Do repeated captures and handling affect phenotype and survival of growing Snow Petrel (Pagodroma nivea)?

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Do repeated captures and handling affect phenotype and survival of growing Snow Petrel (*Pagodroma nivea*)?

Sophie M. Dupont[1] · Christophe Barbraud[1] · Olivier Chastel[1] · Karine Delord[1] · Charline Parenteau[1] · Cécile Ribout[1] · Frédéric Angelier[1]

Abstract

In vertebrates, developmental conditions can affect not only fledging success but also the phenotype of the offspring, with potential long-term consequences on adult performance. However, surprisingly the potential impact of anthropogenic disturbance on developing chicks is rarely investigated, notably in Antarctic wildlife. In this study, we specifically investigated the effects of repeated nest visits, capture, and handling on offspring survival and several complementary offspring phenotypic traits in the Snow Petrel (*Pagodroma nivea*) chicks after thermal emancipation. We did not find any significant effect of our disturbance protocol on the morphology (body size, body mass, body condition), the physiology (breath rate, stress-induced corticosterone levels) and the behaviour (defense behaviour) of developing Snow Petrels. This specific disturbance protocol did not have any significant effect on chick survival, but there was a non-significant trend towards a lower survival for the disturbed group ($p = 0.1006$), which showed an especially high mortality during a period of repeated snow storms. To conclude, investigator disturbance seems to have little effect on Snow Petrel chicks after thermal emancipation, but to remain cautious, we recommend to avoid capture and handling of Snow Petrel chicks during or soon after inclement weather.

Keywords Disturbance · Survival · Growth · Antarctic seabirds

Introduction

In the context of experimental studies, there is a growing interest in understanding the impact of anthropogenic activities on the phenotype of developing birds. In seabirds, many studies focusing on reproduction or development are associated with repeated nest disturbance and handling of chicks. However, the potential impact of such disturbance on developing chicks is often overlooked in such studies (Gjelsvik 2017). In 2009, Carey reviewed the impact of investigator disturbance on physiology, behaviour, reproductive success, offspring quality, and population trends of Procellariforms (e.g. albatrosses, petrels) and no significant detrimental effects on chick growth were detected in most studies although disturbance often results in food regurgitation and meal loss. For example, O'Dwyer et al. (2006) and Saffer et al. (2000) found no evidence of slower growth

rates in Gould's Petrel (*Pterodroma leucoptera*) and Short-tailed Shearwater (*Ardenna tenuirostris*) chicks in response to repeated handling. Similarly, Sagar et al. (2019) found no adverse effect of repeated handling on the growth of Mottled Petrel (*Pterodroma inexpectata*) chicks. However, Carey (2011) recently found that repeated handling was associated with a lower body mass and a smaller body size in Short-tailed Shearwaters. Altogether, these few studies suggest that the sensitivity of Procellariform chicks to disturbance may vary with environmental conditions and between species, but experimental data are lacking. Importantly, most studies have measured fledging success or body condition to assess the impact of disturbance or repeated handling on chicks, often neglecting other relevant behavioural or physiological indices (Carey 2009, but see Fiske et al. 2013; Wheeler et al. 2013). In addition, only a few studies have so far included the sex of the chick in their analyses although even a slight sexual dimorphism may potentially blur a potential impact of disturbance on growth.

Behavioural and physiological stress responses are increasingly used in conservation to monitor the influence of disturbance or environmental constraints on seabirds

✉ Sophie M. Dupont
sophie.dupont93@gmail.com

1   Centre D'Etudes Biologiques de Chizé, CNRS-ULR, UMR 7372, 79360 Villiers en Bois, France

(Brewer et al. 2008; Wheeler et al. 2009; Angelier et al. 2011; Fiske et al. 2013). Indeed, it is relevant to use multiple and complementary proxies of developmental constraints to assess the impact of disturbance on growing seabird chicks. Circulating corticosterone (CORT) levels are especially relevant to study this question because CORT is often classified as the main "stress" hormone in birds. In response to stressors or energetic challenges, there is a rapid and intense secretion of CORT (Sapolsky et al. 2000; Romero 2004). Increased circulating CORT levels mediate morphological, behavioural and physiological adjustments, which aim to promote immediate survival when stressors occur (McEwen and Wingfield 2003; Romero et al. 2009; Angelier and Wingfield 2013). Breath rate is also increasingly used in such ecological studies because it can be another proxy of the stress response in wild birds (Senar et al. 2017; Angelier et al. 2018; Liang et al. 2018). The stress response is usually associated with ventilatory patterns that help modulate cardiovascular activity to cope with the acute stressor (Grossman 1983) and increased breath rate can be a sign of high stress levels in birds (Karlíková et al. 2018). Finally, behavioural indices can also be useful to monitor the stress response of seabirds. In most Procellariiforms, chicks often regurgitate oily stomach contents when feeling threatened (Imber 1976). Such regurgitation is an effective defensive behaviour because it can damage the feather structure of avian predators, impairing therefore their flight and thermoregulatory abilities (Warham 1977). The propensity of several Procellariiform chicks to regurgitate in the presence of investigators can therefore be one easily-observed parameter to assess their sensitivity to disturbance.

Here, we investigated the potential effects of repeated nest visits, capture, and handling on offspring survival and several complementary offspring phenotypic traits in an Antarctic seabird species, the Snow Petrel (*Pagodroma nivea*). We specifically tested the impact of anthropogenic disturbance for free-living chicks that also have to cope with a suite of environmental constraints (food supply, predation risk, inclement weather). Several chicks from the same colonies (i.e. living in the same environment) were captured, handled and measured soon after emancipation (day 13) and before fledging (day 32). Chicks from the disturbed group were then captured, handled and measured every 5 days between these two dates (days 18, 23, 28) while the chicks from the control group were left undisturbed. We determined the impact of these different handling regimes on growth, body condition, anti-predator behaviour, breath rate and baseline and stress-induced CORT levels. In addition, we identified the sex of chicks and tested whether the sensitivity to repeated disturbance differed between males and females. We also tested whether chicks' survival differed between control and disturbed chicks. To further explore the impact of disturbance on survival, we also monitored

chicks' survival at another site where all chicks remained undisturbed from hatching until a few days before fledging (undisturbed group). If Snow Petrel chicks are sensitive to investigator disturbance, we predict that "disturbed" chicks will be characterized by at least one of the following: (1) a slower growth, (2) higher circulating CORT levels (baseline and stress-induced), (3) higher breath rates, (4) a higher probability to express defensive behaviour, (5) and a lower survival relative to control and undisturbed chicks.

Material and methods

Study species

The Snow Petrel is one of the most sexually dimorphic seabird species (Croxall 1982; Fairbairn and Shine 1993), is long-lived with a high survival probability and has low fecundity (only one egg per couple and per year, Chastel et al. 1993). Sexual maturity is acquired on average at 9.9-years of age (Chastel et al. 1993) but the earliest age of first breeding is 6 years (Jenouvrier al. 2005). Snow Petrels attempt to breed annually although a varying proportion of birds skip breeding each year (Chastel et al. 1993; Jenouvrier et al. 2005). This altricial species provides bi-parental care both during incubation and brooding (Brown 1966; Isenmann 1970). At the age of ~ 10 days, the chick becomes thermally emancipated and is left unattended at the nest. Both parents forage at sea to feed their chick and restore their own body reserves (Brown 1966; Barbraud et al. 1999). The chick fledges when approximately 47-days of age (Brown 1966; Barbraud et al. 1999).

Experimental disturbance protocol

The field study was carried out during the austral summer of 2017/2018 at Ile des Pétrels, Pointe Géologie Archipelago, Terre Adélie (66° 40′ S, 140° 01′ E), in East Antarctica. During the last part of the incubation period, 110 nests were monitored and hatching date were determined by daily observation of the nests. A sub-sample of those nests was selected and then divided into three groups: "undisturbed", "control" and "disturbed" (Fig. 1). Both "control" and "disturbed" groups of nests were left undisturbed until the chicks were 13 days old and were left alone in the nest (i.e. thermal emancipation). The "disturbed" nests were visited every 5 days during which the chicks were approached by the field worker, handled and measured from day 13 (hereafter named early stage) to day 32 (hereafter named late stage) after hatching; the "control" nests were left undisturbed between early and late stages and control chicks were only approached, captured, and handled on day 13 and day 32 to allow comparisons in morphometric, behavioural and
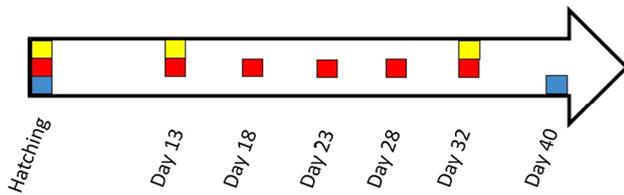
Fig. 1 Experimental disturbance protocol. All nests from all groups were monitored for hatching. Control (Yellow) and Disturbed (Red) chicks were then captured, handled, measured, and bled 13 and 32 days after hatching to monitor growth, behaviour, physiology and survival from day 13 to day 32. Disturbed chicks were additionally captured, handled, and measured 18, 23, and 28 after hatching. Undisturbed (Blue) chicks were part of a long-term monitoring programme, and they were left totally undisturbed from hatching to 40 days after hatching

physiological parameters between the disturbed and the control groups (Fig. 1). Therefore, disturbed chicks ($N=41$) were handled 5 times in total. In contrast, control nests were left undisturbed (no visit) from day 13 to day 32, and thus, control chicks ($N=14$) were manipulated 2 times in total. Experimental handling of disturbed chicks consisted of capturing, measuring and weighing the chick for a total of 10 min. The "undisturbed" chicks ($N=23$) were left totally undisturbed from hatching until day 40 with no visit, capture or handling. After hatching, these chicks were thus only visited once (at day 40) to ring them before fledging. The nests of these chicks are part of a long-term capture-mark-recapture programme conducted since 1964 in Terre Adélie.

Early and late morphological, physiological and behavioural measurements

On days 13 and 32, all chicks were bled according to a standardized stress protocol to measure their CORT stress response (Wingfield et al. 1992). Immediately after capture, an initial blood sample was collected within 3 min of capture by puncturing the alar vein using a 25-gauge needle and heparinized microcapillary tubes (~ 150 μL). This initial sample allowed us to measure baseline CORT levels, which are independent of the stress of capture. When entering the colony, we were as discrete as possible to avoid detection by the chicks. This was easily achieved by the specific configuration of the colony and the nests, which are located into crevices or hidden under and between rocks. After this blood collection, tarsus size was measured for all disturbed and control chicks with a calliper (± 0.1 mm). All chicks were also weighed using a spring balance (± 5 g). These morphometric measurements were chosen because they have been previously demonstrated as robust marker to study the influence of anthropogenic disturbance on chick development and growth (Saffer et al. 2000; O'Dwyer et al. 2006; Carey 2011). We also measured individual breath rate at day 13 and 32 by counting the number of respiratory movements during 30 s while holding the bird in a natural position (i.e. laying on its abdomen). The chick was then kept in a tissue bag until a second blood sample (~ 150 μL) collected 30 min after capture. This second sample allowed us to measure stress-induced CORT levels (called hereafter stress-induced CORT), a proxy of the hormonal sensibility to stress (Angelier and Wingfield 2013). In addition, at day 13 and 32, we recorded whether a chick exhibited defensive spitting behaviour at capture by monitoring whether it regurgitated stomach oil or not (Imber 1976; Warham 1977). Thus, we were able to monitor the behavioural stress response (binomial variable, 1: spit; 0: no spit). The Snow Petrel is an altricial species. Consequently, chicks could not respond to predation risk by escaping from the nest and they instead exhibit this specific defensive behaviour. We used this observation to estimate to what extent disturbed and control chicks felt threatened by human disturbance.

At each nest check, we also noted whether the chick was alive (scored 1) or not (dead body or empty nest, scored 0) at day 13, 18, 23, 28, and 32 for disturbed nests; at day 13 and 32 only for control nests; to monitor the survival between each capture and during the whole study period (from day 13 to day 32). For the undisturbed group, we determined the chick's survival only on day 40.

Body condition and growth rate

Body condition was calculated for all disturbed and control chicks using the scaled mass index (SMI) following Peig and Green (2009). Tarsus length is highly correlated with body mass (tarsus: Pearson rank correlation, $r=0.8872$; $n=55$; $p<0.0001$) and was therefore used to calculate the SMI of the chicks. We determined the change in tarsus length, body mass and body condition between day 13 and 32 for all chicks that survived until day 32 by subtracting the measurement at day 13 from the measurement at day 32.

CORT assay and molecular sexing

Immediately at the end of each field session, we centrifuged blood samples (5000 rpm for 7 min) to separate plasma and red cells. Samples were stored at − 20 °C until analyses at the Centre d'Etudes Biologiques de Chizé laboratory. Plasma CORT concentration was determined by radioimmunoassay as described previously in Angelier et al. (2007). DNA was extracted from red cells samples and the sex of each disturbed/control chick was determined by molecular sexing (see Angelier et al. 2015), based on polymerase chain reaction (PCR) amplification of the CHD gene (see Fridolfsson and Ellegren 1999).

Statistical analyses

Firstly, we tested if morphometric, behavioural and physiological variables differed between disturbed and control chicks and between sexes (males and females) at day 13 (i.e. at the early stage of our experimental protocol); at day 32 (i.e. at the late stage of our experimental protocol) by using linear models (LM, morphology and physiology) or general linear models (GLM with a binomial distribution, behaviour) with sex of the chick and experimental disturbance procedure as the explanatory variables (Table 1). Note that at day 13, almost all chicks regurgitated stomach oil in both groups, and therefore, we did not incorporate these data in the analyses. Secondly, we studied the influence of our experimental protocol (disturbed and control) and sex (males and females) on the morphological and physiological changes from day 13 to day 32 by using linear models (LM) with sex of the chick and experimental disturbance procedure as the explanatory variables (Table 1). Thirdly, we tested the influence of our experimental protocol (disturbed and control) and sex (males and females) on chick's survival between day 13 and day 32 (1 and 0 represent respectively alive and dead chicks) by using GLM with a binomial distribution (Table 1) with sex of the chick and experimental disturbance procedure as the explanatory variables. Finally, we tested whether chicks' survival differed between the three groups (disturbed, control and undisturbed) by using GLM with a binomial distribution and our experimental disturbance procedure as the explanatory variable. We modelled the main effect of our experimental protocol using Tukey's range tests. In addition, we conducted a sensitivity analysis and reported the effect sizes (Hedges' *g*) for all comparisons. All statistical analyses were performed with R 3.4.2 (R core Team 2015).

Results

Morphometric, behavioural and physiological measurements on day 13 (early stage)

On day 13, all morphometric measurements were similar between the disturbed and the control chicks (Table 2). In both groups, tarsus length did not differ between males and females, but males were heavier and in better condition than females (ANOVA, $F_{1,52} < 0.01$, $p = 0.9114$; $F_{1,53} = 7.30$, $p = 0.0092$; $F_{1,53} = 10.22$, $p = 0.0023$, respectively for tarsus length, body mass and SMI). Breath rate was similar for disturbed and control chicks (Table 2) and for sexes (ANOVA, $F_{1,52} < 0.01$, $p = 0.9585$). Finally, baseline and stress-induced CORT levels neither differ significantly between disturbed and control chicks (Table 2) nor between sexes (ANOVA, baseline, $F_{1,52} < 0.01$ $p = 0.9845$; stress-induced, $F_{1,53} = 3.64$, $p = 0.0617$).

Morphometric, behavioural, physiological variables and survival on day 32 (late stage)

On day 32, all morphometric measurements between the disturbed and the control chicks were similar (Table 2). Tarsus length, and body mass significantly differed between males and females; males were larger and heavier than females (ANOVA, $F_{1,23} = 6.63$, $p = 0.0169$; $F_{1,23} = 6.01$, $p = 0.0223$, respectively for tarsus length and body mass). SMI did not differ between males and females (ANOVA, $F_{1,23} = 2.22$, $p = 0.1502$). Defensive behaviour differ neither between disturbed and control chicks (Table 2) nor between sexes (Chi-square test, $\chi^2_{23} = 1.51$, $p = 0.2190$). Breath rate was not significantly different between disturbed and control chicks (Table 2) and between sexes (ANOVA, $F_{1,53} < 0.01$, $p = 0.9855$). Baseline CORT levels did not differ between the sexes (ANOVA, $F_{1,22} = 0.26$, $p = 0.6124$) but those of disturbed chicks were significantly lower than those of control chicks (Table 2). This was however mainly due to 3

Table 1 Description of the models, which were built to test the influence of the experimental disturbance protocol on morphological, behavioural and physiological variables at day 13 and 32 as well as on chick's survival between these two stages

Biological assumption	Dependent variable	Independent variable	Model type
Effects of disturbance on chicks' morphology and condition	Tarsus length	Disturbance, sex of the offspring, interaction	LM
	Body mass	Disturbance, sex of the offspring, interaction	LM
	Body condition	Disturbance, Sex of the offspring, interaction	LM
Effects of disturbance on chicks' physiology	Baseline CORT level	Disturbance, sex of the offspring, interaction	LM
	Stress-induced CORT level	Disturbance, sex of the offspring, interaction	LM
	Breath rate	Disturbance, sex of the offspring, interaction	LM
Effect of disturbance on chicks' behaviour	Defensive behaviour (spit or no spit)	Disturbance, sex of the offspring, interaction	GLM
Effect of disturbance on chicks' survival	Survival (alive or dead)	Disturbance, Sex of the offspring, interaction	GLM

Table 2 Effects of the experimental disturbance protocol (disturbed and control) on morphometric, behavioural and physiological variables at day 13 and 32 respectively and on the survival rate between day 13 and 32 (mean ± SE for all variables)

	Day 13					Day 32				
	N (disturbed, control)	Disturbed chicks	Control chicks	p	Hedges' g	N (disturbed, control)	Disturbed chicks	Control chicks	p	Hedges' g
Tarsus length (mm)	41, 14	29.05±0.22	29.16±0.51	0.8130	0.0716	16, 9	37.43±4.63	37.19±6.19	0.8250	0.0129
Body mass (g)	41, 14	255.61±7.39	248.57±12.16	0.6685	0.1447	16, 9	429.06±54.18	401.11±70.08	0.6830	0.1303
Body condition (g)	41, 14	329.75±49.64	320.81±16.47	0.6229	0.1692	16, 9	320.91±40.06	302.40±51.02	0.3887	0.1173
Baseline CORT level (ng mL^{-1})	41, 14	3.04±0.41	2.96±0.72	0.9246	0.0303	16, 9	3.43±0.54	7.02±1.92	**0.0353**	0.9419
Stress-induced CORT level (ng mL^{-1})	41, 14	39.67±2.24	41.76±2.93	0.6523	0.1540	16, 9	43.96±5.94	42.75±8.02	0.9350	0.0507
Breath rate	41, 14	50.49±1.88	51.36±3.40	0.8188	0.0713	16, 9	54.00±7.16	48.56±8.31	0.3776	0.1985
Defensive behaviour occurrence (%)						16, 9	68.75±11.21	77.78±17.29	0.4280	0.1893
Survival between day 13 and 32 (%)						16, 9	39.02±7.71	64.28±13.29	**0.1006**	0.5066

The bold value indicates the significant factors

All parameters were also tested for sex-differences (presented in the Results part of the manuscript). The interactions 'disturbance × sex' were non-significant for all variables (all $p > 0.2355$). p values were found using LM and GLM as previously described in Table 1. Hedges' g were calculated by adjusting the calculation according to the sample size of each group. N (disturbed, control) denoted the number of individuals in the disturbed and control groups

control birds, with very high baseline CORT levels (> 12 ng/mL while all other control chicks were below 6 ng/mL). Stress-induced CORT levels were similar between disturbed and control chicks (Table 2) and between sexes (ANOVA, $F_{1,23} = 0.24$, $p = 0.6257$). Finally, survival rates did not significantly differ between disturbed and control chicks although the former had a slightly lower survival probability from day 13 to day 32 (Table 2; Figs. 1, 2). Survival of males and females was similar (Chi-square test, $\chi^2_{22} = 0.16$, $p = 0.6845$). Regarding the disturbed group, most chicks died during the second phase of the study when the chicks were > 23 days old (mortality from 13 to 23 days after hatching: 19.5%; mortality from 23 to 32 days after hatching: 51.5%, Fig. 2). This increased mortality coincided with major snow storms which covered most nests and chicks with snow (Fig. 3).

Changes in morphometric, behavioural and physiological variables between days 13 and 32

From day 13 to day 32, changes in morphometric variables did not differ between disturbed and control chicks (Table 3) but tarsus grew larger and faster in males than females (ANOVA, $F_{1,23} = 9.08$, $p = 0.0062$). However, changes in body mass and SMI did not differ between sexes (ANOVA, $F_{1,23} = 2.01$, $p = 0.1696$; $F_{1,23} = 0.43$, $p = 0.5168$, for body mass and SMI, respectively). From day 13 to day 32, changes in breath rate differed neither between disturbed and control chicks nor between sexes (ANOVA, Table 3, $F_{1,22} = 0.18$, $p = 0.6732$). Finally, over the same period, changes in baseline or stress-induced CORT levels were similar for disturbed and control chicks as well as for males and females (ANOVA, Table 3, $F_{1,22} = 0.17$, $p = 0.6875$; $F_{1,23} = 1.11$, $p = 0.3035$, for baseline and stress-induced CORT levels, respectively).

Comparison with the undisturbed group

Survival rate did not significantly differ between undisturbed, control and disturbed chicks (Chi-square test, $\chi^2_{75} = 4.25$, $p = 0.1196$; Tukey test, $z_{\text{undisturbed – control}} = -0.21$, $p = 0.8350$; $z_{\text{undisturbed – disturbed}} = 1.66$, $p = 0.0962$).

Discussion

In this study, we demonstrated that based on our protocol, repeated nest visits, captures, and handlings had little effect on the growth, the behaviour and the physiology of Snow Petrel chicks. We did not find any significant effect of investigator disturbance on body size, body mass, and body condition of developing Snow Petrels. In addition, defensive behaviour, breath rate and stress-induced corticosterone
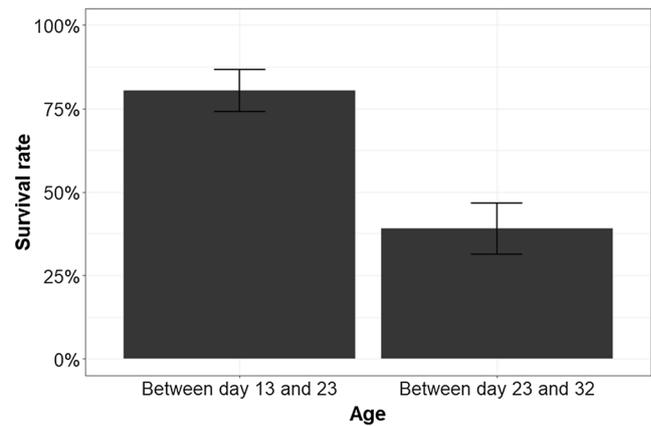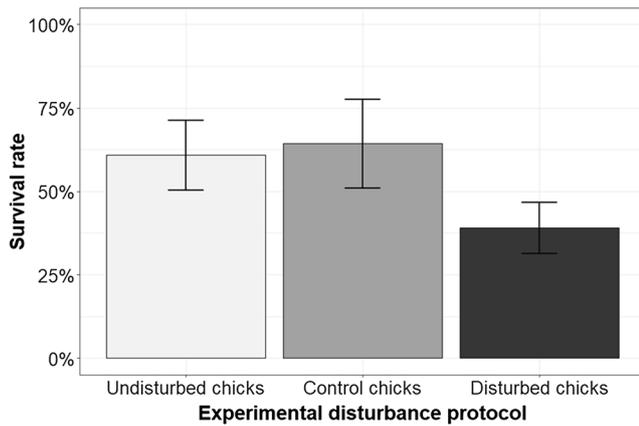
Fig. 2 Survival of Snow Petrel (*Pagodroma nivea*) chicks in relation with nest disturbance (*disturbed*: 3 visits, captures, and handling events between early (day 13) and late (day 32) measurements; *control*: no visit, capture and handling event between early and late measurements): (A) between day 13 and day 32 (control and disturbed chicks) and between hatching and day 40 (undisturbed group) and (B) only for *disturbed* chicks, survival between day 13 and day 23 (before massive snowstorms) and between day 23 and day 32 (during and after snowstorms). Results are given as means ± standard deviation

Fig. 3 Picture of a 26-days old Snow Petrel (*Pagodroma nivea*) chick under the snow. Picture by Sophie M. Dupont

levels were similar in the two groups. Finally, we did not find any significant effect of our disturbance protocol on survival probability. Altogether, these results support the idea that repeated investigator disturbance did not significantly affect fledging success and the development of the chicks, at least when such disturbance is conducted after thermal emancipation and as long as it is not more frequent than every 5 days.

In our study, we specifically evaluated the impact of different handling regimes on the chicks' morphology and growth. Our design allowed us to precisely monitor morphological and physiological changes between early and late development. However, all control chicks were handled at least once before the final measurements (at 13 days old) and, consequently, we were only able to compare the impact of repeated captures and handling vs. the impact of a single capture and handling event. We included a third group of birds (undisturbed group), which were not captured, handled during the study period (only on day 40), and not bled. Therefore, we could not compare their morphology, physiology, and behaviour with our control and disturbed groups.

Survival

In our study, we found that repeated handling did not have a significant effect on chick survival. However, there was a non-significant trend towards a lower survival probability for disturbed chicks and we also found that most of this mortality (i.e. observation of dead bodies) occurred towards the end of the study period when the chicks were > 23 days old. This result was unexpected because in Procellariiforms most chick mortality usually occurs soon after hatching or at the time of thermal emancipation (Prince et al. 1994; Catry et al. 2006; Wheeler et al. 2013). In 2018, food appears to

Table 3 Effects of the experimental disturbance protocol (disturbed and control groups) on morphometric and physiological changes between day 13 and 32 (mean ± SE for all variables)

	Changes between day 13 and 32				
	N (disturbed, control)	Disturbed chicks	Control chicks	p	Hedges' g
Tarsus length (mm)	16, 9	8.07 ± 1.04	8.14 ± 1.43	0.4427	0.0167
Body mass (g)	16, 9	165.31 ± 24.22	168.33 ± 33.46	0.6866	0.0306
Body condition (g)	16, 9	− 12.77 ± 10.11	− 2.14 ± 17.56	0.8085	0.2365
Baseline CORT level (ng mL^{-1})	16, 9	0.79 ± 0.55	3.56 ± 2.28	0.1921	0.6273
Stress-induced CORT level (ng mL^{-1})	16, 9	5.13 ± 3.14	− 3.17 ± 6.55	0.4429	0.5389
Breath rate	16, 9	2.44 ± 4.42	− 3.00 ± 5.47	0.3796	0.0324

All parameters were also tested for sex-differences (presented in the Results part of the manuscript). The interactions 'disturbance × sex' were non-significant for all variables (all $p > 0.2355$). p values were found using LM as previously described in Table 1. Hedges' g were calculated by adjusting the calculation according to the sample size of each group. N (disturbed, control) denoted the number of individuals in the disturbed and control nests

have been at least sufficient during the study period as supported by the information on Table 2. However, the end of January was characterized by extremely harsh weather with multiple snowstorms. At this period, the occurrence of such events is unusual (Wingfield et al. 2011a). Most nests were covered and filled with snow and several chicks became wet and probably died from hypothermia when the snow melted (S. Dupont, personal observations). Importantly, these weather events may have exacerbated a potentially detrimental impact of repeated handling on chick survival. Because Snow Petrel chicks are usually agitated at capture and release, disturbed chicks may have come into contact with melted snow and this is known to increase the risk of mortality (Wingfield et al. 2011a, b, 2017). In addition, the handling of the chicks and their post-handling agitation may have drawn attention of Skuas to their nests and then increased the predation risk of these disturbed chicks. However, chicks were carefully replaced to their nests and their movements were monitored at distance for 15 min to ensure that they remained in their nests. Moreover, predation on Snow Petrels by South Polar Skuas is very rarely recorded at this study site where Skuas preferentially feed on Adélie Penguins, *Pygoscelis adeliae* (Isenmann 1970; Jouventin and Bried 2001). Survival rates were also not entirely comparable between the undisturbed group and the two other groups (control and disturbed) because the survival among undisturbed chicks was checked between hatching and day 40 while the survival of control and disturbed chicks was monitored between days 13 and 32. Note that between hatching and day 13 mortality was very low, i.e. 5.62% for all the nests that were monitored for hatching and then visited on day 13. Although the difference in survival between disturbed and either control or undisturbed chicks was not significant, this trend and this slight difference in methodology between the three groups must alert us to be cautious and we recommend to avoid any chick handling and nest visit soon after snow storms or/and when chicks are covered with snow.

Growth, body size and body condition

Our experimental disturbance protocol did not affect chick growth and morphology. These results are in line with several studies of other Procellariform species (Saffer et al. 2000; Brewer et al. 2008; Fiske et al. 2013), although another study found detrimental effects of investigator disturbance on growth (Carey 2011). This discrepancy may be explained by several factors. Firstly, in our study, we did not measure several environmental variables that could affect growth and fledging success (e.g. food availability). However, our experiment was conducted during a reproductive season during which the growth, body mass and body condition were relatively good (Sauser et al. 2018). In addition, no chick starved to death before the snowstorm. This suggests that nutritional conditions were relatively good during the study period. These good nutritional conditions are important to emphasize because it means that our results could have been totally different in another context. More precisely, we could expect a stronger effect of anthropogenic disturbance on growth, body condition and fledging success during a year of poor nutritional conditions. Secondly, some species may be more sensitive than others and the same disturbance may have detrimental effects on a specific species, but no or little measurable effect on another (Götmark 1992; Nisbet 2000; Phillips et al. 2003; Ellenberg et al. 2006; Seddon et al. 2008; Carey 2009; Villanueva et al. 2012; Fiske et al. 2013). Thirdly, disturbance protocols can vary between studies sometimes to a large extent (Caughley 1977; Götmark 1992; Phillips et al. 2003). Some studies were conducted just after hatching while others were conducted after thermal emancipation or even later. In addition, the frequency and the duration of handling also varied among studies. Finally,

the same disturbance may have various effects on chicks depending on various environmental conditions (e.g. food availability, predation risk, weather, Walker et al. 2005; Carlini et al. 2007; Bertellotti et al. 2013; Palacios et al. 2018).

Our study was among the first to test whether female or male chicks may be more sensitive to repeated disturbance in a Procellariform species. Energy requirement has been positively correlated to body size, and male chicks are often thought to be more costly to produce and consequently more sensitive to environmental constraints (Teather and Weatherhead 1994; Weimerskirch et al. 2000). In our study, neither males nor females were significantly impacted by the disturbance protocol but we found a strong sexual morphometric dimorphism in Snow Petrel chicks. Body size (i.e. tarsus length) was not significantly different between males and females at day 13 but males had become significantly larger than females by day 32, highlighting a significantly faster tarsus growth for males. In addition, males were heavier than females on days 13 and 32. Thus, future studies should assess sex and include it as an explanatory factor when assessing the impact of disturbance on growth performances, particularly in sexually dimorphic species.

Physiology and behaviour

We did not find any impact of our disturbance protocol on defensive behaviour and some proxies of stress in Snow Petrel chicks. Specifically, breath rate, stress-induced CORT levels and the probability to show defensive behaviour did not vary between disturbed and control chicks. This study focused on multiple physiological stress-related variables (breath rates, CORT levels) that were measured several minutes after the capture except for baseline CORT levels. These stress-induced measurements are definitely affected by handling and capture, which similarly occurred for both the experimental and the control groups. Therefore, they may not provide information on the impact of our disturbance protocol on the baseline degree of stress of the chicks. However, they are very useful to understand how disturbed and control chicks react to a disturbing event (i.e. their sensitivity to stress), and more specifically to test whether chicks become sensitized or, in contrast, habituated to the stress of capture/handling (Angelier and Wingfield 2013; Dickens and Romero 2013; Angelier et al. 2018; Karlíková et al. 2018). We did not find any evidence that our experimental protocol affected breath rates and stress-induced CORT levels, suggesting that repeated disturbance did not have a deep impact on the sensitivity to stress of Snow Petrel chicks. In contrast to those stress-induced variables, baseline CORT levels were measured within 3 min of capture and were therefore independent of the stress of capture/handling (Romero and Reed 2005; Angelier et al. 2010). Therefore, these baseline CORT levels allowed us to test whether the experimental protocol

affected circulating CORT levels independently of the stress of capture. Interestingly, we found that baseline CORT levels of disturbed chicks were lower than those of control chicks. Classically, chronic stress is thought to result in elevated baseline and/or stress-induced CORT levels (Busch and Hayward 2009), suggesting unexpectedly that control birds could be more stressed than disturbed birds. This difference in baseline CORT levels between disturbed and control chicks was driven by three control chicks, which had noticeably high baseline CORT levels (> 12.00 ng/mL while all others were below 6.00 ng/mL; excluding these three outliers, control and disturbed chicks do not differ anymore, $p = 0.8856$). It is possible that these three chicks were confronted with a stressor prior to capture and blood sampling (presence of a predator or a conspecific nearby), explaining these abnormally elevated baseline CORT levels and the difference between disturbed and control chicks. In some studies, chronically stressed birds had lower baseline CORT levels (Cyr and Romero 2009; Dickens and Romero 2013), and this could suggest that disturbed chicks were chronically stressed. However, this interpretation is very unlikely because no other morphological, behavioural, and physiological variables differed between disturbed and control chicks. Importantly, we also did not detect any significant difference in the change in baseline CORT levels from day 13 to day 32 between control and disturbed chicks. Overall, these results suggest that repeated captures and handling had no major effect on stress-induced behaviour and physiology, or at least that a recovery period of 5 days between sampling events is enough to limit the impact of disturbance on Snow Petrel chicks. However, our study only focused on short-term effect of anthropogenic disturbance on the phenotype and the survival of Snow Petrel chicks (i.e. before fledging) and we cannot rule out that anthropogenic disturbance has long-term effects, which subsequently affect the fitness of the Snow Petrels (juvenile or adult survival, ability to successfully reproduce).

Conclusion

Our results show that our specific protocol (i.e. repeated nest visits, captures, and handling) had little, if any, detrimental impact on the phenotype of developing Snow Petrels (stress physiology and behaviour, growth, body condition). Although no morphological, behavioural, and physiological effect of disturbance were detected, repeated captures and handling events were associated with a non-significant trend towards higher mortality for disturbed chicks relative to control ($p = 0.1006$) and undisturbed ($p = 0.0962$) chicks. This trend may result from extremely harsh weather conditions towards the end of the study period (repeated snowstorms), which were associated with increased mortality

rates in disturbed chicks. To conclude, investigator disturbance appears to have little effect on Snow Petrel chicks after thermal emancipation, but we remain cautious and recommend to avoid capture and handling of Snow Petrel chicks soon after snow storms or/and when the nests are covered with snow. Scientific data are obviously crucial to obtain robust and relevant data to monitor the impact of our changing world on Antarctic seabird populations. Therefore, the development of alternative and less invasive methodologies should be a priority in the coming decade in order to reduce or minimize exposure of wildlife to human contact.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval Licences and permissions were granted by the Ethic Committee of the Institut Polaire Francais (IPEV) and by the Préfet of Terres Australes et Antarctiques Francaises (MP/09/13/04/09).

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