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Drosophila

- A Population Study in Southern Scania

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Abstract

Most studies done on *Drosophila* are made in labs and focus on genetics, something which has formed a somewhat one-sided field of studies. To counter this bias, this report was based on fieldwork. From a classical ecological aspect, *Drosophila* populations in southern Scania were investigated in order to put more clinical *Drosophila* insights in a proper context. According to previous studies, urban and rural factors together with temperature were thought to have an impact on sex ratios and species composition. This was tested for by sampling wild populations in Lund and Beddingestrand. Although no findings of greater importance were brought to light since none of the hypothesizes could be proven statistically true, convincing evidence for the summer 2017 being a bad year for *Drosophila* was found. No relation between temperature, seasonality, sex ratio, or species distribution was found, neither was any exotic species found.

Keywords: Drosophila, community ecology, Scania

Table of Content

Introduction

Drosophila is a genus of flies consisting of over 1,500 species with an estimate of around 60 of those species living in Sweden (Bächli *et al*. 2004). Most known of all these is presumably *Drosophila melanogaster*, a species used widely throughout modern research, mainly genetics. Since its introduction as a model species in the early 1900s it has given us enormous insights in heredity, disease and biological systems in general due to the fact that a large percent of our genes is believed to be homologous with genes in *D. melanogaster* (Pandey & Nichols 2011, Markow 2015). Recently, work on *Drosophila* helped gaining insights about circadian rhythms, which led to the Nobel prize in medicine this year, 2017 (Hardin *et al*. 1990). However, in contrast to the wide knowledge from lab work, *Drosophila* in an ecological context is poorly understood. In order to really understand the vast amount of findings originating from the lab, one has to also understand their ecological background which can give insights about how the observed mechanics interact in a broader sense. However, studies that take different ecological aspects into account use these to analyze wild populations of *Drosophila* are scarce. This issue was addressed already 1956 by Carson, Knapp and Phaf "... Flies of the genus *Drosophila* provide unexcelled material for genetic studies but ecological data on natural populations are sparse and qualitative " resulting in an upswing of studies within this field. Unfortunately, interest died of quickly and the latest study producing ecological data on natural populations was published 1978 by Begon & Shorrocks, where the feeding- and breeding-sites of *Drosophila obscura* and *Drosophila subobscura* were investigated. This study aims to once again shed some light on a forgotten but important topic.

D. melanogaster originates from sub-Saharan Africa (Lachaise *et al.* 1988) but is now found worldwide and together with *Drosophila simulans* among others form a group of cosmopolitic species. What define them is their close relation to human activity and broad niches. (Atkinson & Shorrocks 1977, Bächli 2004). Even though these species are synanthropic and can be found inside all year round, they are also present out in nature together with other species. Except for *D. melanogaster* and *D. simulans,* one more species was found in this study; *Drosophila Obscura*. *D. Obscura* is very common and described as a forest species and should therefore be less dependent on humans (Frydenberg 1956, Begon & Shorrocks 1978).

Purpose and hypothesis

Studies suggests that both temperature and seasonal variation have an effect on *Drosophila* communities, both regarding sex ratio and species structure (Bonnier 1926, Shorrocks 1975, Schnebel and Grossfield 1984). Studies have also shown that urban habitats are warmer than rural ones (Oke 1973). Based on these facts, this study will investigate if noticeable changes in *Drosophila* population structure can be seen during summer 2017 in Scania and if those will differ between an urban site in Lund and a rural in Beddingestrand. Since data about population structure will be collected one may also be interested in the potential catch of the invasive fly, *Drosophila suzukii*. *D. suzukii* is a fly species which originates from Japan and has proven very harmful to local fruit farms (Walsh et al. 2011). Recent studies suggest that it is now vastly spreading, and it has been found in several European countries where it might pose as a serious threat against the farming industry (Cini et al. 2012). Questions this study will investigate are the following:

- How does temperature affect sex and species structure?
- Does population structure differ between urban and rural populations?
- Do populations change during season?
- Is *Drosophila suzukii* present in the sampling area?

Methods

Sample and Measures

Data collecting took place during the summer of 2017, between the 25/6-2017 and 31/8-2017, see table 1. Due to low temperatures and small *Drosophila* populations, sampling had to end late August.

Table 1 – Dates of collecting data in Lund and Beddingestrand.

	Lund		Beddingestrand	
Collection Nr	Date	Days from start	Date	Days from start
1	26/6–2017	2	25/6–2017	1
2	3/7–2017	9	2/7–2017	8
3	30/7–2017	36	31/7–2017	37
4	7/8–2017	44	6/8–2017	43
5	13/8–2017	50	15/8–2017	52
6	31/8–2017	68	27/8–2017	64

Figure 1 - Placement of the sampling sites. Beddingestrand to the left; Site 1 (55°22'08.2"N 13°26'39.5"E), Site 2 (55°22'06.9"N 13°26'23.6"E), Site 3 (55°22'30.7"N 13°27'19.8"E), Site 4 (55°22'38.2"N 13°27'00.7"E). Lund to the right; Site 1 (55°42'49.5"N 13°12'24.6"E), Site 2 (55°43'34.3"N 13°13'26.9"E), Site 3 (55°43'46.5"N 13°12'52.3"E), Site 4 (55°43'25.2"N 13°11'37.6"E).

Flies, from the genus *Drosophila*, were collected with generally the same method as described in DrosEU Fly Sampling Call for 2017 Season (EDPGCM, 2017), although somewhat changed to fit our terms. Traps were built from plastic Erlenmeyer flasks, 95x65mm, by blocking the original opening with a cork and adding a 1,5ml Eppendorf tube on the side, offering a new way in, roughly 10mm wide. These were filled with 60ml of a 50/50 beer and banana solution together with 5ml bakers' yeast, acting as bait.

Sampling took place in two areas, Lund city and the small community of Beddingestrand in the south of Scania. This in order to resemble urban and rural conditions. In each area, four sites were used which all resembled the same kind of habitat, deciduous, shady boskets, see figure 1. Common tree species were, wild cherry *(Prunus avium*), small-leaved lime (*Tillia cordata*), Hawthorns (*Crataegus laevigata* and *monogyna*) and wych elm (*Ulmus glabra*). At each site, five traps were placed and left there for 24 hours. Maximum and minimum temperature were measured during the collection time and notes were made regarding other weather conditions such as wind and the general weather situation. Since this project, at least in the beginning, were helping the *European Drosophila Population Genomics Consortium* (EDPGC) with collecting *Drosophila melanogaster* genetic material, together with some ecological concerns, sites distance to grocery stores played a role. With regards to the influx of exotic and cosmopolitan species from foreign deliveries to these stores (Bächli *et al*., 2004), a minimum distance of 500m between site and grocery store was decided.

Sex and species was determined in the lab with help of "*The Drosophilidae (Diptera) of Fennoscandia and Denmark and Drosophila - a guide to species identification and use*", "*Drosophila - a guide to species identification and use*" and the key in "*The Danish Species of Drosophila (Dipt.)*".

Statistical methods

To be able to test for differences in species composition in different communities, an exponential Shannon Wiener biodiversity index was calculated for each sample:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

$$^{1}D = e^{H'}$$

To determine a collection optimum for all *Drosophila*, a one-way ANOVA post hoc Tukey was used. T-tests were used to find differences in the means of female ratio, exponential Shannon Wiener biodiversity index and maximum/minimum temperature between Lund and Beddingestrand. In the case of finding correlation between exponential Shannon Wiener biodiversity index and temperature, sex ratio and temperature, regressions were used. Data was processed with IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA)

Even though some of the data were not independent, tests with dependency limitations were still used due to time restriction and the difficulties with more advanced models. This will be further discussed in sources of error.

Results

Differences in temperature

Mean maximum and minimum temperature were compared between Lund and Beddingestrand with T-tests showing significant difference for minimum temperature but not maximum (df = 568, Std Error Difference = 0,17137, *p* = 0,000 and df = 568, Std Error Difference = -0,00886, *p* = 0,979).

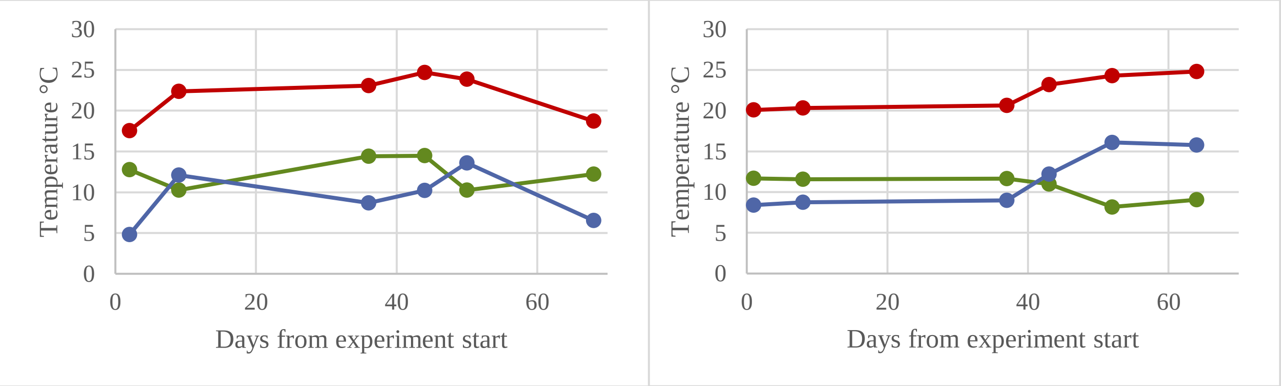

Figure 2 - Mean Maximum, minimum and delta Temperature. Urban Lund to the right, rural Beddingestrand to the left. Red; Max. Blue; Min. Green; Delta.

Differences in species distribution

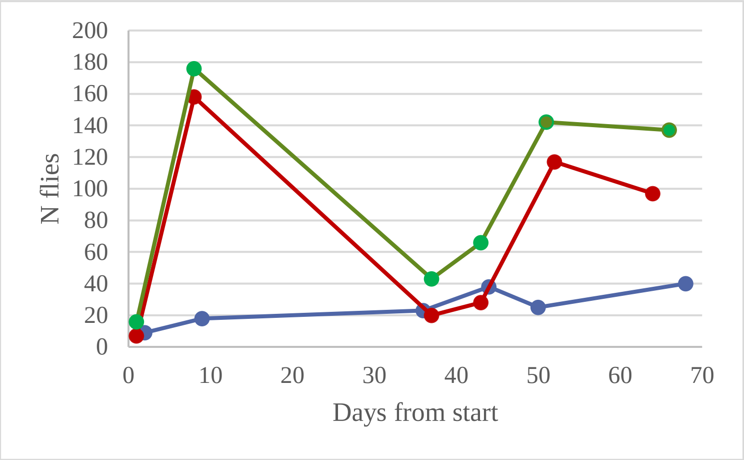

Figure 3 - Total amount of flies collected. Blue; Beddingestrand. Red; Lund. Green; Total.

Sampling resulted in n=570 flies in total, n=153 in Lund and n=417 in Beddingestrand, see figure 3. Two optimum, sampling number 2 and 5, could be found in collection rate in Beddingestrand, none in Lund (One-way ANOVA, df = 23, F = 3,863, *p* = 0,000 and df = 23, F = 1,772, *p* = 0,169). The results from regression A and B, see figure 4, suggests an increase in the exponential Shannon Wiener diversity index for the two areas together with maximum temperature, however it could only be proven significant in A (A; y = 0,1212x - 1,2262, R^2 = 0,2919, df = 18, p = 0,017. B; y = 0,0181x + 0,7056, R^2 = 0,1302, df = 20, p = 0,108). Regression C and D, D being significant (C; y = 0,1804x - 0,8072, R^2 = 0,2763, df = 18, p = 0,021. D; y = -0,0705x + 1,8423, R^2 = 0,2909, df = 20, p = 0,012), revealed no correlation of importance between minimum temperature and diversity. Mean exponential Shannon Wiener diversity index was also compared between Lund and Beddingestrand and a greater species diversity in Lund was proven significant (T-test, df = 38, Std Error Difference = 0,151, p = 0,027). Figure 5 illustrates this by showing species distribution over time, note however that species frequency is assigned to the y-axis, not diversity.

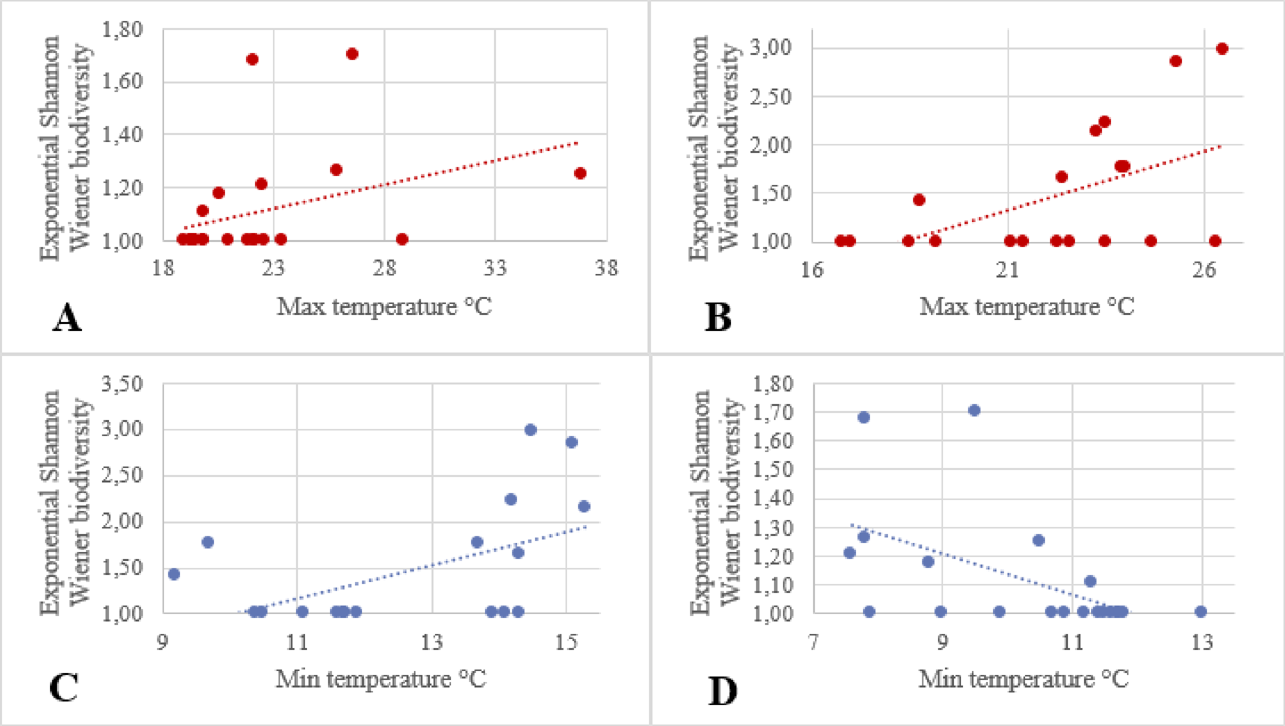

Figure 4 – The exponential Shannon Wiener biodiversity index for Lund (A and C) and Beddingestrand (B and D) compared with regard to maximum and minimum temperature in a linear regression. A; y = 0,1212x - 1,2262, R^2 = 0,2919, df = 18, *p* = 0,017. B; y = 0,0181x + 0,7056, R^2 = 0,1302, df = 20, *p* = 0,108. C; y = 0,1804x - 0,8072, R^2 = 0,2763, df = 18, *p* = 0,021. D; y = -0,0705x + 1,8423, R^2 = 0,2909, df = 20, *p* = 0,012.

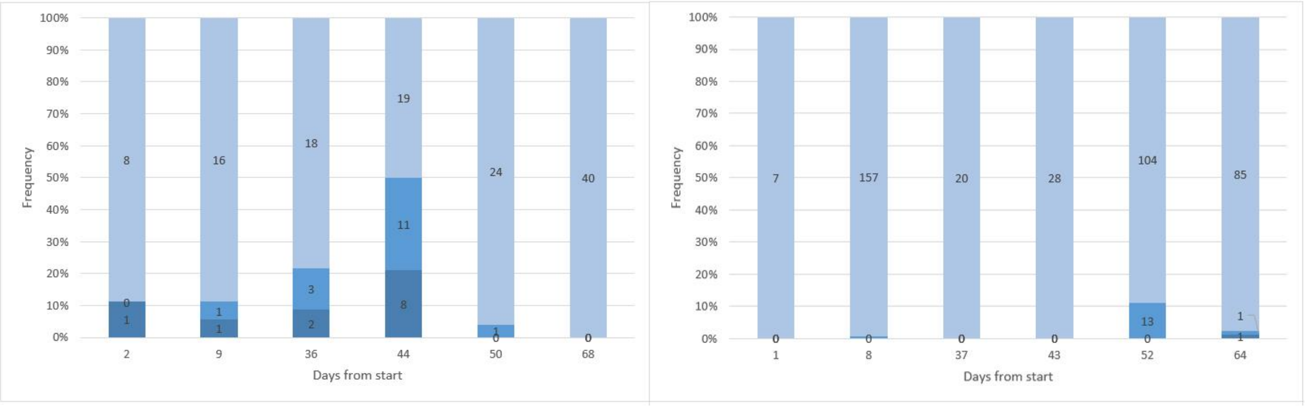

Figure 5 – Species distribution in frequencies, Lund to the right, Beddingestrand to the left. Light; *Drosophila obscura*. Medium; *Drosophila simulans*. Dark; *Drosophila melanogaster*.

Differences in sex ratio

No obvious relationship through regression could be shown between sex ratio and temperature, see appendix 1; figure 1 (Lund maximum temperature; $y = 0{,}0322x + 0{,}0243$, $R^2 = 0{,}1236$, df = 23, $p = 0{,}09$. Lund minimum temperature; $y = -0{,}0105x + 0{,}8518$, $R^2 = 0{,}0043$, df = 23, $p = 0{,}762$. Beddingestrand maximum temperature; $y = -0{,}0165x + 1{,}0655$, $R^2 = 0{,}0452$, df=23, $p = 0{,}319$. Beddingestrand minimum temperature; $y = 0{,}0212x + 0{,}4757$, $R^2 = 0{,}0115$, df = 23, $p = 0{,}617$). To check for effects during egg and larval stages, regressions were made between sex ratio and the maximum temperature one or two sampling dates before that (One date; $R^2 = 0{,}000$, df = 43, $p = 0{,}948$. Two dates; $R^2 = 0{,}011$, df = 39, $p = 0{,}520$) but nothing was found. Using a Pearson's chi-square test, sex ratios between Lund and Beddingestrand were compared suggesting some slightly larger amount of females in the population in Lund ($p = 0{,}041$), see appendix 1; figure 2.

Discussion

Diversity

To begin with the small amount of species and the uniform distribution was not expected. In total three species were found which is considerable fewer than previous studies (Basden 1954, Frydenberg 1956, Shorrocks 1975). Basden suggest that *Drosophila* frequencies varies between years, implying that good and bad *Drosophila* years exist. Although the variance of species was not that great, the total number of flies caught, n = 570 was in line, or even better, than the studies already mentioned. Collection peaked in the beginning of July and mid-August, both times due to increased *D. obscura* frequencies, why this peak occurs remains yet to investigate but Shorrocks (1975) observed the same patterns. Temperature from the date sampled nor earlier measurements (one and two measurements before) could not explain the increase. Earlier temperatures were tested for to find possible effects during egg and larval stages (Markow & O'Grady, 2005). Neither could other weather factors as it was raining with an intermediate amount of wind, just like all other sampling occasions. *D. obscura* is also the reason why more flies were collected in the rural area compared to the urban. This was expected since *D. obscura* is described as a forest species (Frydenberg 1956, Begon & Shorrocks 1978). Even though high *D. obscura* abundancies was expected, it still does not explain the low frequencies of the other two species found.

Although weak, tendencies for a higher diversity based on a higher maximum and minimum temperature was found in the urban setting. Further work to understand the explanation rate of temperature and locality needs to be done. The higher diversity implies higher frequencies of *D. melanogaster* and *D. simulans*, two cosmopolitan species (Bächli *et al.* 2004). Due to uncertainty regarding the explanation rate this can have two reasons. Studies have suggested that cosmopolitan *Drosophila* species have a higher tolerance against thermal stress (Levins 1969, Stanley *et al.* 1980). It could also be due to these two species connection to humans. With increased human population, rotting food and orchards come a larger amount of cosmopolitan species. Also, immigration of exotic species from import via grocery stores might facilitate faster dispersion. Perhaps the 500m minimum distance between sample sites and grocery stores was completely unnecessary?

Only three species were found in total, and *D. suzukii* was not one of them, which is a good thing for local fruit farmers. However, this does not mean that *D. suzukii* has not spread to Sweden and is not present in the area. Proper surveillance should be imposed in the future in order to detect and quickly react to the threat this species imply for the food industry.

Sex ratio

Except for a slightly higher ratio of females in Lund compared to Beddingestrand, no other correlation regarding sex could be found. However, interesting enough a constant higher rate of females was caught throughout the sampling period, 71% females in total. Even though previous studies have shown that females of other *Drosophila* species react different to temperature than males in terms of survivability (Worthen & Haney 1999, Tochen *et al.* 2014), no such conclusions can be made from this dataset. Indications of higher correlation to precipitation instead of temperature are supported by Frydenberg (1956), Begon & Shorrocks (1978) and Kellermann *et al.* (2013). Shorrocks (1975) experienced the same thing when catching *D. obscura* and he suggested differences in food preference or different late life stage mortality as possible reasons. Basden (1954) found a variation in sex ratios between collection with open bait and collection with traps. A larger number of females was found in the semi-enclosed traps. Since it was raining more or less constantly when the summer sampling took place, the last findings mentioned make the most sense. However, the reason behind females having a greater affinity for traps or males having a greater aversion is beyond the scope of this report.

Sources of error

Measurements of temperature could have been affected by the inferior quality of the thermometers. Being cheap and of low standard made synchronization between the devices hard and consistency over time even harder. In an attempt to tackle this, the same thermometer was used at the same sampling site each time.

This summer we experienced low temperatures and a lot of precipitation, this could perhaps be the factors that represent a bad *Drosophila* year as theorized by Basden (1954). On a better year, both a greater sample sizes and a greater species diversity would have been expected.

Both Frydenberg (1956), Begon & Shorrocks (1978) and Kellermann *et al.* (2013) suggest that dryness explains more variation than higher temperature. Perhaps other results could have been found if that data was collected.

Due to the authors' working situation summer 2017 measurements could not be done throughout the whole summer. This resulted in a data gap between the 3/7 and 30/7. Except for better consistency, a larger dataset would have been achieved if sampling had kept on going.

Much of the data collected are repeated measures from the same sites. Due to dependency, in order to proper deal with this data a repeated measures analysis should have been made instead of many of the regressions. Time restrictions and the scientific level of this report made that impossible. The author is well-aware of what consequences this have had for the statistical analysis and report in general.

Future studies

After this report, some questions remain unanswered. The underlaying causes to the peaks in *D. obscura* population during late summer can not be explain and requires proper research. Perhaps an all-year study where both food sources, breeding grounds and predation are observed in order to find seasonal changes that might affect the fly population. Although harder to explain and design research for, the issue of the skewed sex ratio also need an answer. Why are females, compared to males, in a higher degree drawn to traps? One possible hypothesis to be tested is differences in the olfactory system between the sexes. Since scent compounds have a harder time getting outside the trap, the smell of the bait is harder to detect, thus demanding a better olfactory system. This could be combined with experiments on different kind of baits to detect possible food preferences.

Temperatures are globally rising due to climate change and this affects all living species on earth (Addo-Bediako *et al.* 2000, IPCC 2007). Animals like *Drosophila* are especially susceptible to these changes since they are small soft bodied ectotherms with strict temperature niches optimums (David *et al.*1983, Atkinson 1994). Therefore, both for a greater general knowledge regarding the effects of climate change but also for deeper knowledge of genus that been so beneficial for mankind, further broad research with an ecological approach needs to be done.

Conclusion

A slightly higher value species diversity was found in the urban area, suggesting that human associated species are more abundant there. A uniform result, both in regard to sex ratio and species ratio, might imply some kind of hidden preference which this experimental set up was not able to find, further studies is required. No invasive species were found. Overall, the summer of 2017 indicates on a bad year for *Drosophila* populations in general.

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Appendix 1

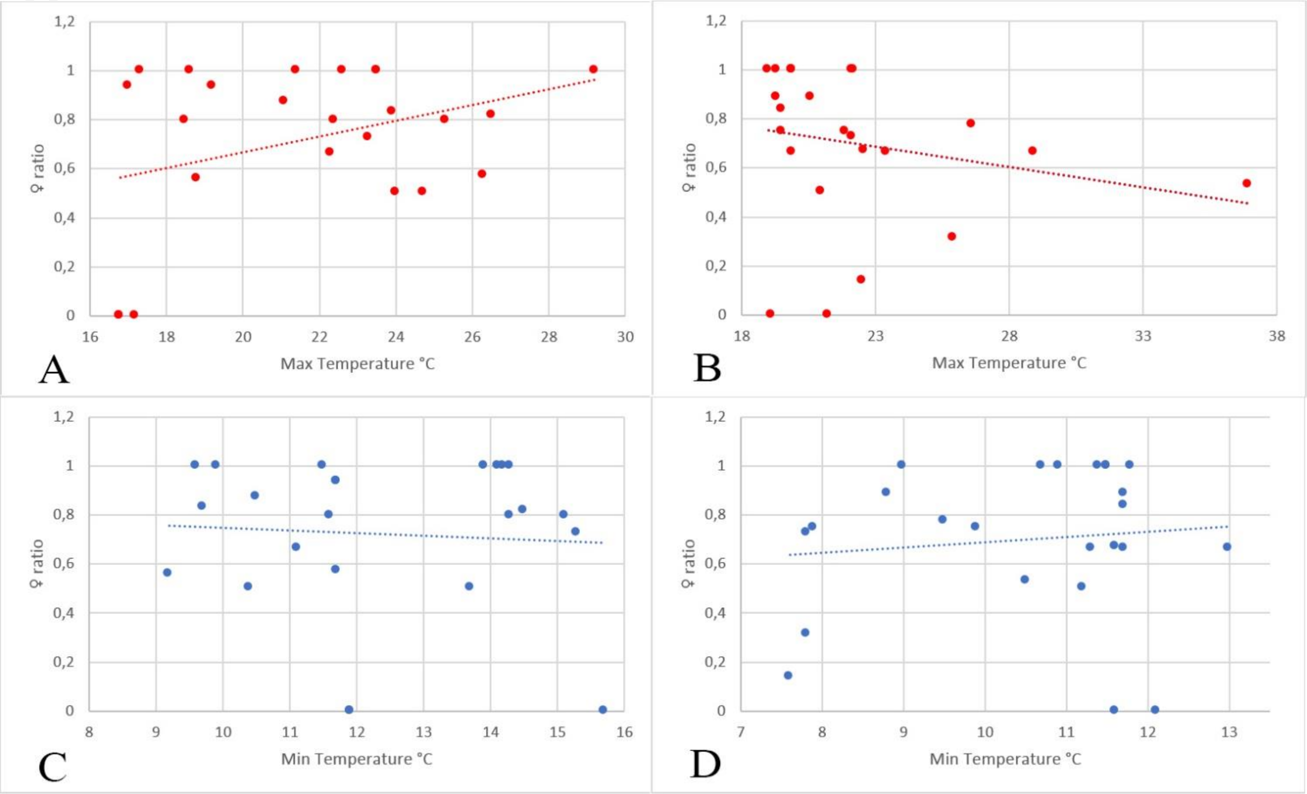

Figure 1 – No relationship between female ratio and temperature was found. A Lund maximum temperature; y = 0,0322x + 0,0243, R^2 = 0,1236, df = 23, p = 0,09. B Beddingestrand maximum temperature; y = -0,0165x + 1,0655, R^2 = 0,0452, df=23, p = 0,319. C Lund minimum temperature; y = -0,0105x + 0,8518, R^2 = 0,0043, df = 23, p = 0,762. D Beddingestrand minimum temperature; y = 0,0212x + 0,4757, R^2 = 0,0115, df = 23, p = 0,617.

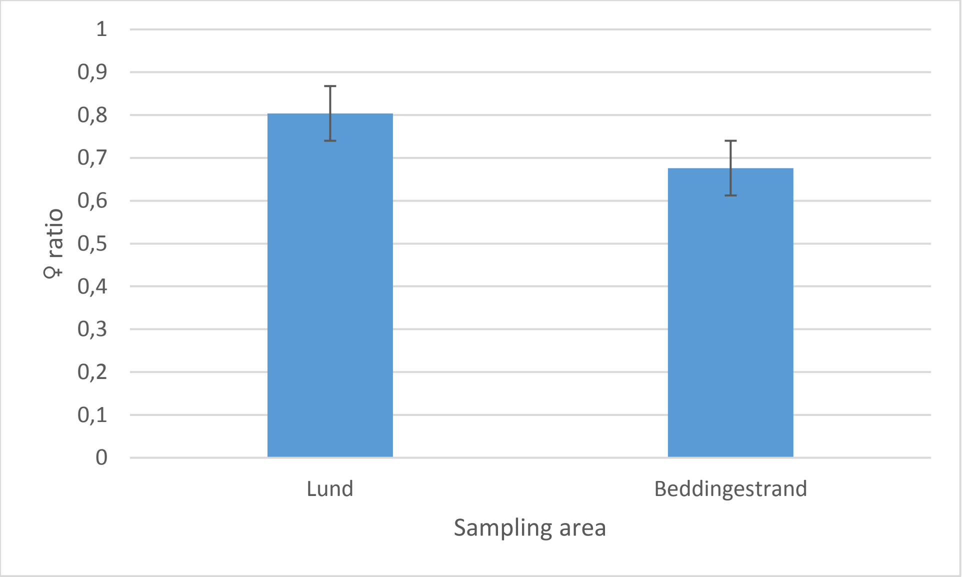

Figure 2 – Sex ratio in Lund and Beddingestrand (Pearson's chi-square, *p* = 0,041).