterone levels came from the monoterritorial groups, while the three highest values measured came from the polyterritorial group. This is in accordance with Silverin and Wingfield (1982) that found higher baseline corticosterone levels in polyterritorial males during this stage of the breeding. Silverin and Wingfield (1982) showed that the baseline corticosterone level was higher in polyterritorial males than for monoterritorial males through all of the breeding stages, from early nest building until the

nestlings were about 8-14 days old, followed by the corticosterone level evening out. However, during the nestling period (feeding 1-7 days old) the difference in baseline corticosterone level between mono- and polyterritorial males was small. Therefore, because the difference between mono- and polyterritorial males at early nestling period was expected to be small, the tendency in this study supported Silvering and Wingfield (1982). This accordingly sustain that polyterritorial strategy is an energetically demanding behavior.

Also, when the difference between two groups is small, and hard to detect, a large sample size is needed to show that the difference is significant (Hill and Lewicki, 2007). This illustrates the problem in this study with small sample size, not only for the baseline corticosterone measurements, but also for all the stress response measurements the sample size was small. If stress is just one of the many factors explaining pied flycatchers territorial behavior, then a large sample would be needed to see any significant difference between those two groups.

4.3 Corticosterone increase in relation to breathing rate

The results in this study showed no significant correlation between breathing rate and corticosterone increase. However, a small tendency for a negative correlation was revealed. To my knowledge there are no studies that have shown a negative correlation. Torne-Noguero (2014) correlated their results with previous studies to show that there was a positive correlation between high breathing rate and high levels of corticosterone increase. The small negative correlation could be because of the large range in the values of the corticosterone rate of increase measured, and the small sample size. Also, the baseline corticosterone level was slightly higher for polyterritorial males. The slightly lower corticosterone increase for polyterritorial males could therefore be because they already had high levels of corticosterone. Corticosterone maximum (the highest level of corticosterone increase during stress) may therefore be a better stress response indicator than “corticosterone rate of increase” in further studies. The small tendency for a negative correlation is most likely therefore a random result. However, this needs more research.

4.4 Investigating territorial status

There are still possibilities that some males that were registered as monoterritorial had some degree of polyterritorial activity that was not observed, due to the limited areas that can be covered at the same time with the resources that were available for this field study. It is also a possibility that some males can be polyterritorial in other areas outside the study site. However, if this is the case they have shown a very low grade of polyterritorial activity since

the males observed were seen at their territories on several occasions. Also the results are strengthened by the fact that pied flycatcher males rarely find other territories outside the study site (Sinober), as pied flycatchers usually do not leave their primary territory too far (Slagsvold et al., 1992) and Sinober is a more attractive area than areas around because of the extensive access to nest boxes. The males that were identified as polyterritorial are on the other hand very likely to be so, because they were seen several times at their secondary territories.

4.5 Stress response in territorial establishment period versus feeding nestlings period

Both groups showed a significantly higher breathing rate in the feeding period. It is known that birds are able to modulate their response to acute stress, and Wada and Shimizu (2004) have shown in a study that bush warblers (*Cettia diphone*) have lower capture induced corticosterone levels during territory establishment and higher capture induced corticosterone levels during the incubating, feeding hatchling, or nestling period. This might be because suppressed stress response during territory establishment will allow for greater territorial defense. The acute stress response during the feeding period may be affected by the age of the parents and the size of the brood, also known as the "brood value hypothesis" (Schmid et al., 2013).

Another explanation for higher breathing rate measured at the second time capture may be because this time a blood sample was collected from the brachial vein of the bird before breathing rate measurements were taken. Although, to my knowledge, there are no direct studies on how blood sampling affects the breathing rate in birds, so it cannot be excluded that this explains some of the stress reaction (Stangel, 1986, Voss et al., 2010). However, since the slope of the decrease in breathing rate is the same for the first and the second time capture, it is conceivable that the difference in breathing rate between territorial establishment and feeding nestling period is the fact that the bird is in different breeding stages and not the performed blood sample. This is because we would probably expect a steeper decreasing curve in breathing rate if this was due to the blood sample that was taken.

Habituation could be an explanation for the difference in breathing rate measured at the period feeding nestling. If the birds were habituated to the capture procedure the breathing rate is lower than it would be if they were not captured once before in the season (Walker et

al., 2006). However, if habituation is the case, this would make the difference between territorial establishment phase, and period feeding 3-7 days old nestling even bigger.

4.6 Confounding variables

Body size and age might be important because they have shown to affect the baseline corticosterone level, and individuals sensitivity to acute stress (Carere et al., 2003, Silverin, 1998a, Silverin, 1998b, Silverin and Wingfield, 1998). The body size measurements weight and tarsus length had a significant influence on breathing rate, with decreasing breathing rate as body size increased. Nonetheless, in the present study this was not corrected for in the statistical analyzes, due to the small sample size, and also body condition (when body mass is calculated for size) was not significant correlated with breathing rate.

4.7 Conclusion

The results suggest that there is no difference in stress response between mono- and polyterritorial males. This suggests that sensitivity to stressors is not an important factor to determinate which males will become polyterritorial. Another factor or a combination of factors most likely determinate which males will become polyterritorial. The result also suggests that polyterritorial males do not suppress their stress response in order to stay polyterritorial through the breeding, and that therefore suppressed stress response is not important to perform this behavior. The results suggest a tendency for polyterritorial males to have higher baseline corticosterone levels during the feeding nestling period, in accordance with previous studies (Silverin and Wingfield 1992). This suggests that polyterritorial behavior is an energetically demanding strategy.

Interesting results were also discovered by investigating the difference in the breathing rate measurement between the territorial establishment period and the nestling period, indicating highly significantly larger breathing rates in the nestling period. It could be interesting to investigate for future studies if the stress response difference is due to the pied flycatcher modulating its stress response in different breeding stages or if the difference is due to the performed blood sample taken before breathing rate measurement.

References

ALATALO, R. V., GOTTLANDER, K. & LUNDBERG, A. 1987. Extra-pair copulations and mate guarding in the polyterritorial pied flycatcher, (*Ficedula hypoleuca)*. *Behaviour,* 101**,** 139-155.

ALATALO, R. V. & LUNDBERG, A. 1984. Polyterritorial polygyny in the pied flycatcher *(Ficedula hypoleuca)* - evidence for the deception hypothesis. *Annales Zoologici Fennici,* 21**,** 217-228.

ALATALO, R. V. & LUNDBERG, A. 1990. Polyterritorial polygyny in the pied flycatcher. *Advances in the Study of Behavior,* 19**,** 1-27.

ASTHEIMER, L. B., BUTTEMER, W. A. & WINGFIELD, J. C. 1995. Seasonal and acute changes in adrenocortical responsiveness in an arctic-breeding bird. *Hormones and Behavior,* 29**,** 442-457.

CARERE, C., GROOTHUIS, T. G. G., MOSTL, E., DAAN, S. & KOOLHAAS, J. M. 2003. Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress. *Hormones and Behavior,* 43**,** 540-548.

CARERE, C. & VAN OERS, K. 2004. Shy and bold great tits *(Parus major)*: body temperature and breath rate in response to handling stress. *Physiology & Behavior,* 82**,** 905-912.

CLASS, B., KLUEN, E. & BROMMER, J. E. 2014. Evolutionary quantitative genetics of behavioral responses to handling in a wild passerine. *Ecology and Evolution,* 4**,** 427-440.

COCKREM, J. F. & SILVERIN, B. 2002. Variation within and between birds in corticosterone responses of great tits *(Parus major)*. *General and Comparative Endocrinology,* 125**,** 197-206.

DALE, S., AMUNDSEN, T., LIFJELD, J. T. & SLAGSVOLD, T. 1990. Mate sampling behavior of female pied flycatchers - Evidence for active mate choice. *Behavioral Ecology and Sociobiology,* 27**,** 87-91.

DROST, R. 1936. Uber das brutkleid männlicher trauer- fliegenfänger *(Ficedula hypoleuca). Vogelzug,* 6, 179-186.

FOLSTAD, I. & KARTER, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *American Naturalist,* 139**,** 603-622.

GARAMSZEGI, L. Z., ROSIVALL, B., RETTENBACHER, S., MARKO, G., ZSEBOK, S., SZOLLOSI, E., EENS, M., POTTI, J. & TOROK, J. 2012. Corticosterone, Avoidance of Novelty, Risk-Taking and Aggression in a Wild Bird: No Evidence for Pleiotropic Effects. *Ethology,* 118**,** 621-635.

GOYMANN, W., TRAPPSCHUH, M., JENSEN, W. & SCHWABL, I. 2006. Low ambient temperature increases food intake and dropping production, leading to incorrect estimates of hormone metabolite concentrations in European stonechats. *Hormones and Behavior,* 49**,** 644-53.

HILL & LEWICKI 2007. STATISTICS: Methods and applications., Statsoft.

KETTERSON, E. D., NOLAN, V., JR., WOLF, L., ZIEGENFUS, C., DUFTY, A. M., JR., BALL, G. F. & JOHNSEN, T. S. 1991. Testosterone and avian life histories: the effect of experimentally elevated testosterone on corticosterone and body mass in dark-eyed juncos. *Hormones and Behavior,* 25**,** 489-503.

KREBS, J. R. 1982. Territorial Defence in the Great Tit *(Parus major)*: Do Residents Always Win? *Behavioral Ecology and Sociobiology,* 11**,** 185-194.

LAIOLO, P., BANDA, E., LEMUS, J. A., AGUIRRE, J. I. & BLANCO, G. 2009. Behaviour and stress response during capture and handling of the red-billed chough *(Pyrrhocorax pyrrhocorax)* (Aves: Corvidae). *Biological Journal of the Linnean Society,* 96**,** 846-855.

LANDYS, M. M., RAMENOFSKY, M. & WINGFIELD, J. C. 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology,* 148**,** 132-149.

LANDYS, M. M., GOYMANN, W., RAESS, M. & SLAGSVOLD, T. 2007. Hormonal responses to male-male social challenge in the blue tit *(Cyanistes caeruleus)*: Single-broodedness as an explanatory variable. *Physiological and Biochemical Zoology,* 80**,** 228-240.

LI, A. & NATTIE, E. 2006. Catecholamine neurones in rats modulate sleep, breathing, central chemoreception and breathing variability. *The Journal of Physiology,* 570**,** 385-396.

LIFJELD, J. T. & SLAGSVOLD, T. 1986. The function of courtship feeding during incubation in the pied flycatcher *(Ficedula hypoleuca)*. *Animal Behaviour,* 34**,** 1441-1453.

LIFJELD, J. T. & SLAGSVOLD, T. 1989. Allocation of parental investment by polygynous pied flycatcher males. *Ornis Fennica,* 66**,** 3-14.

LUNDBERG, A. & ALATALO, R. 1992. The pied flycatcher. AD poyser Ltd, London.

MARKO, G., AZCARATE, M., HEGYI, G., HERCEG, G., LACZI, M., NAGY, G., CARLOS SENAR, J., TOEROEK, J. & ZSOLT GARAMSZEGI, L. 2013. Behavioural responses to handling stress in the Great Tit: within-individual consistency and the effect of age, sex and body condition. *Ornis Hungarica,* 21**,** 12-25.

MCEWEN, B. S. & WINGFIELD, J. C. 2003. The concept of allostasis in biology and biomedicine. *Hormones and Behavior,* 43**,** 2-15.

POIANI, A., GOLDSMITH, A. R. & EVANS, M. R. 2000. Ectoparasites of house sparrows *(Passer domesticus)*: an experimental test of the immunocompetence handicap hypothesis and a new model. *Behavioral Ecology and Sociobiology,* 47**,** 230-242.

ROMERO, L. M. & ROMERO, R. C. 2002. Corticosterone responses in wild birds: The importance of rapid initial sampling. *Condor,* 104**,** 129-135.

SCHMID, B., TAM-DAFOND, L., JENNI-EIERMANN, S., ARLETTAZ, R., SCHAUB, M. & JENNI, L. 2013. Modulation of the adrenocortical response to acute stress with respect to brood value, reproductive success and survival in the Eurasian hoopoe. *Oecologia,* 173**,** 33-44.

SIEGEL, H. S. 1980. Physiological stress in birds. *Bioscience,* 30**,** 529-534.

SILVERIN, B. 1980. Effects of long-acting testosterone treatment on free-living pied flycatchers, *(Ficedula hypoleuca)*, during the breeding period. *Animal Behaviour,* 28**,** 906-912.

SILVERIN, B. 1986. Corticosterone binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period in the pied flycatcher. *General and Comparative Endocrinology,* 64**,** 67-74.

SILVERIN, B. 1998a. Behavioural and hormonal responses of the pied flycatcher to environmental stressors. *Animal Behaviour,* 55**,** 1411-1420.

SILVERIN, B. 1998b. Stress responses in birds. *Poultry and Avian Biology Reviews,* 9**,** 153-168.

SILVERIN, B. 1998c. Territorial behaviour and hormones of pied flycatchers in optimal and suboptimal habitats. *Animal Behaviour,* 56**,** 811-818.

SILVERIN, B. & WINGFIELD, J. C. 1982. Patterns of breeding behavior and plasma levels of hormones in a free living population of pied flycatchers, *(Ficedula hypoleuca)*. *Journal of Zoology,* 198**,** 117-129.

SILVERIN, B. & WINGFIELD, J. C. 1998. Adrenocortical responses to stress in breeding Pied Flycatchers *(Ficedula hypoleuca)*: relation to latitude, sex and mating status. *Journal of Avian Biology,* 29**,** 228-234.

SILVERIN, B., ARVIDSSON, B. & WINGFIELD, J. 1997. The adrenocortical responses to stress in breeding Willow Warblers *(Phylloscopus trochilus)* in Sweden: Effects of latitude and gender. *Functional Ecology,* 11**,** 376-384.

SLAGSVOLD, T., AMUNDSEN, T., DALE, S. & LAMPE, H. 1992. Female-female agression explains polyterritoriality in male pied flycatchers. *Animal behavior,* 43**,** 397-407.

SLAGSVOLD, T. & LIFJELD, J. T. 1988. Why are some birds polyterritorial. *Ibis,* 130**,** 65-68.

SOL, D., LAPIEDRA, O. & GONZALEZ-LAGOS, C. 2013. Behavioural adjustments for a life in the city. *Animal Behaviour,* 85**,** 1101-1112.

STANGEL, P. W. 1986. Lack of effect from sampling blood from small birds. *Condor,* 88**,** 244-245.

STENMARK, G., SLAGSVOLD, T. & LIFJELD, J. T. 1988. Polygyny in the pied flycatcher, *(Ficedula hypoleuca)*: a test of the deception hypothesis. *Animal Behaviour,* 36**,** 1646-1657.

SVENSSON, L. 1992. Identification Guide to European Passerines. Fingraf AB, Södertälje.

TORNE-NOGUERA, A., PAGANI-NUNEZ, E. & SENAR, J. C. 2014. Great Tit *(Parus major)* breath rate in response to handling stress: urban and forest birds differ. *Journal of Ornithology,* 155**,** 315-318.

VAN OERS, K. & CARERE, C. 2007. Long-term effects of repeated handling and bleeding in wild caught Great Tits *(Parus major)*. *Journal of Ornithology,* 148**,** 185-190.

VON HAARTMAN, L. 1956. Territory in the pied flycatcher. *International journal of avian species,* 98**,** 460-475.

VONHAARTMAN, L. 1951. Successive polygamy. *Behaviour,* 3**,** 256-274.

VOSS, M., SHUTLER, D. & WERNER, J. 2010. A hard look at blood sampling in bird. *Auk,* 127**,** 704-708.

WADA, M. & SHIMIZU, T. 2004. Seasonal changes in adrenocortical responses to acute stress in polygynous male bush warblers *(Cettia diphone)*. *General and Comparative Endocrinology,* 135**,** 193-200.

WALKER, B. G., BOERSMA, P. D. & WINGFIELD, J. C. 2006. Habituation of adult magellanic penguins to human visitation as expressed through behavior and corticosterone secretion. *Conservation Biology,* 20**,** 146-154.

WINGFIELD, J. C. 1994. Modulation of the adrenocortical response to stress in birds. Perspectives in comparative endocrinology. Ottawa: National Research Councilof Canada. p 520–528.

WINGFIELD, J. C., DEVICHE, P., SHARBAUGH, S., ASTHEIMER, L. B., HOLBERTON, R., SUYDAM, R. & HUNT, K. 1994. Seasonal-changes of the adrenocortical responses to stress in redpolls, *(Achantis flammea)*, in Alaska. *Journal of Experimental Zoology,* 270**,** 372-380.

WINGFIELD, J. C. & ROMERO, L. M. 2010. Adrenocortical Responses to Stress and Their Modulation in Free-Living Vertebrates. *Comprehensive Physiology.* John Wiley & Sons, Inc.

WINGFIELD, J. C. & SILVERIN, B. 2009. Ecophysiological studies of hormone-Behavior relation in birds, Elsviser Inc.

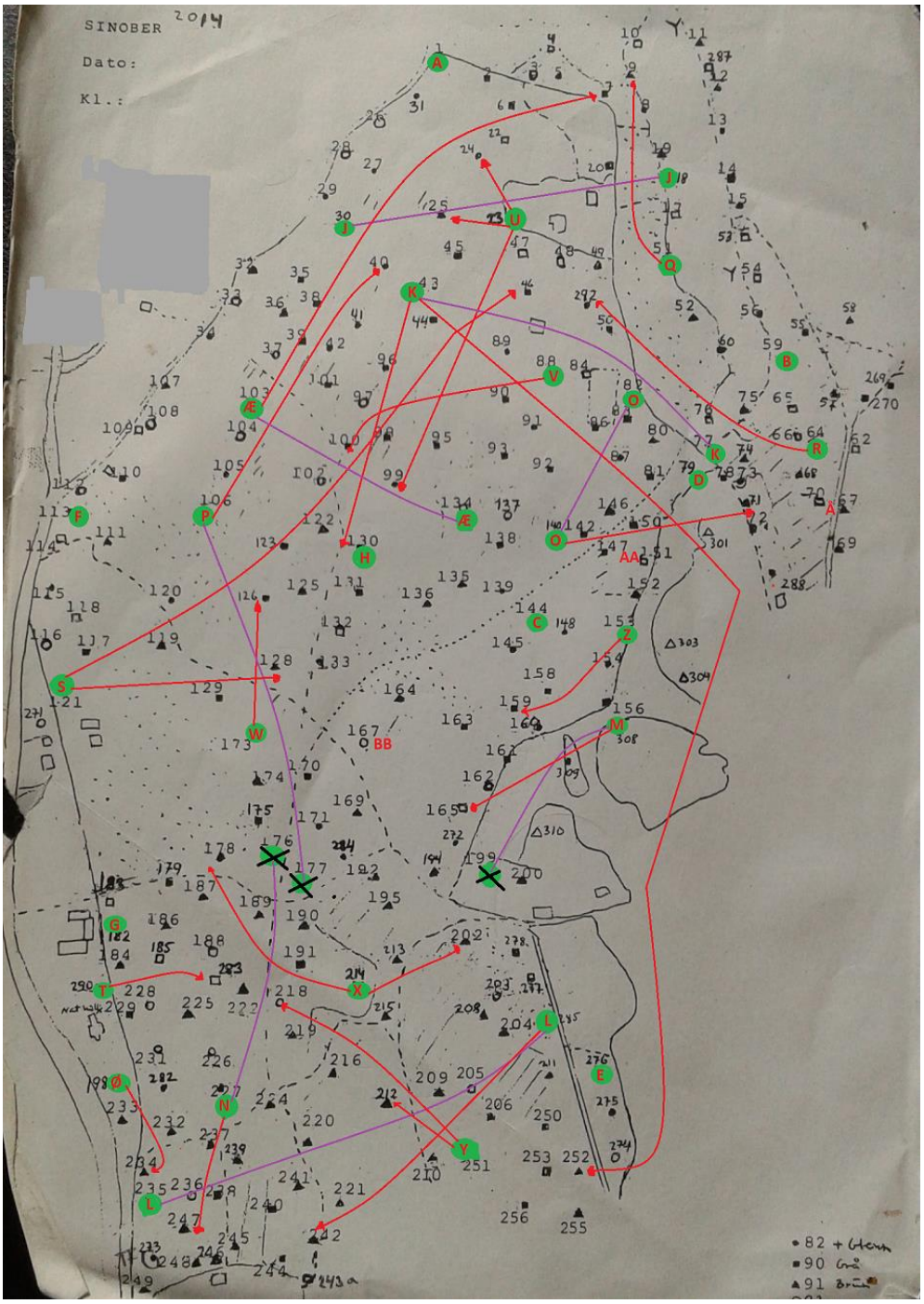
Appendix

Table A1:The table provides information for all male pied flycatcher males observed in the Sinober area spring/summer 2014. (Continues on the next page).

Individual	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P
Territorial status	Mono	Mono	Mono	Mono	Mono	Mono	Mono	Mono	Poly	Poly	Poly	Poly	Poly	Poly	Poly
Number of mates	One	One	One	One	One	One	One	One	Two	Two	Two	Two (One lost)	Two (One lost)	Two	Two (One lost)
Date and time; first time captured	18/5-08^{15}	14/5-08^{55}	18/5-10^{45}	17/5-09^{15}	15/5-08^{55}	15/5-12^{30}	14/5-10^{35}	16/5-10^{20}	16/5-09^{20}	-	13/5-09^{05}	-	-	15/5-10^{05}	-
Breathing rate; first time captured (1min)	81	71	76	78	71	77	82	73	79	-	76	-	-	74	-
Breathing rate; first time captured (10min)	78	72	76	73	64	74	83	74	78	-	76	-	-	69	-
Date and time; second time captured	16/6-12^{50}	16/6-11^{50}	18/6-10^{00}	-	-	25/6-09^{30}	16/6-09^{50}	12/6-10^{30}	14/6-10^{45}	11/6-10^{00}	15/6-10^{10}	11/6-11^{00}	12/6-11^{50}	14/6-12^{50}	17/6-10^{35}
Breathing rate; second time captured (3min)	83	82	76	-	-	82	73	81	87	82	79	72	82	82	82
Breathing rate; second time captured (10min)	76	75	71	-	-	79	80	83	90	78	81	73	75	79	74
Baseline corticosterone (ng/ml)	8.28	6.30	11.20	-	-	1.32	2.55	3.13	-	-	6.38	-	-	3.47	3.51
Corticoseterone Maximum. 15 min (ng/ml)	-	34.80	32.71	-	-	18.89	22.34	15.00	-	-	-			21.61	22.36
Corticosterone increase (ng/ml) (maximum – baseline)	-	28.5	21.51	-	-	17.57	19.79	11.87	-	-	-			18.14	18.85

Individual	Q	R	S	T	U	V	W	X	Y	Z	Æ	Ø	Å	AA	BB
Territorial status	Poly	Poly	Poly	Poly	Poly	Poly	Poly	Poly	Poly	Poly	Poly	Poly	-	-	-
Number of mates	One	One	One	One	One	One	One	One	One	One	Two	One	-	-	-
Date and time; first time captured	22/5-11^{00}	16/5-12^{55}	28/5-13^{20}	-	13/5-14^{00}	-	13/5-. 10^{15}	-	13/5-11^{30}	14/5-09^{15}	19/5-09^{30}	-	29/5-10^{00}	30/5-09^{45}	23/5-11^{40}
Breathing rate; first time captured (1min)	73	75	72	-	73	-	78	-	71	73	60	-	72	72	55
Breathing rate; first time captured (10min)	67	72	70	-	74	-	76	-	74	63	70	-	69	70	60
Date and time; second time captured	12/6-09^{30}	17/6-12^{40}	17/6-11^{15}	16/6. 09^{20}	14/6-11^{25}	14/6-12^{15}	17/6-09^{50}	16/6-10^{40}	15/6-09^{35}	17/6-12^{00}	12/6-10^{30}	17/6-09^{00}	-	-	-
Breathing rate; second time captured (3min)	75	81	82	73	78	81	96	79	87	78	72	74	-	-	-
Breathing rate; second time captured (10min)	73	76	80	78	80	79	94	84	84	78	71	75	-	-	-
Baseline corticosterone (ng/ml)	21.61	3.45	-	15.99	4.52	-	12.25	-	6.47	8.93	-	5.80	-	-	-
Corticoseterone Maximum. 15 min (ng/ml)	37.80	24.02	-	-	27.57	-	-	-	17.11	-	-	-	-	-	-
Corticosterone increase (ng/ml) (maximum – baseline)	16.19	20.57	-	-	23.05	-	-	-	10.64	-	-	-	-	-	-

Figure A1: Map with an overview of all male pied flycatcher males in the Sinober area spring/summer 2014.



Nest Box with nestlings. Letters indicates which male that occupies the nest box.

Nest box were nestlings were eaten by a woodpecker.

------→ Indicates were the male pied flycatcher have traveled for a second territory.

------→ Indicates the distance between two nest boxes were the male have successfully been able to have nestlings with two different females.