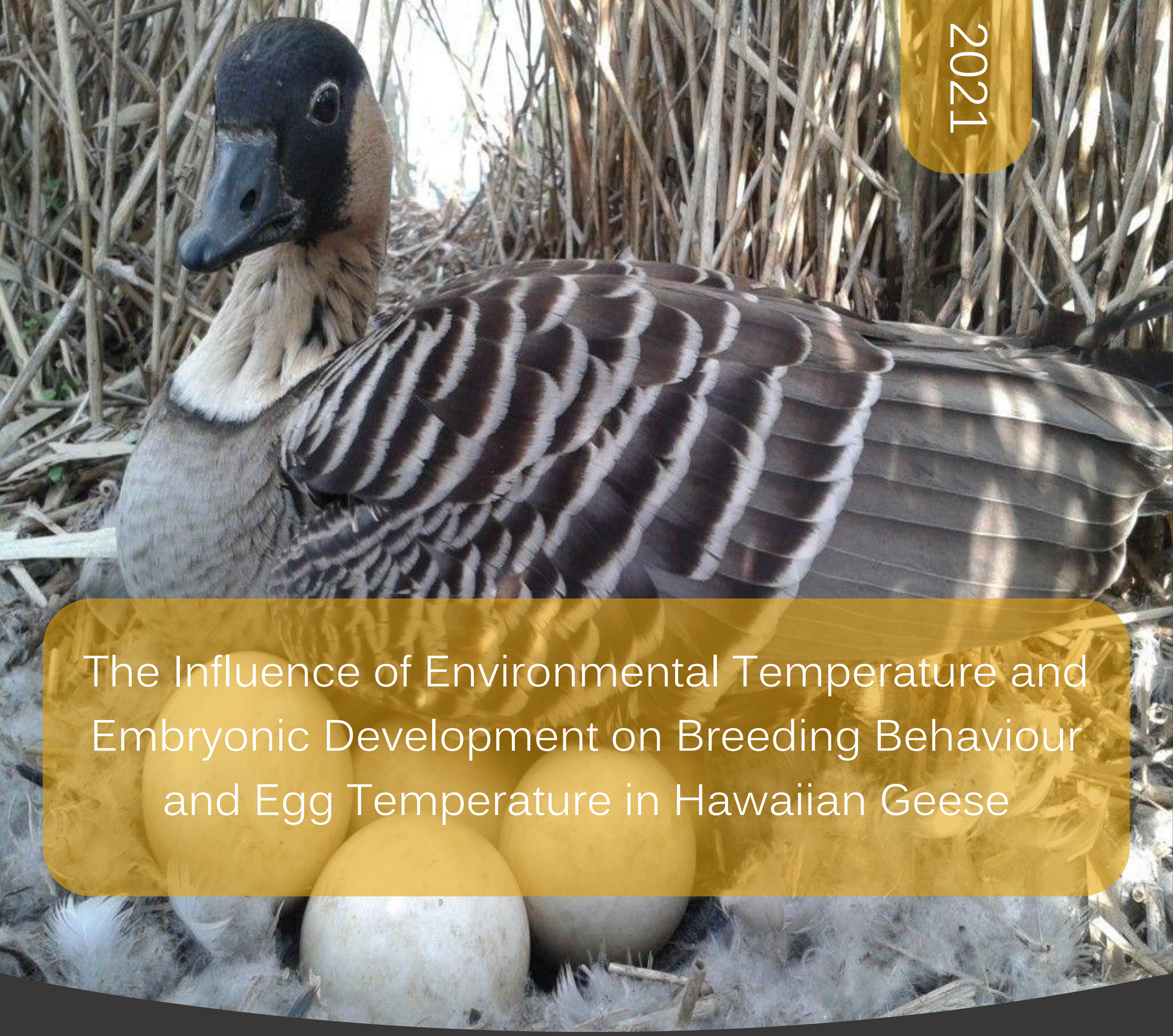


The Influence of Environmental Temperature and Embryonic Development on Breeding Behaviour and Egg Temperature in Hawaiian Geese

MELINDY DIRKS | WAGENINGEN UNIVERSITY | 960428184040

RESOURCE ECOLOGY GROUP | SUPERVISOR: JENTE OTTENBURGHS | THESIS CODE: REG-80436

ABSTRACT

Background: The incubation period is considered an essential component of parental care and reproduction in birds. Species-specific quantifications of the incubation period can be helpful in improving captive breeding strategies, *e.g.* by altering artificial incubator settings. For many species it is unknown how environmental temperatures and embryonic development affect breeding behaviour and subsequently the egg temperature.

Aim: During this research, I investigated the effect of environmental temperature and embryonic development on both on- and off-bout frequency and duration and the egg-turning frequency.

Organism: The vulnerable Hawaiian Goose, *Branta sandvicensis*, held in captivity in the Netherlands.

Place of research: The Netherlands

Methodology: Specialised EggLoggers and camera traps were used to collect data during the incubation period.

Principal findings: Results show that on-bout duration increases when environmental temperatures decline while off-bout duration and on- and off-bout frequency do not change. Thereby, the egg temperature is not affected by environmental temperature. However, egg temperature increases with incubation day. As the incubation period progresses, off-bout duration declines while on-bout duration and on- and off-bout frequency do not change. Moreover, egg-turning frequency is not affected by environmental temperature but increases with incubation day.

Conclusion: On-bout duration does not decrease when environmental temperatures decline or when the incubation period progresses. The fact that the mother in captivity has a sufficient amount of food and water in close range, thus enables her to use off-bouts more efficiently and stay for prolonged time on the nest if required, could explain this result. Thereby, egg temperature and egg-turning frequency increase when the incubation period progresses. Additional research on the hatching success when applying these results in captive breeding of *B. sandvicensis* will aid in understanding breeding behaviour during captive breeding and might help finding the solution to save vulnerable species.

Correspondence: Jente Ottenburghs; jente.ottenburghs@wur.nl and Bert Kleijer; a.c.kleijer@aeres.nl.

INTRODUCTION

Captive breeding is an important strategy in species conservation programs. This *ex-situ* breeding is used to increase the number of a certain threatened species outside its natural habitat (Bowkett, 2009). To ensure successful breeding, sufficient knowledge about the breeding process of a particular species is required. For example, some breeders are of the opinion that hatching success in an artificial egg incubator is lower compared to natural broods as there are no species-specific guidelines for artificial incubation, (personal communication B. Kleijer). Studying the incubation period by quantifying characteristics that are assumed to increase hatchability, *e.g.* egg temperature, humidity and optimal egg turning rates, could give insight in how to increase hatchability of eggs of threatened species in artificial incubators (Shaffer *et al.*, 2014). Quantifying the incubation period could thereby identify how environmental conditions, such as environmental temperature, influence the breeding behaviour of parent birds.

The incubation period is a vital component of parental care and reproduction in birds (DuRant *et al.*, 2013). For proper embryonic development and successful hatching, eggs need to be incubated under a narrow range of temperatures (Huggins, 1941; Morgan *et al.*, 2004; Webb, 1987). If the egg temperature falls below a certain temperature called the physiological zero temperature (PZT), no embryonic development takes place. Many studies have focused on how incubation behaviour in birds is affected by environmental temperature under the assumption that a mother bird balances the thermal needs of her eggs with her own thermal and energy needs, *e.g.* foraging, especially in case only the mother physically incubates the eggs, *i.e.* single-sex incubation (Amininasab *et al.*, 2016; Conway & Martin, 2000; Lombardo *et al.*, 1995). Conway & Martin (2000) hypothesised that off-bout durations should decline when the environmental temperature falls below the PZT to prevent eggs from cooling to temperature levels that negatively affect embryonic development. As a result of a declining off-bout duration with lower temperature, the frequency of off-bouts was assumed to increase, because the mother still requires her time off the nest to forage. However, an increased frequency of off-bouts should lead to a decline of continuous on-bout durations. Therefore, both on -and off-bout durations were expected to decline with temperatures below the PZT. Above the PZT, risk of eggs cooling too much is lower which should enable the female to maximize her on- and off-bout duration allowing her to optimize foraging and embryo development. The hypothesis of Conway & Martin (2000) was confirmed in orange-crowned warblers (*Vermivora celata)* as both on- and off-bout durations correlated positively with environmental temperatures between 9° and 26°C, with 26°C being the PZT, while no correlations were found at temperatures <9° and >26°C. The hypothesis of Conway & Martin (2000) can also be tested in other bird species that apply single-sex incubation of which scientific knowledge about the incubation period of *ex situ* breeding is lacking and artificial incubators still perform less successful compared to natural broods.

Beside the influence of environmental temperature on on- and off-bout duration and frequency, these variables could also be influenced by the progress of embryonic development, *i.e.* the stage of incubation. Due to embryonic development, embryos produce more heat themselves during incubation resulting in increasing egg temperatures (Cooper & Voss, 2014; Tullet, 1989; Weathers & Sullivan, 1989). Heat in an egg can move by either conduction in the tissues or convection by blood flow. Heat flow by conduction within the egg at the start of incubation is gradually amplified by a developing circulatory system, *i.e.* flowing of blood, of the embryo itself later in the incubation progress (Turner, 1987). However, this increased heat flow by convection within an egg during embryonic development also results in increased cooling rates during off-bouts as heat is more easily lost to the environment (Cooper & Voss, 2014; Turner, 1987). Cooper & Voss (2014) suggested, during their research on Black-capped Chickadees (*Poecile atricapilla*), that birds respond to this increased cooling rate by decreasing off-bout duration and increasing off-bout frequency as a shorter off-bout duration allows eggs to have a shorter time to cool.

Another aspect that might influence internal egg temperature is the position of the egg in the nest. In many bird species, heat is transferred from mother to egg with aid of a developed brood patch that is in direct contact with the egg's surface (Turner, 1997). However, the brood patch size might limit the area in which heat is transferred to the eggs in case of nests with larger clutches. Eggs furthest from the centre of the nest might receive less heat and have a higher cooling rate when the mother is off the nest compared to eggs more central to the nest (Boulton & Cassey, 2012). A hypothetical solution for keeping all eggs within the narrow range of temperatures for proper embryonic development, is egg turning. While egg turning behaviour is initially assumed to prevent embryos to stick to the inner shell membrane and to enhance the utilization of albumen during incubation, another assumption is that egg turning relocates eggs to equally distribute heat in time (Ferguson & Deeming, 1991; Tullet, 1990).

During this research, I will investigate how egg temperature is influenced by environmental temperature and how the breeding behaviour of the mother, *i.e.* the duration and frequency of on- and off-bouts and egg-turning frequency, is related to the environmental temperature. This research will be conducted on the species *Branta sandvicensis*. Species-specific information about how breeding variables such as egg temperature, egg-turning frequency and on- and off-bout frequency and duration vary during incubation is still lacking for this species. Therefore, I will also look at the influence of incubation day on these variables. *B. sandvicensis*, better known as the Hawaiian Goose or Nene, is a species native to the islands of Hawaii of which the number of mature individuals is estimated to be 250 to 1.000 (Birdlife International, 2017). However, during the 1940s, the number of individuals was estimated to be less than 50 whereas before the 20th century, numbers were estimated to be up to 25.000 (Baldwin, 1945; Paxinos *et al*., 2002). Multiple causes might explain this drastic decline during the previous century such as reduction of habitat due to agri- and aquaculture and recreational activities, the introduction of IAS-species (Invasive Alien Species) and excessive drought due to climate change (Baldwin, 1945; Kear & Berger, 2010). Since this decline, restoration -and conservation actions, both *in situ* and *ex situ* were initialised leading to the current, still increasing, number of Nenes (Smith, 1952; Birdlife International, 2017). However, the Nene is still classified as 'Vulnerable' according to the IUCN Red List (Birdlife International, 2017). In order to enhance recovery, more knowledge is required about the Nene such as behaviour and ecology in captivity. For example, learning more about the breeding behaviour regarding *ex situ* breeding might improve captive breeding strategies and might optimize artificial incubator settings used for this specific species.

Therefore, the first part of this research will focus on the frequency and duration of on- and off-bouts of the *B. sandvicensis* mother during the incubation period in relation with environmental temperature. Thereby, I will investigate how egg temperature is affected by this on- and off-bout behaviour and whether egg temperature is indeed kept approximately constant by performing this behaviour. Following the hypothesis of Conway & Martin (2000), I expect the frequency and continuous duration of both on- and off-bout of *B. sandvicensis* to show a positive relationship up to a certain environmental temperature point which should represent the PZT (Figure 1abc). Thereby, I expect egg temperature to be kept within a narrow range under all circumstances as this is required to ensure embryonic development (Figure1d). Because of embryonic development, *i.e.* developing blood system and metabolic activities, I hypothesise that egg temperature of *B. sandvicensis* will increase during the incubation period (Figure 1h). However, because cooling rate of eggs are assumed to increase during embryonic development, I hypothesise that off-bout duration will decrease and off-bout frequency will increase during the incubation period (Figure 1eg). As a response to an increased off-bout frequency, I thereby expect continuous on-bout duration to decrease (Figure 1f).

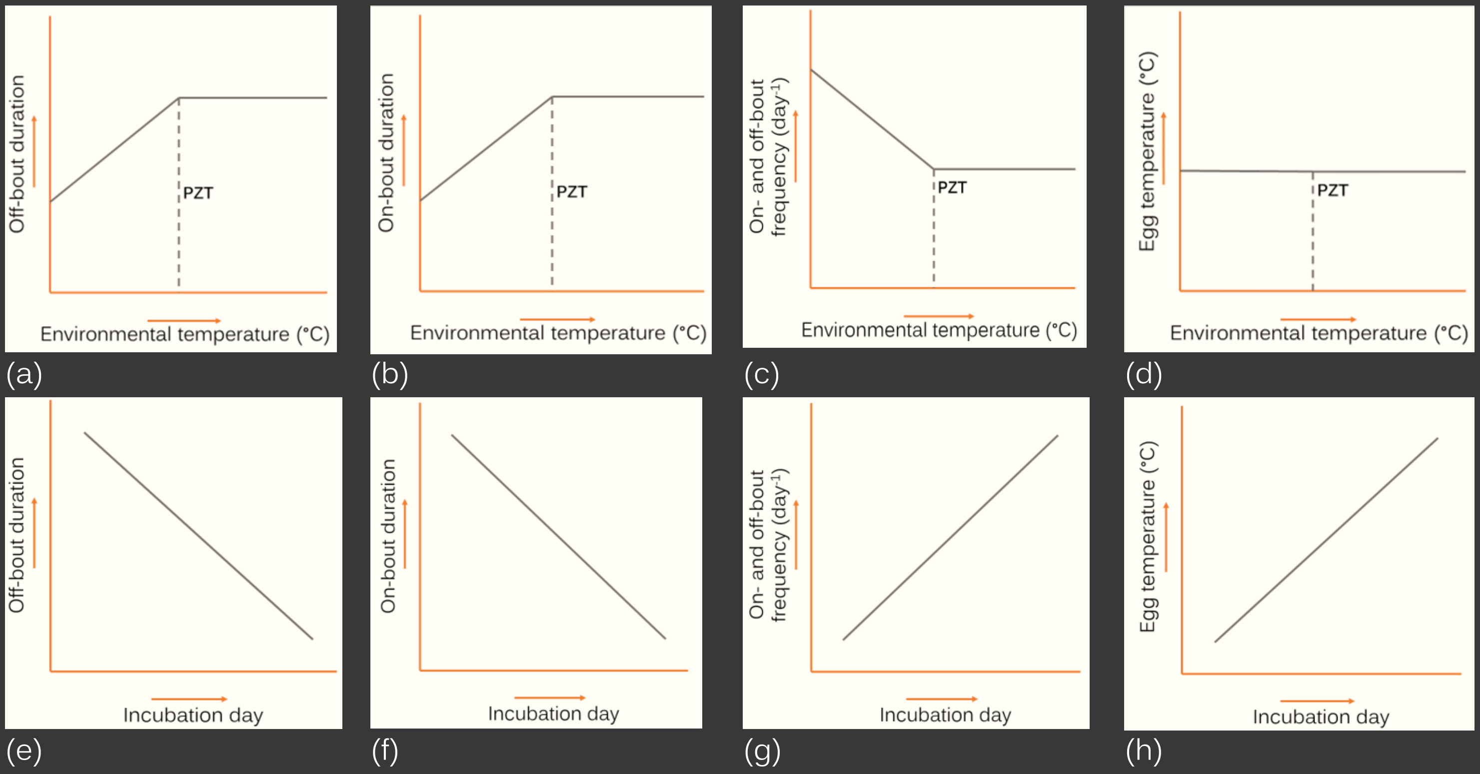


Figure 1. Hypotheses concerning the effect of environmental temperature on off-bout duration (a) and on-bout duration (b), the on- and off-bout frequency (c) and the average egg temperature (d) and how off-bout duration (e), on-bout duration (f), on- and off-bout frequency (g) and egg temperature (h) are influenced by incubation day during the incubation period of *Branta sandvicensis*. PZT refers to the physiological zero temperature.

In a previous study, Weathers & Zaun (2010) observed egg turning behaviour in the Nene. Given this knowledge, I will investigate how egg-turning behaviour is influenced by the environmental temperature. I expect the cooling rate of eggs to be negatively correlated with environmental temperatures. I also expect eggs that are located further from the centre of the nest to receive less heat from the brood patch and to have a higher cooling rate compared to eggs more centrally in the nest. Therefore, I hypothesise that the frequency of egg-turning bouts, *i.e.* the distribution of heat along the eggs in time, is negatively correlated with environmental temperature (Figure 2a). Egg-turning is also assumed to enhance the utilization of albumen during incubation and it is assumed that utilization of albumen increases with embryonic development (Deeming, 1989; Romanoff, 1960). I therefore hypothesise that egg-turning frequency will increase during the incubation period (Figure 2b).

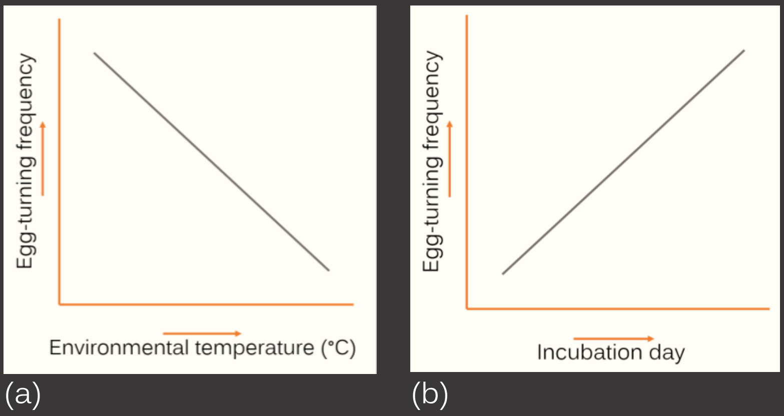


Figure 2. Hypotheses concerning how egg-turning frequency is affected by the environmental temperature (a) and incubation day (b).

RESEARCH METHODS

Study area – The research was conducted during the breeding season of Nenes held in captivity (February-March) in 2021 among multiple locations in The Netherlands where the Nene is kept for conservation or pleasure purposes (Figure 3). The average temperature in The Netherlands is 4.7 and 6.7°C in February and March respectively with a minimum of -8.5°C and a maximum of 18.9°C in February and a minimum of -8.3°C and a maximum of 21.8°C in March during the years 2016-2020 (KNMI, 2020). *B. sandvicensis* individuals used in this research are all kept outside with an adequate amount of food and water within close range.

Figure 3. An overview of locations in The Netherlands where one or more couples of Hawaiian geese are studied during this research. Research is conducted in Barneveld (n=1), Beneden-Leeuwen (n=1) and Groot-Ammers (n=4).

Study species – The species studied during this research is the Hawaiian Goose (*Branta sandvicensis*). *B. sandvicensis* is a non-migratory species native to the Hawaiian Islands Kauai, Maui, Molokai and Hawaii and thought to be derived from the Canada Goose, *Branta canadensis* (Kear & Berger, 2010) although this is still debated (Ottenburghs *et al.*, 2016). Female *B. sandvicensis* start laying eggs at the age of two to three years old and clutch size varies from three to five eggs. Research of *ex situ* incubation in Hawaii shows that eggs are incubated for approximately 28 days by the female, *i.e.* single-sex incubation (Smith, 1952; Kear & Berger, 2010).

Data collection – During this research, 6 nests of *B. sandvicensis* were monitored during the breeding season. An EggLogger covered by a 3D-printed egg (75 x 55 mm, 110 gram) was placed at the start of the breeding season, *i.e.* when only one or two eggs were laid, within the nest to measure internal egg temperature (°C) during the incubation period. I indicated the start of an incubation period from the moment the egg temperature increased from environmental temperature to temperatures above approximately 25 °C from this point on. The EggLogger was set to store measurements 10 minutes after initiation and save measurements when the egg temperature differs 0.1 °C from the previous saved data point. Simultaneously with placing the EggLogger, a camera trap (Reconyx HC500) was installed near the nest of which I could determine at what time of the day and how long the mother is on or off the nest (Figure 4). The settings of the Reconyx HC500 were set to medium-high sensitivity with a 2 pictures 1 sec interval. Environmental temperature was retrieved from nearby weather stations with a maximum distance of 28 km between nest and weather station (KNMI, 2021). Every two weeks, data of both EggLogger and camera trap were collected and batteries of the camera trap were refreshed.

A review of the use of the EggLogger can be found in Appendix 3.

Statistical analysis – Statistical analysis was performed with the program *R* (R Core Team, 2018). Variables that were retrieved during the incubation period of multiple nests were: on- and off-bout frequency (day^{-1}), on- and off-bout duration (s), egg-turning frequency (day^{-1}) and egg- and environmental temperature (°C). Embryonic development is approximated by the incubation day. From the egg temperature and the retrieved times of off-bouts, the cooling rate of an egg during an off-bout is calculated (°Cs^{-1}). First, a Generalized Linear Mixed Model (GLMM), with 'Poisson' set as family and nest number set as a random effect, was used to test whether both egg-turning frequency and on- and off-bout frequency can be explained by both environmental temperature and incubation day. A Linear Mixed Model (LMM), with nest number set as a random effect, was used to test whether on- and off-bout duration and egg temperature can be explained by both environmental temperature and incubation day. In addition, egg temperature was aggregated by mean per incubation day per nest to test for a possible trend. At last, a LMM, with nest number set as a random effect, was also used to test whether the cooling rate of eggs during an off-bout can be explained by the environmental temperature. An overview of dependent and independent variables is given in Table 1.

Figure 4. Research setup of a camera trap near a nest of *B. sandvicensis*.

Table 1. An overview of dependent (Y) and independent (X) variables and which type of test was used to identify a relation between these variables.

Dependent variable (unit)		Independent variable (unit)	Random effect	Statistical test		
				Linear Mixed Model (LMM)	Generalized Linear Mixed Model (GLMM)	Data aggregated
$Y_{\text{egg-turning frequency}}$ (day^{-1})	~	$X_{\text{environmental temperature}}$ (°C)	Nest number		x	
	+	$X_{\text{incubation day}}$ (#)				
$Y_{\text{on/off-bout frequency}}$ (s)	~	$X_{\text{environmental temperature}}$ (°C)	Nest number		x	
	+	$X_{\text{incubation day}}$ (#)				
$Y_{\text{egg temperature}}$ (°C)	~	$X_{\text{environmental temperature}}$ (°C)	Nest number	x		x
	+	$X_{\text{incubation day}}$ (#)				
$Y_{\text{duration on/off-bout}}$ (s)	~	$X_{\text{environmental temperature}}$ (°C)	Nest number	x		
	+	$X_{\text{incubation day}}$ (#)				
$Y_{\text{cooling rate egg}}$ (Δ °C)	~	$X_{\text{environmental temperature}}$ (°C)	Nest number	x		

RESULTS

General information - This research was conducted on six nests of *B. sandviscensis* of which four nests were followed from start of incubation until hatching.

One nest was aborted after an incubation period of 10 days. After these 10 days, one of the two eggs in this nest contained a crack in the shell while the other egg contained a small but dead embryo. An explanation why these eggs were not succesful could be the 2-hour off-bout during the 8th day of incubation where environmental temperatures were <0°C. Because this nest only contains data of the first 10 days of incubation and there is too much data missing, this nest is not included in further analysis. Another nest only contained unfertilized and poorly developed embryos and of which the EggLogger was rejected from the nest on the 27th day. However, this nest will be included in further analysis due to a sufficient amount of data and no deviant data compared to other nests. Incubation starting data ranged from the 5th of February to the 12th of March and incubation period ranged from 31 to 34 days. Clutch size varied from 2 to 6 eggs. During this research, the environmental temperature ranged from -9 to 24 °C (Table 2).

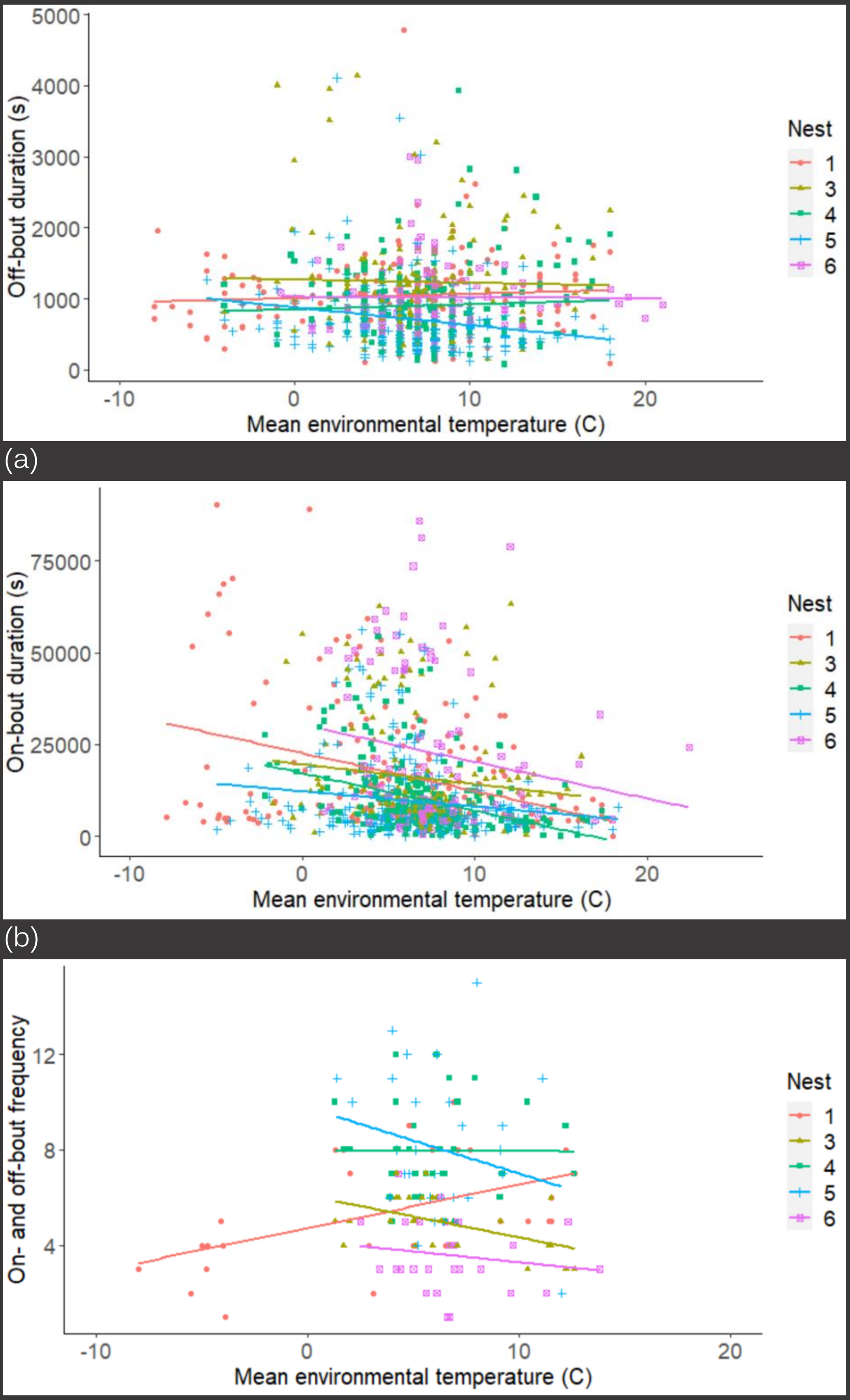
Table 2. General nest overview with the incubation start date, incubation period, environmental temperature range and clutch size per nest of *B. sandviscensis*. Note that for clutch size, '*+1*' represents the EggLogger.

Nest	Incubation start date (dd-mm)	Incubation period (days)	Environmental Temperature range (°C)	Clutch size
1	05-02	34	-9 - 18	5 + 1
2	12-02	NA*	-8 - 18	2 + 1
3	18-02	34	-4 - 18	3 + 1
4	23-02	34	-4 - 18	6 + 1
5	01-03	31	-5 - 19	5 + 1
6	12-03	NA**	-2 - 24	5 + 1

*Nest was aborted after 10 days of incubation and will not be used for further statistical analysis

**Nest was contained unfertilized and poorly developed eggs and EggLogger was abandoned from the nest on day 27

On- and off-bout duration and frequency - I hypothesized that there will be a positive relation between both on- and off bout duration and environmental temperature. The statistical test shows that on-bout duration has a significantly negative relation with the mean environmental temperature during the on-bout (LME; $t = -7.378$, $p < 0.001$). In contrast, off-bout duration has no significant relation with the average environmental temperature during to off-bout (LME; $t = 0.523$, $p = 0.601$) (Figure 5ab). Regarding on- and off-bout frequency, I hypothesised that there will be a negative relation with environmental temperature. The statistical test shows that there is no significant effect of the average environmental temperature on daily on- and off-bout frequency (GLMM; $z = 0.701$, $p = 0.483$) (Figure 5c).



(a)

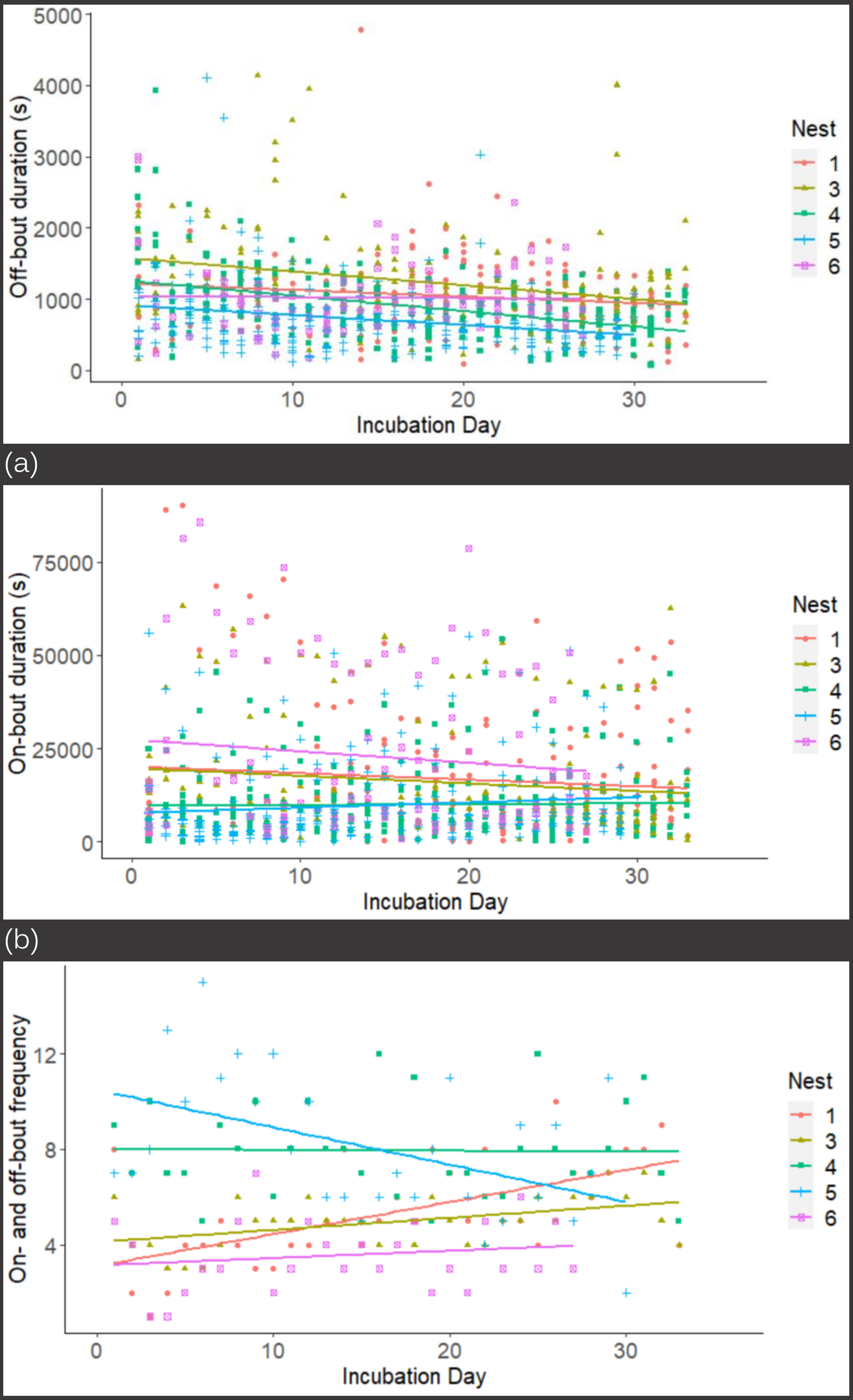
(b)

(c)

Figure 5. The relation of environmental temperature on off-bout duration (a), on-bout duration (b) and on- and off-bout frequency during the incubation period of *Branta sandvicensis*.

Furthermore, I hypothesised that on- and off-bout duration will show a negative relation with incubation day. The statistical test show that there is no significant relation between on-bout duration and incubation day (LME; t = -0.183, p = 0.855). However, off-bout duration is significantly negatively affected by incubation day (LME; t = -8.302, p < 0.001)

(Figure 6ab). Regarding on- and off-bout frequency, I hypothesised that there will be a positive relationship with incubation day. The statistical test showed that there is no significant effect of incubation day on daily on- and off-bout frequency (GLMM; z = 0.788, p = 0.431) (Figure 6c).



(a)

(b)

(c)

Figure 6. The relation of incubation day and off-bout duration (a), on-bout duration (b) and on- and off-bout frequency (c) during the incubation period of *Branta sandvicensis*.

Egg temperature – Mean egg temperature was 31.2 ± 2.1 °C. An overview of the mean egg temperature during on-bouts per nest is given in Table 3.

Table 3. Mean and standard deviation of the egg temperature during on-bouts of the 6 nests of *B. sandvicensis*.

Nest	Mean egg temperature (°C)	Standard deviation
1	28.5	2.3
2	29.5	4.0
3	32.7	3.3
4	33.4	2.2
5	33.2	2.2
6	30.1	2.9

I hypothesised that there will be no relation between egg temperature and environmental temperature. The statistical test shows that egg temperature is not significantly influenced by the environmental temperature (LME; t = -0.337, p = 0.736). However, I hypothesised that egg temperature will increase during the incubation period. The statistical test indeed shows that egg temperature significantly increases during incubation period (LME; t = 9.826, p < 0.001) (Figure 7).

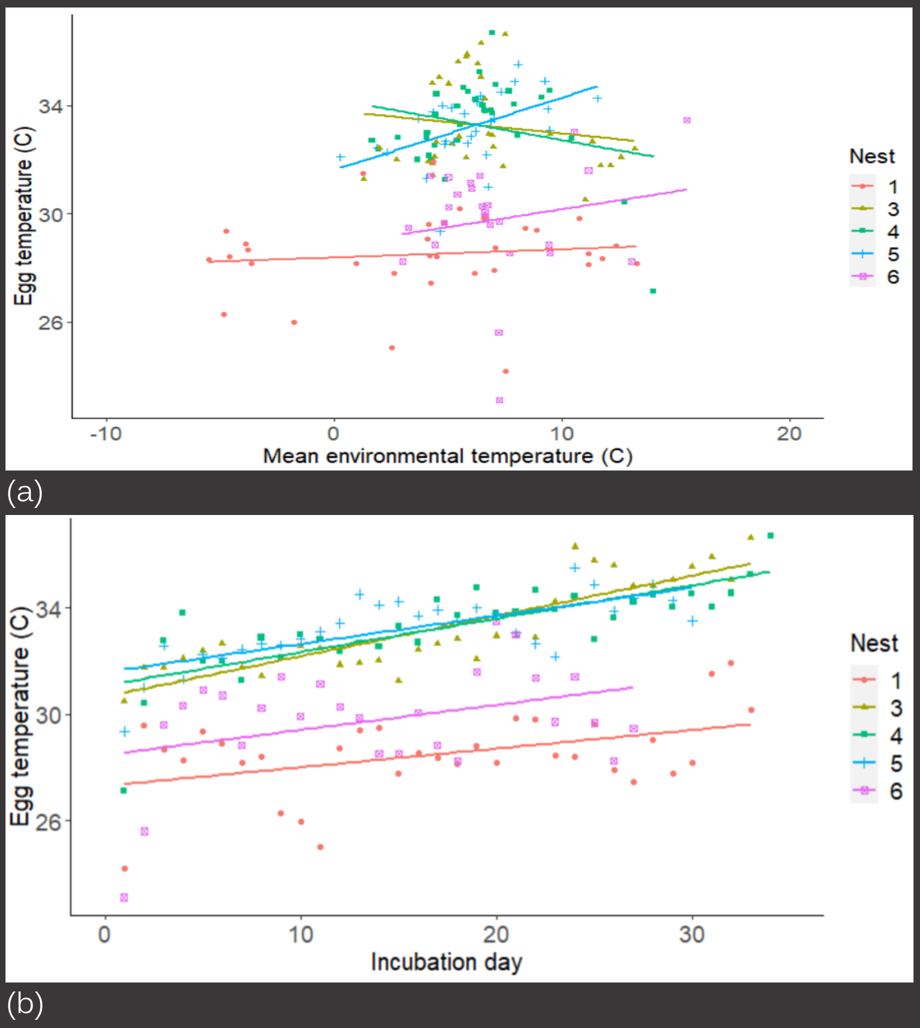


Figure 7. The relation between egg temperature and environmental temperature (a) and incubation day (2) during the incubation period of *Branta sandvicensis*.

Egg-turning – During off-bouts, I expected cooling rates of eggs to be negatively related to environmental temperature. The statistical test shows that the cooling rate of eggs during off-bouts is significantly negatively related with increasing environmental temperatures (LMM; t = -5.056, $p < 0.001$) (Figure 8).

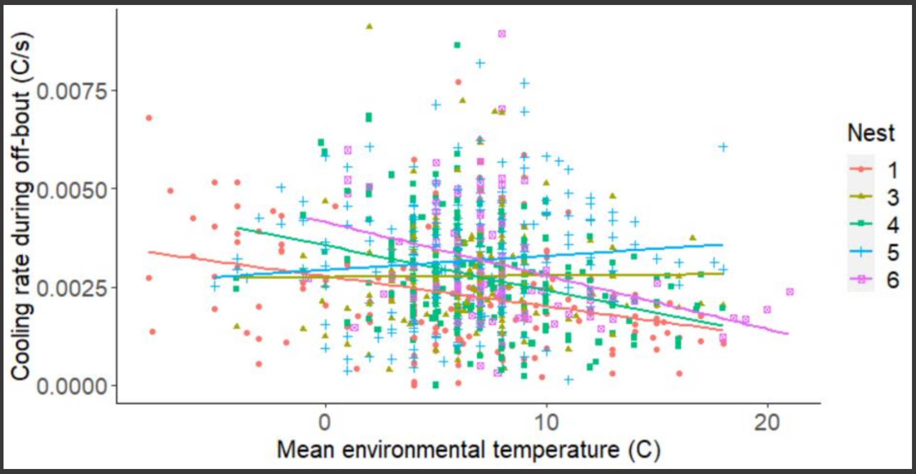
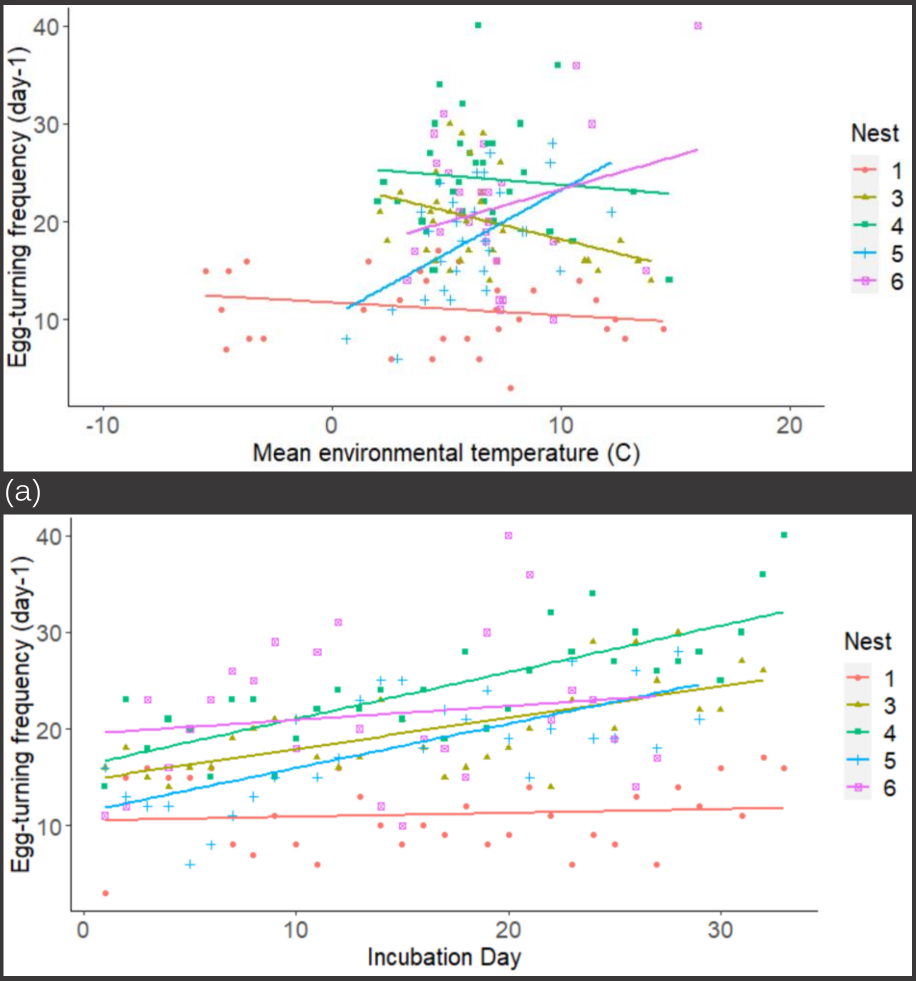


Figure 8. The relation between cooling rate of eggs during an off-bout and incubation day.

Further, I hypothesised that egg-turning frequency will be negatively related to environmental temperature. The statistical test shows that egg-turning frequency is not significantly influenced by the environmental temperature (GLMM; z = -0.377, p = 0.706). I thereby hypothesised that egg-turning frequency will show a positive relation with incubation day. The statistical test shows a positive relation between egg-turning frequency and incubation day (GLMM; z = 7.443, $p < 0.001$) (Figure 9).



(a)

(b)

Figure 9. The relation between egg-turning frequency and environmental temperature (a) and incubation day (b).

An overview of the outputs of the statistical analysis can be found in Appendix 1. Overviews of the egg temperature along the incubation period for each successful nest can be found in Appendix 2.

DISCUSSION

General information - This research was conducted in The Netherlands which has on average much cooler environmental temperatures during the breeding season compared to the native breeding grounds on the Islands of Hawaii. This might explain the much longer incubation periods observed in this research compared to the approximately 28 days described by research performed in Hawaii (Smith, 1952; Kear & Berger, 2010). Moreover, environmental temperatures did not exceed 24 °C and dependent variables did therefore most likely not reach the PZT that was discussed in the introduction. Therefore, we only see, if present, a linear relationship with the explanatory variable instead of a curve approaching an asymptote.

On- and off-bout behaviour and egg temperature - On-bout duration is negatively related to environmental temperature while off-bout duration and on- and off-bout frequency show no relationship with environmental temperature. This means that during low environmental temperatures, off-bout duration is not divided into shorter and more frequent off-bouts as I hypothesised, and as was shown by Conway & Martin (2000), but that only continuous on-bouts are increased. This increase in on-bout duration could mean that mother geese spend less time and thus answer less to their own energetic needs when temperatures are lower. However, this might be a risky strategy when cooler temperatures continue for a longer period. This switch of mother-to-egg energetic investment for prolonged times has not been described in literature yet, but heating of eggs is costly for a mother and this behaviour will most likely have direct consequences for the mother's health and indirect consequences for embryonic development in the eggs due to reduced energetic imput in the eggs (Monaghan & Nager, 1997; Tinbergen & Williams, 2002). However, this observed behaviour could be the result of breeding in captivity where food and water are sufficiently provided within close range of the nest which enables the mother to answer to her own energetic needs in a shorter time and thus stay on the nest longer compared to the wild.

I expected that breeding behaviour of the mother would aim at keeping egg temperature within a narrow range as this is required for proper embryonic development. Although the results of duration and frequency of on- and off-bouts differ from my hypotheses, these strategies do seem to keep egg temperature within a narrow range, *i.e.* not to be influenced by environmental temperatures. However, my hypothesis that egg temperature increases with incubation day was supported, suggesting that embryonic development produces more heat, *e.g.* by a developing metabolic- and blood circulatory system. Although the EggLogger itself does not produce more heat during the incubation period, I assume it showed an increasing temperature because of received heat produced by the surrounding eggs. Because of a developing blood circulatory system of the embryo and therefore increased heat flow, I hypothesised that cooling rates of eggs would increase with embryonic development, approximated by incubation day, as was suggested by Cooper & Voss (2013). Because I used an EggLogger to measure egg temperature which, obviously, did not contain a developing embryo, I cannot directly test my increased cooling rate hypothesis. However, this increased cooling rate should, according to my hypothesis, lead to behavioural changes, such as an increasing on- and off-bout frequency and decreasing on- and off-bout duration. The results show that off-bout duration indeed decreases with incubation day. In contrast, on- and off-bout frequency and on-bout duration do not significantly change with incubation day. Again, I could explain this result with the suggestion that mother geese can shift energetic investment more towards her eggs than towards herself. Because of the fact that the mother does not always performs this breeding behaviour, *i.e.* decreasing off-bout duration while not changing off-bout frequency, suggests that this is not an optimal strategy and is only applied when environmental- and/or embryonic conditions demand it. It would be interesting for future research to study the consequences of this shifted energetic investment for the mother's health, *e.g.* by tracking her body mass, during the incubation period and to investigate how long a mother can continue with this strategy without severe consequences.

Egg-turning behaviour - Furthermore, I hypothesised that egg-turning frequency would be negatively related to environmental temperatures as the distribution of heat among the eggs would be increased when environmental temperatures would decrease. I expected that this distribution of heat is required as eggs furtest from the centre of the nest will receive less heat from the brood patch of the mother and will subsequently have a higher cooling rate during off-bouts compared to more centrally located eggs. Egg-cooling shows a negative relationship with environmental temperature. However, egg-turning frequency does not show a negative relationship with environmental temperature. An explanation for this non-significant result could be that the brood patch of the mother is large enough to cover all eggs so that environmental temperature does not influence eggs located furtest from the centre of the nest. Thereby, in order to perform egg-turning behaviour, a mother needs to elevate herself above her eggs which even allows her eggs to be in more contact with cold air. Thus, egg-turning behaviour could even have negative consequences for egg temperature although this has not been studied yet. Another explanation of this non-significant result could be that the assumption that egg-turning behaviour acts to distribute heat among the eggs is not valid. This assumption is also suggested to be wrong when looking at incubators where heat is approximately equally distributed and eggs are yet turned in order to ensure a larger hatching success (Deeming, 2009, Tullet & Deeming, 1987).

Considering the other assumption why egg-turning behaviour occurs, *i.e.* preventing the embryo to stick to the inner shell membrane and to enhance the utilization of albumen, I hypothesised that this behaviour would show a positive relation with incubation day since the utilization of albumen is assumed to increase with embryonic development (Deeming, 1989; Romanoff, 1960). The results show that egg-turning frequency indeed increases with incubation day. This result therefore supports the assumption that egg-turning frequency functions to enhance this albumen utilization more compared to the assumption that it functions as heat distribution among the eggs.

Conclusion – Overall, this research contributed to general breeding knowledge of *B. sandviscensis* and could help improve incubation settings specifically for this species, *e.g.* by altering the turning-frequency and egg temperature during embryonic development in artificial incubators. However, as this research was conducted on captive geese in The Netherlands, more research is required to ensure whether the same results can be obtained in the native breeding grounds in Hawaii and whether the application of these findings on incubators truly increases hatching success. Subsequently, by continuing this research on other endangered or vulnerable bird species for which captive breeding is used as concervation strategy, we might find a solution to increase the number of individuals that could be re-introduced in the wild.

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APPENDIX 1

Table A1.1. Statistical outcome of the LME test that investigates a relationship between on-bout duration and both environmental temperature and incubation day.

Fixed effects	Estimate	Std. Error	df	t-value	p-value
Environmental temperature	-825.026	111.823	936.448	-7.378	3.54e-13
Incubation day	-9.116	49.9317	935	-0.182575	0.8552
Random effects	**Variance**	**Std. dev**			
Nest number	30369860	5511			

Table A1.2. Statistical outcome of the LME test that investigates a relationship between off-bout duration and both environmental temperature and incubation day.

Fixed effects	Estimate	Std. Error	df	t-value	p-value
Environmental temperature	1.122	4.179	929.573	0.268	0.788
Incubation day	-15.395	1.873	930.614	-8.219	6.87e-16
Random effects	**Variance**	**Std. dev**			
Nest number	43810	209.3			

Table A1.3. Statistical outcome of the GLMM test that investigates a relationship between on- and off-bout frequency and both environmental temperature and incubation day.

Fixed effects	Estimate	Std. Error	df	t-value	p-value
Environmental temperature	0.006746	0.009617	152	0.701	0.483
Incubation day	0.002830	0.003591	152	0.788	0.431
Random effects	**Variance**	**Std. dev**			
Nest number	0.08721	0.2953			

Table A1.4. Statistical outcome of the LME test that investigates a relationship between egg temperature and both environmental temperature and incubation day.

Fixed effects	Estimate	Std. Error	df	t-value	p-value
Environmental temperature	-0.01008	0.02989	150	-0.337	0.736
Incubation day	0.11208	0.01141	150	9.826	< 2e-16
Random effects	**Variance**	**Std. dev**			
Nest number	5.066	2.251			

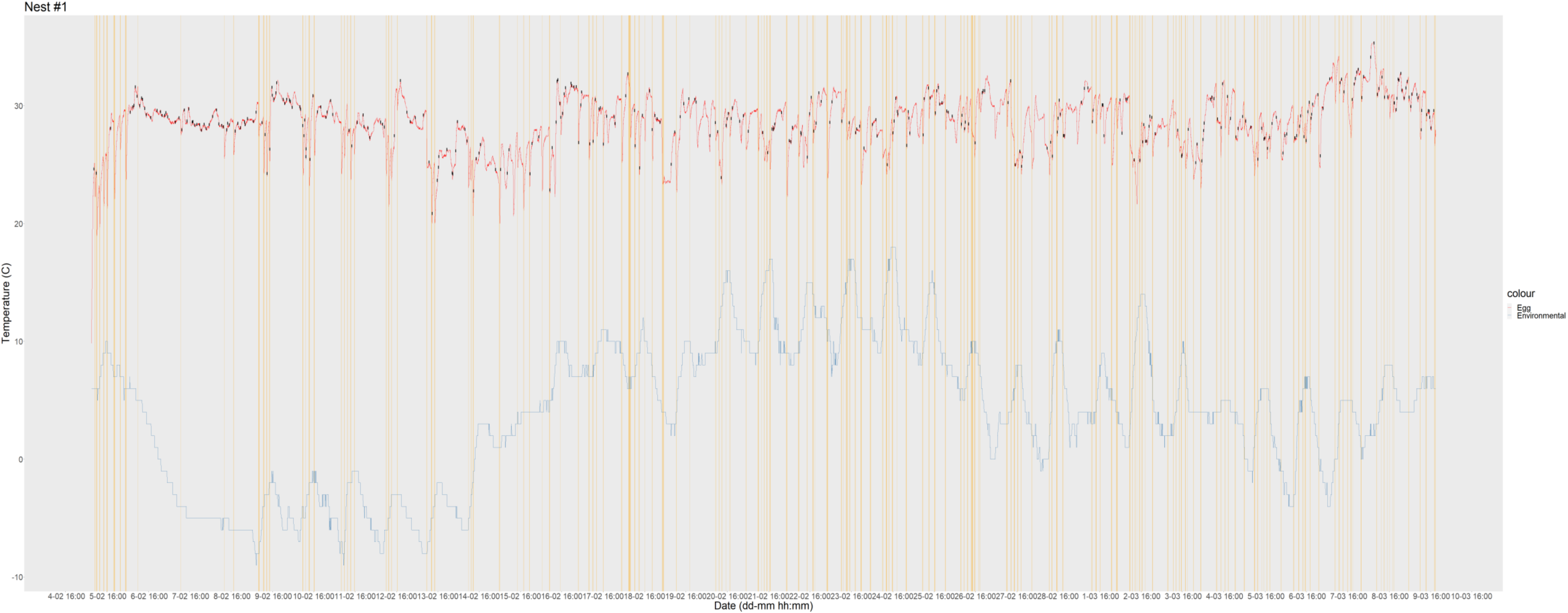
Table A1.5. Statistical outcome of the GLMM test that investigates a relationship between egg-turning behaviour and both environmental temperature and incubation day.

Fixed effects	Estimate	Std. Error	df	t-value	p-value
Environmental temperature	-0.002182	0.005782	150	-0.377	0.706
Incubation day	0.015538	0.002088	150	7.443	9.82e-14
Random effects	**Variance**	**Std. dev**			
Nest number	0.07428	0.2725			

Table A1.6. Statistical outcome of the LMM test that investigates a relationship between cooling rate of eggs during an off-bout and the duration of the off-bout.

Fixed effects	Estimate	Std. Error	df	t-value	p-value
Environmental temperature	-5.290e-05	1.046e-05	9.359e+02	-5.056	5.15e-07
Random effects	**Variance**	**Std. dev**			
Nest number	1.132e-07	0.0003365			

APPENDIX 2 – OVERVIEWS OF EGG TEMPERATURE ALONG INCUBATION PERIODS



Nest #1

Figure A2.1. Overview of egg temperature (red line) during the incubation period of nest 1 (location Groot-Ammers). The blue line represents the environmental temperature, the black dots on the red line represent the observations of egg-turning behaviour and the yellow vertical lines represent off-bouts.

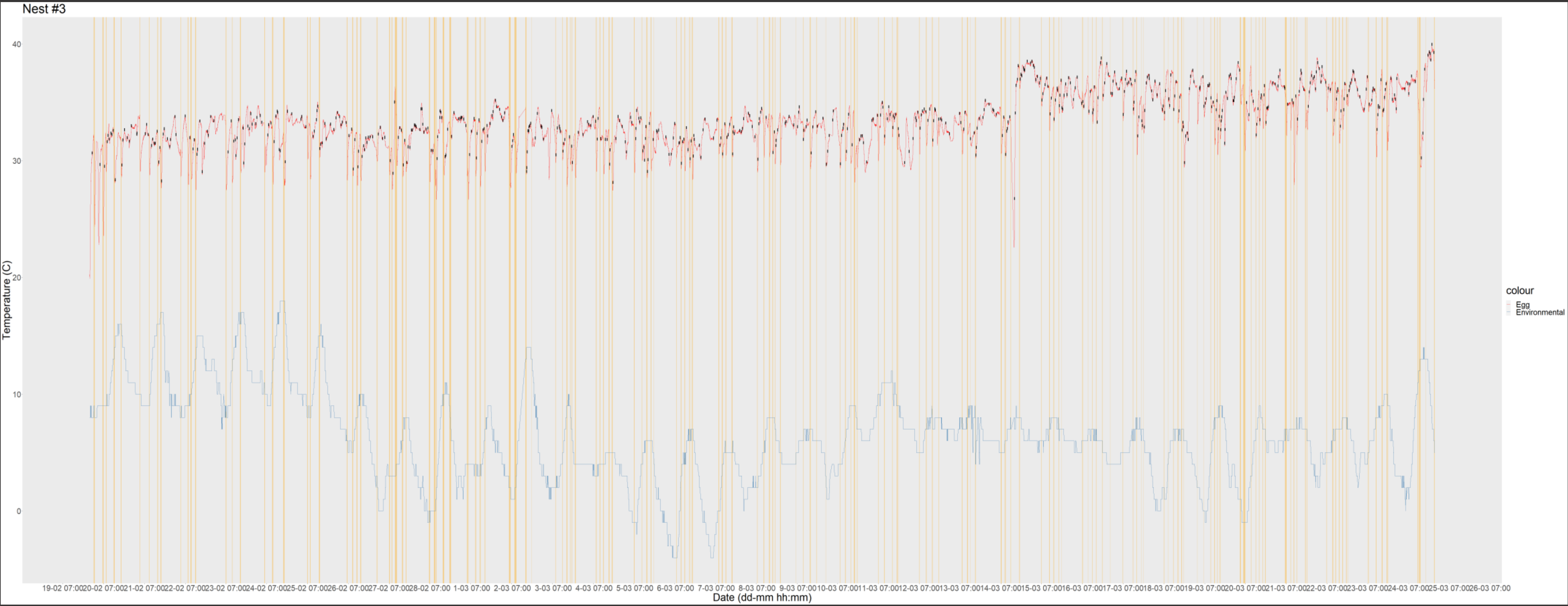


Figure A2.2. Overview of egg temperature (red line) during the incubation period of nest 3 (location Groot-Ammers). The blue line represents the environmental temperature, the black dots on the red line represent the observations of egg-turning behaviour and the yellow vertical lines represent off-bouts.

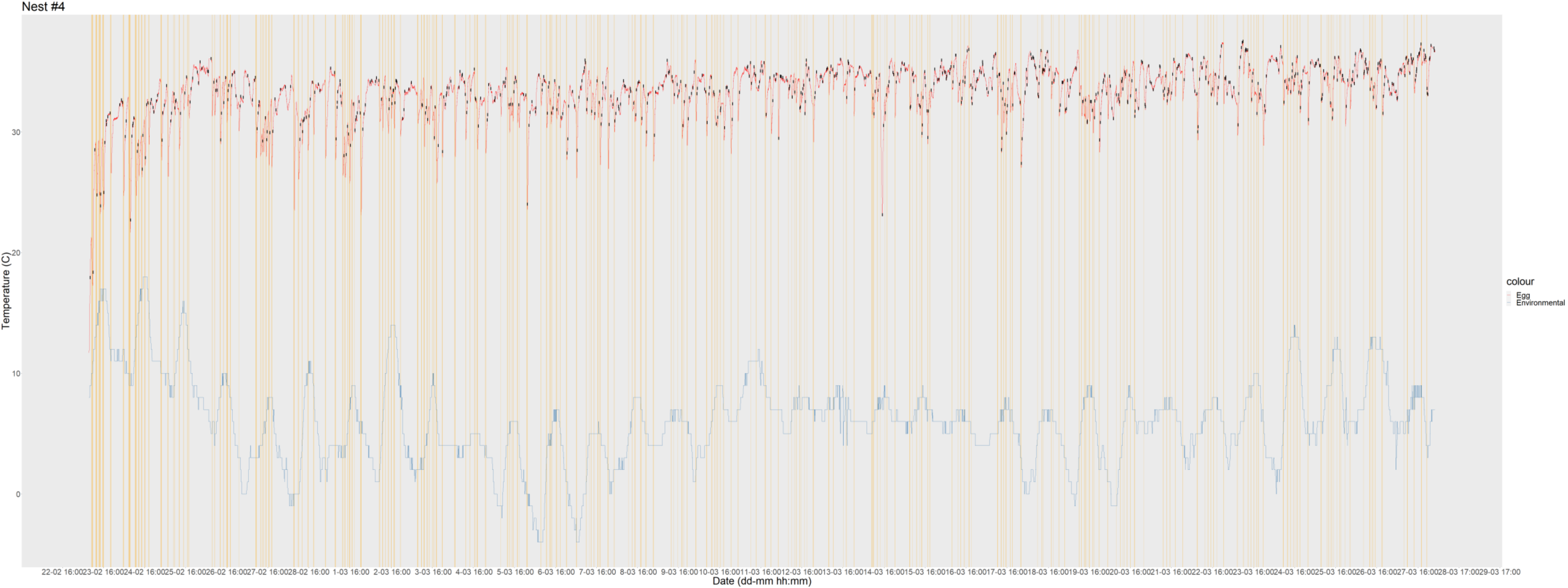


Figure A2.3. Overview of egg temperature (red line) during the incubation period of nest 4 (location Groot-Ammers). The blue line represents the environmental temperature, the black dots on the red line represent the observations of egg-turning behaviour and the yellow vertical lines represent off-bouts.

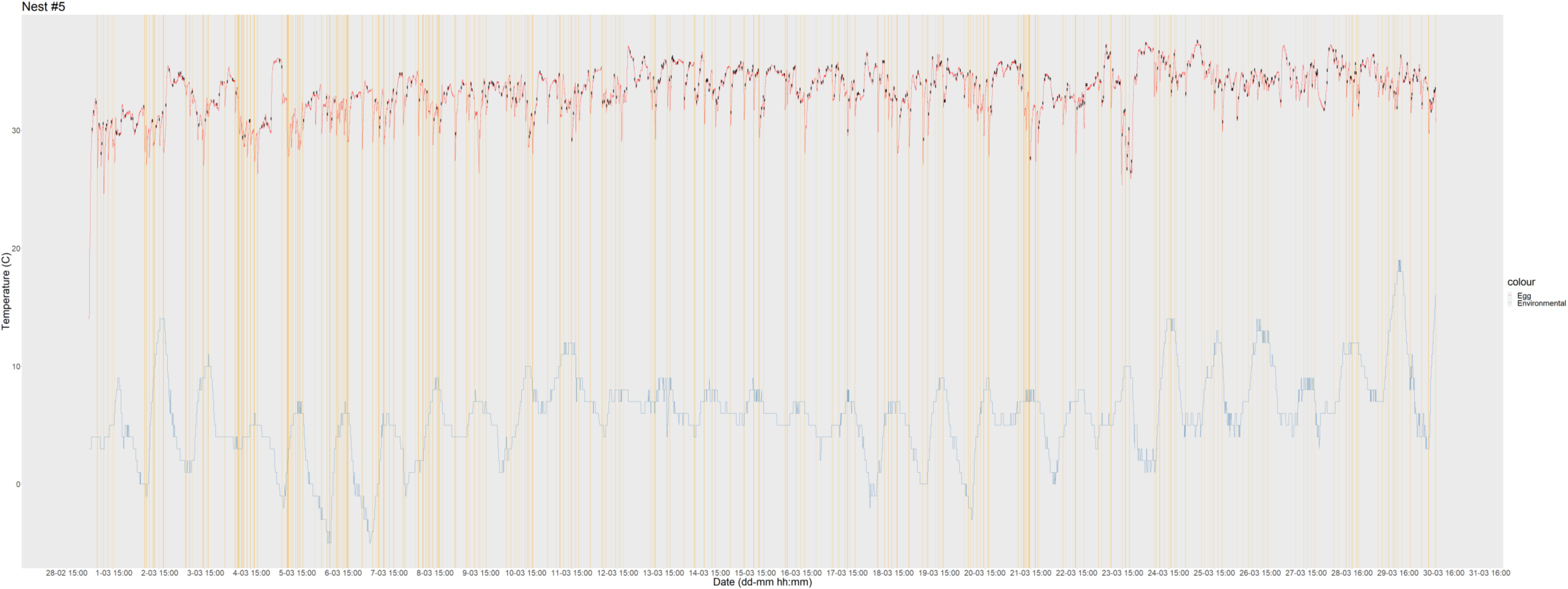


Figure A2.4. Overview of egg temperature (red line) during the incubation period of nest 5 (location Beneden-Leeuwen). The blue line represents the environmental temperature, the black dots on the red line represent the observations of egg-turning behaviour and the yellow vertical lines represent off-bouts.

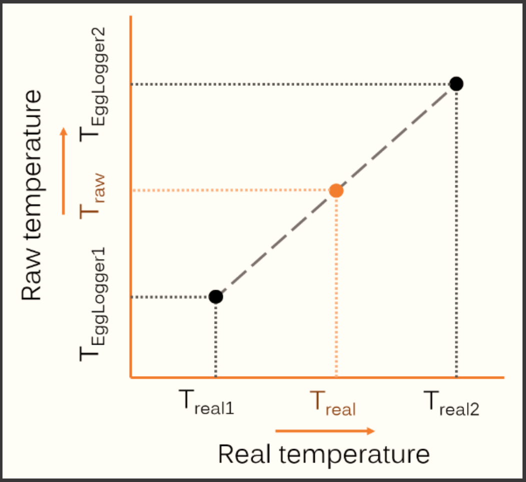
APPENDIX 3: REPORT ON THE USE OF THE EGGLOGGER

The EggLogger used during this research was produced by Marcel Ruizenaar as a private initiative. The EggLogger is a small electronic device covered by a 3D-printed egg and measures the egg temperature and turning of the egg *i.e.* the pitch and roll. The minimum egg size the logger will fit in is 25 x 19 mm. The eggs used in this research have a size of 75 x 55 mm and weigh 110 gram. The EggLogger has to be activated and stored data needs to be collected by the EggLogger interface software (https://www.ruizenaar.eu/egglogger/).

In order to activate, use and read-out the EggLogger, the following materials are required:

- An EggLogger Printed Circuit Board
- A 3D-printed egg (different egg sizes, weights and colours are possible)
- A USB-interface
- A USB-to-micro-USB cable
- A Coin-cell battery typ BR1225 or CR1225
- EggLogger interface software (Microsoft Windows 7 or higher)

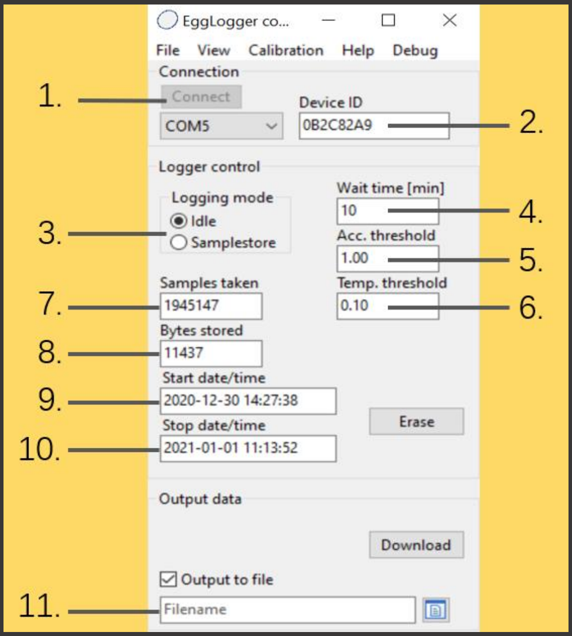
Calibration of the EggLogger - Before the EggLogger was used in this research, it was callibrated in a temperature chamber where the temperature is accurately (0.25 °C) monitored by sensors. During callibration, the temperature chamber was set to two distinctive temperatures T_{real1} and T_{real2}. During these two distinctive temperatures, raw temperature data of the EggLogger was also collected and averaged resulting in $T_{EggLogger1}$ and $T_{EggLogger2}$. With these four obtained values, a simple linear interpolation formula can be derived to calculate the real temperature from a raw temperature data point of the EggLogger.



$$\frac{Treal - Treal1}{Treal2 - Treal1} = \frac{Traw - Tegglogger1}{Tegglogger2 - Tegglogger1}$$

$$Treal - Treal1 = \frac{Traw - Tegglogger1}{Tegglogger2 - Tegglogger1} * (Treal2 - Treal1)$$

$$Treal = \frac{Traw - Tegglogger1}{Tegglogger2 - Tegglogger1} * (Treal2 - Treal1) + Treal1$$



COM5

Device ID: 0B2C82A9

● Idle
○ Samplestore

Wait time [min]: 10

Acc. threshold: 1.00

Temp. threshold: 0.10

Samples taken: 1945147

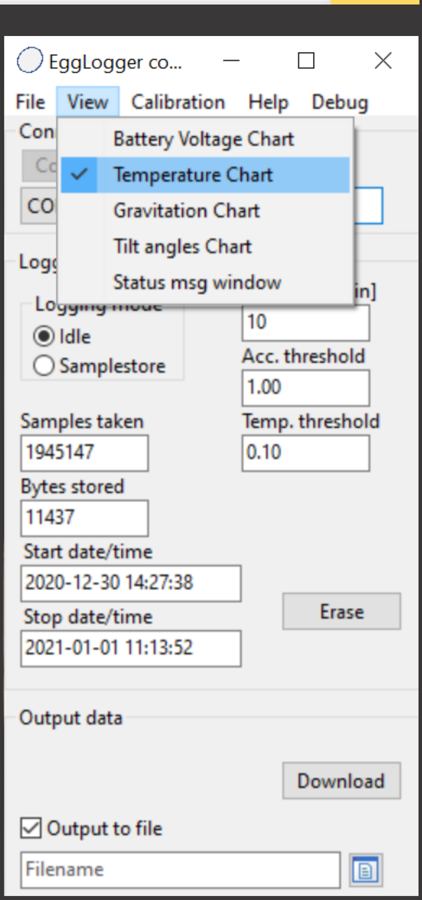
Bytes stored: 11437

Start date/time: 2020-12-30 14:27:38

Stop date/time: 2021-01-01 11:13:52

☑ Output to file

Filename



● Idle
○ Samplestore

10

Acc. threshold: 1.00

Temp. threshold: 0.10

Samples taken: 1945147

Bytes stored: 11437

Start date/time: 2020-12-30 14:27:38

Stop date/time: 2021-01-01 11:13:52

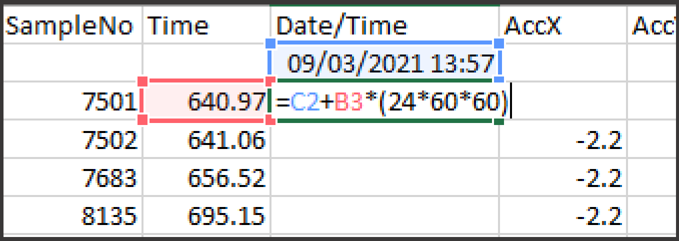
☑ Output to file

Filename

(De)activation and data collection – Before handling the EggLogger, it is important to mention that the EggLogger, like most low-power electronic devices, is very sensitive to static electricity. Therefore, care should be taken to avoid discharging electricity to or from the device. It is common practice to ground your body before handling the EggLogger to prevent static electricity from you hands to flow to or from the EggLogger. Thereby, the EggLogger should be handled with care at all times and should not be in direct contact with water. To start recording, the EggLogger needs to be connected to the computer by means of the dedicated USB-interface and USB-to-micro USB cable. Next, the EggLogger interface software needs to be opened. By pressing the *Connection*-button (1), the EggLogger is recognised by the program and the Device ID (2) of the EggLogger will appear. The *Idle*-option (3) beneath *Logging mode* will be selected when the EggLogger is not activated. By selecting *Samplestore* (3), the EggLogger will be activated and will start taking samples. Before activating, a waiting time before the EggLogger actually stores data can be entered under *Wait time [min]* (4). This means that when the wait time is set to 10 minutes, the EggLogger will not save the samples that are taken during the first 10 minutes after activation. Before activation, there are also two thresholds that can be filled in; the accelaration threshold *Acc. threshold* (5) and the temperature threshold *Temp. threshold* (6). When entering an accelation threshold of 1.00, the EggLogger will save data samples when a sample differs by for instance 1.00 ms^{-2} or more from value of the previously stored data sample. Likewise, when entering a temperature threshold of for instance 0.10 °C, the EggLogger will store temperature data samples only when te value of a sample differs by 0.10 °C or more from value of the previously stored data sample. Under *Samples taken* (7), the number of taken samples can be seen. Under *Bytes stored* (8), the number of actual stored bytes can be seen. *Start date/time* (9) represents the starting date and time when the EggLogger was activated in yyyy-mm-dd hh:mm:ss. *Stop date/time* (10) represents the end date and time when the EggLogger was de-activated (by pressing

Idle (3)). This stop date/time is important to note down and use later to compensate for small errors in the time measurements of the EggLogger. At last, by ticking *Output to file* (11) and entering a file name, the data can be downloaded to the computer as a text file. To make sure all data is downloaded, open the *Temperature Chart* on the *View* option before pressing *Download* to follow the graph in time. When the graph is finished and shows the correct number of seconds, minutes, hours or days, the downloaded file (found in the Download folder of your computer) can be opened by other programs, *e.g.* Microsoft Excel. If the graph is not finished yet when you try to open the downloaded file, there is a high chance some of the data is missing. When the EggLogger is re-used to make new measurements, any existing data in the memory of the EggLogger needs to be erased first by clicking the *Erase*-button. After doing so, logging mode should be set to *Samplestore* (3) and the EggLogger is activated again.

Processing data – When analysing raw data that is retrieved from the EggLogger, the real egg temparture must be calculated according to the formula given above. Thereby, time is counted in seconds after activation of the EggLogger. It is important to have noted down the start date and time to check whether the data is complete and if there are no time-errors. When using Microsoft Excel as a processing program of the data, time in seconds that is measured by the EggLogger can be added to the start date/time as illustrated below:



SampleNo	Time	Date/Time	AccX	Acc
		09/03/2021 13:57		
7501	640.97	=C2+B3*(24*60*60)		
7502	641.06		-2.2	
7683	656.52		-2.2	
8135	695.15		-2.2	

Therefore, the TIME() function in Microsoft Excel should not be used (which only introduces errors in the time clock).

Addtional comments – During this research, I did not look at the specific roll- and pitch values (only to confirm that egg-turning behaviour occured). Therefore, additional information on how to handle roll- and pitch data is not included in this review.