Seabird Ontogeny in an Antarctic Environment

Threats, Coping Strategy and its Consequences for Wilson's Storm-petrel Chicks

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Seabird Ontogeny in an Antarctic Environment
Threats, Coping Strategy and its Consequences for Wilson's Storm-petrel Chicks

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Abstract

In the extreme environments of polar areas, time for offspring rearing is limited by environmental conditions for many animals. The completion of all body structures and functions in time as well as juvenile survival is linked to efficient development. Additionally, poor weather or foraging conditions may force long-live species with high adult survival rates like procellariiform seabirds to abandon their egg or chick to ensure their own survival in case of elongated breeding periods. Yet, seabird chicks are not only dependent on their parents' care and environmental conditions. During the last decades several anthropogenic threats were detected that may impair chick development and survival.

In my thesis I studied anthropogenic, environmental and biological influences on the ontogeny of a procellariiform seabird, the Wilson's Storm-petrel (*Oceanites oceanicus*). Chicks of this species are known to be spontaneously reversibly able to reduce their body temperature and metabolism (facultative hypothermia). This is considered as a survival strategy to preserve energy. I identified body condition for unfed chicks as crucial factor to determine if they could maintain a constant body temperature between 36 °C and 41 °C or not (Chapter 1). After periods of unpredictable feeding that followed snowstorms, facultative hypothermia occurred almost in all chicks for several days until they could recover. Next, I examined the potential consequences of facultative hypothermia use to preserve energy resources, on developmental processes (Chapter 2). Low body temperatures had negative influences on growth, immune parameters, and fat physiology. Growth rates, that were reduced by hypothermia, recovered soon after rewarming. To evaluate if a chick could recover from effects of facultative hypothermia on other physiological parameters or if its development with regard to these might be slowed down, the available data was insufficient. Lastly, I evaluated anthropogenic pollutants that accumulated in embryos through transfer of nutrients from yolk and albumen, which the mother collects during a prey-laying exodus and deposits to the egg (Chapter 3). Embryos were contaminated with legacy pollutants like polychlorinated biphenyls (PCBs), metabolites of dichlorodiphenyltrichloroethane (DDT), and mercury (Hg), and their PCB concentrations were higher than species with similar diets. Among samples collected during different years, those had higher PCB and Hg concentrations that were about ten years older. Concentrations of DDT metabolites remained similar among the differently aged samples.

Concluding, in the current environmental conditions, Wilson's Storm-petrel chicks can cope with weather extremes and unpredictable feeding by using facultative hypothermia, and seem able to recover from physiological setbacks regarding growth during hypothermia. In addition, they face high concentrations of pollutants from the very beginning. As seabird populations are declining worldwide, awareness of threats at all life-history stages is crucial to act successfully on their protection.

Zusammenfassung

In extremen Regionen wie den Polargebieten ist die Aufzuchtphase vieler Tiere durch Umwelteinflüsse begrenzt. Die rechtzeitige und vollständige Ausbildung aller Körperstrukturen und -funktionen sowie das Überleben der Jungtiere ist an eine effiziente Entwicklung gekoppelt. Dazu kommt, dass es für Tiere mit hoher Alterserwartung und niedriger Sterblichkeit im Erwachsenenalter – wie bei Seevögeln der Ordnung Procellariiformes – sinnvoll sein kann, die Brut in einem schlechten Jahr aufzugeben, wenn eine durch Wetter- oder Futterbedingungen verlängerte Brutperiode das eigene Leben gefährden würden. Doch nicht nur die Pflege der Eltern und die Umweltbedingungen beeinflussen das Überleben der Seevogelküken. In den letzten Jahrzehnten wurden immer mehr Gefahren menschlichen Ursprungs bekannt, die sich negativ auf die Entwicklung der Küken auswirken.

In meiner Dissertation untersuchte ich biologische und anthropogene Einflüsse sowie die der Umwelt auf die Kükenentwicklung der Buntfuß-Sturmschwalbe (*Oceanites oceanicus*), die zur Ordnung der Procellariiformes gehört. Für diese Art ist bekannt, dass Küken ihre Körpertemperatur und ihren Metabolismus kurzfristig und reversibel reduzieren können (optionale Hypothermie). Dies gilt als Überlebensstrategie, wenn der Energiebedarf nicht ausreichend gedeckt werden kann. Ob Küken der Buntfuß-Sturmschwalbe ihre Normaltemperatur von 36 °C bis 41 °C aufrechterhielten oder nicht, hing vor allem bei ungefütterten Küken maßgeblich von ihrer körperlichen Verfassung ab (Kapitel 1). Nach Schneestürmen, die zu ausbleibenden und unregelmäßigen Fütterungen führten, nutzten fast alle Küken die optionale Hypothermie für mehrere Tage, bevor sie wieder konstante Körpertemperaturen aufwiesen. Um mögliche Konsequenzen der

Hypothermie auf die Küken zu untersuchen, betrachtete ich als nächstes verschiedene physiologische Entwicklungsparameter (Kapitel 2). Niedrige Körpertemperaturen beeinflussten das Wachstum, immunologische Parameter und die Fettphysiologie negativ. Die während der Hypothermie reduzierten Wachstumsraten stiegen jedoch kurz nach dem Aufwärmen der Küken wieder an. Ähnliche Erkenntnisse, ob sich Küken von Einflüssen der optionalen Hypothermie auf andere physiologische Parameter erholen oder ihre Entwicklung bezüglich dieser langfristig gehemmt wird, ließ die Datengrundlage nicht zu. Zuletzt betrachtete ich im Embryo angereicherte Schadstoffe, die über Nährstoffe, welche das Weibchen während der Futtersuche direkt vor der Eiablage aufnimmt, in das Ei gelangen (Kapitel 3). Die Embryonen wiesen langlebige Schadstoffe wie Polychlorinierte Biphenyle (PCBs), die Metaboliten von Dichlordiphenyltrichlorethan (DDT), sowie Quecksilber (Hg) auf und ihre PCB Konzentrationen waren höher als in Eiern anderer Seevögel mit gleichem Futterspektrum. Bei Proben unterschiedlichen Alters wiesen die etwa zehn Jahre älteren Proben höhere PCB und Hg Konzentrationen auf als die aktuelleren. Die Konzentrationen der DDT Metaboliten waren vergleichbar zwischen den unterschiedlich alten Proben.

Zusammenfassend lässt sich sagen, dass Küken der Buntfuß-Sturmschwalbe bei extremen Wetterlagen und unregelmäßigen Fütterungen durch optionale Hypothermie Ressourcen sparen können und sie sich von den physiologischen Folgen in Bezug auf Wachstum erholen können. Zusätzlich sind sie direkt zu Beginn ihres Lebens hohen Mengen an Schadstoffen ausgesetzt. Ein Überblick über Gefahrenpotentiale auf allen Entwicklungsstufen ist besonders heute, da Seevogelpopulationen weltweit abnehmen, wichtig, um funktionale Schutzmaßnahmen zu ergreifen.

Synthesis

1 General Introduction

The reproductive period is a challenging time in most endotherm species. Egg-laying, incubation and chick rearing put breeding birds in challenging situations, especially from an energetic view (H. I. Ellis & Gabrielsen, 2001). Among others, weather conditions and prey accessibility will influence how well parents can care for their young (Schreiber, 2001), and already before hatching bird embryos often suffer from cooling during egg neglect (Boersma & Wheelwright, 1979). After hatching, precocial birds have a high degree of independence, and a main challenge is to become efficient at where and what to forage or prey on, and to become capable of escaping (Starck & Ricklefs, 1998). Altricial offspring additionally have to grow feathers, acquire active thermoregulation, and start locomotor activity (Starck & Ricklefs, 1998). They depend essentially on their parents for food, protection and warmth until, step by step they develop capabilities to care for themselves. Depending on their life-history and ecology challenges during ontogeny differ between species. Next to natural challenges like weather and weather related prey availability or predation pressure, adults and juveniles in our anthropogenic world suffer from pollution, climate change, exploitation of foraging grounds, or destruction of habitats (Boersma, Clark, & Hillgarth, 2001). In the following I will focus on the specifics of seabird ontogeny in an Antarctic environment.

1.1 Seabirds of the Order Procellariiformes

The term seabird describes birds from different orders that exclusively feed at sea (Schreiber & Burger, 2001). Species of the order Procellariiformes are pelagic birds that can spend weeks to months on the open sea. They are highly mobile and cover huge distances to find promising feeding grounds. During their time off shore most species are considered high up in the food chain with only few predators. For breeding they come to land where they breed in colonies, and nests are in burrows, cavities or on pedestals. Procellariiform species have particularly long rearing periods compared to birds of similar sizes, and most often have only one brood consisting of one egg per year (Coulson, 2001). After copulation ashore, females leave for a pre-laying exodus. Here they gather sufficient nutrients at sea to produce the egg and build some reserves for fasting times during incubation (Coulson, 2001). Procellariiform chicks are categorized as semiprecocial, which means they hatch with open eyes, a downy plumage, and have locomotor abilities, but will rely on their parents for provisioning and have to develop flight feathers (Hamer, Schreiber, & Burger, 2001; Starck & Ricklefs, 1998). Both parents share care of incubating, brooding, guarding and feeding their chick (Nelson & Baird, 2001).

Among the Procellariiformes, the Wilson's Storm-petrel *Oceanites oceanicus* has a special place: it is the smallest species of the order breeding in Antarctica, which makes it at the same time the smallest endotherm breeding there (Quillfeldt, 2006). The species uses ice-free areas of the Antarctic continent, the Antarctic islands of the Scotia Arc, as well as subantarctic Islands. With a high adult survival and maximum ages of more than 20 years, Wilson's Storm-petrels can reproduce for many years (Quillfeldt, 2006). A breeding pair raises its single chick in cavities under big rocks, which form the only protections against the severe climate (Quillfeldt, 2006; Roberts, 1941). As chances of another opportunity to breed successfully are high in this long-lived species, it is often beneficial during bad years for adults to abandon their offspring to ensure their own survival. Late spring snowfalls blocking nest sites can prevent birds from breeding or delay breeding so long that chicks may not fledge in time before birds start migrating north during the southern winter (Beck & Brown, 1972; Büßer, Kahles, & Quillfeldt, 2004). Storms during the breeding season impact brooding and feeding, when the rough sea negatively impacts foraging at sea or does not allow a fast return to the colony (Boersma & Wheelwright, 1979). Whole breeding seasons can be at risks if warm temperatures during the southern winter prevent sea ice, which impacts the reproduction and survival of Antarctic krill *Euphausia superba*, one of the most important prey species of Wilson's Storm-petrels (Quillfeldt, 2001, 2006). Next to unpredictable environmental threats, those of anthropogenic origin became apparent during the last decades: pollution in form of plastic, pesticides, flame retardants, heavy metals or remains from oil spills enter the seabirds' environment and food web (Fisk, Hobson, & Norstrom, 2001; Jarman et al., 1996; Wilcox, Van Sebille, Hardesty, & Estes, 2015). Risks range from chick or adult death through starvation, egg shell breaking, malformation of embryos or behavioral changes impacting breeding success and pose a threat to whole seabird colonies or populations (Burger & Gochfeld, 1997; Elliott, Norstrom, & Keith, 1988; Pierce, Harris, Larned, & Pokras, 2004; Tartu et al., 2015).

1.2 Ontogeny - from Egg to Fledging

1.2.1 The Embryo - In the Egg

Avian life starts in the fertilized egg, formed by the mother. The composition of nutrients for embryonic development is determined at this point it is influenced by the mother's fitness and health as well as the environmental conditions during its production (Mousseau & Fox, 1998). Gas exchange of metabolized carbon dioxide and oxygen is possible through the eggshell's pores, and water evaporates through the same structures causing water loss during incubation (Starck, 1998). The yolk of an egg contains fat, proteins, carbohydrates and water, which are the main nutrient sources for the embryo. The albumen consists majorly of water, but contains additional proteins (Starck, 1998). An extra-embryonic membrane, the yolk sac, transfers nutrients to the embryo. Another membrane, the chorioallontois, covers after a few days the entire internal surface of the egg, and forms the respiratory organ of the embryo, mediating the gas exchange with the environment (Starck, 1998). Embryonic development in birds is highly determined and different species share the same set of developmental stages (Cordero & Werneburg, 2022; Starck & Ricklefs, 1998). Even though no studies investigated embryonic development specifically in Wilson's Storm-petrels, this high similarity among bird species suggests that the normal stages of embryonic development described by Hamburger and Hamilton (1951) are also suitable for Wilson's Storm-petrel embryos. According to these normal steps, among the first visible structures are head folds and the first neural folds, followed by the development of somites, that will later differentiate into skin, muscles, ligaments and vertebrates. Later, the parts of the central nervous system and sensory organs form, wing and leg buds show, and the embryo becomes step by step recognizable as a bird (Hamburger & Hamilton, 1951). During this time the embryo is supplied constantly with the energy and nutritional components it needs from yolk and albumen, and its parents provide warmth by brooding. While adult birds maintain a constant body temperature, embryos are poikilotherm and cannot generate warmth by themselves (Dawson & Whittow, 1994; Visser, 1998). To maintain growth and development and to avoid lethal chilling, most embryos depend on their parents, especially in cold breeding areas like Antarctica. In Procellariform birds egg chilling through neglect by the parents elongates incubation times (Boersma, 1982; Boersma & Wheelwright, 1979). While other species often do not hatch after cold exposure in the second half of incubation, in Procellariiform species even older embryos seem adapted to regular chilling intervals due to their parents' feeding scheme (Gaston & Powelo, 1989; Pefaur, 1974;

Roby & Ricklefs, 1984). As the embryo is growing, nutrients are used up and through respiration the egg becomes lighter with incubation time (Furness & Furness, 1981). As a last step, the embryo consumes the remains of the yolk and the pipping phase begins briefly prior hatching. Now, after hatching, new challenges await the young bird.

1.2.2 The Hatchling - Thermoregulation

One of the most important developmental steps for hatchlings of many seabirds is the acquisition of endothermy. Without being able to maintain a stable high body temperature, the chick has to be brooded and guarded closely. As soon as chicks are capable of active thermoregulation both parents can leave for foraging without risking lethal cooling of the chick, especially in cold and temperate areas. Several species show abrupt thermoregulatory abilities after hatching within a maximum of 24 hours (Laysan (*Phoebastria immutabilis*) and Black-footed Albatross (*Phoebastria nigripes*): Dawson & Whittow, 1994). In others, thermoregulatory development and hence, brooding by parents requires more than a week (e.g. eleven days in Antarctic Petrel (*Thalassoica antarctica*): Bech, Mehlum, & Haftorn, 1991). After establishing active thermoregulation and homeothermy, chicks of some species show heterothermic abilities during development. This means metabolic rate and body temperature can be reduced by a few degrees or as low as ambient temperature to reduce energetic demands of normothermia (Geiser, 2008). Birds show this behavior to escape energy shortages, for example during bad weather conditions or migration (Schleucher, 2004; Wojciechowski & Pinshow, 2009). Different terms have been used in discussions about this topic in the attempt to clarify between passive and active use of metabolic reduction. In general, torpor describes a state where animals seem lifeless, and show low body temperatures and metabolic rates (Bartholomew, Howell, & Cade, 1957; Kronfeld-Schor & Dayan, 2013; Reinertsen, 1996). To discriminate between active torpor and passive hypothermia it is often necessary to asses both, body temperature and metabolic rate (Geiser, Currie, O'Shea, & Hiebert, 2014). One important difference between the two is the ability of animals to overcome the hypotherm state actively by increasing their metabolic rate, which leads to increased body temperatures, instead of e.g. passive rewarming by rising ambient temperatures (Geiser et al., 2014). This requires an animal to develop from a poikilothermic stage at hatching to an independent endothermic stage where it is able to maintain a constant high body temperature (Geiser, 2008). The term facultative hypothermia or hypothermic response is often used to describe the pattern of reduced body temperature as well as the actively regulated mechanism (McKechnie & Lovegrove, 2002). This way patterns of reduced metabolism and body temperatures can be analyzed without limiting findings to more narrow definitions that might be difficult to assess precisely in the field.

Wilson's Storm-petrel chicks were observed in cold, lifeless states, from which they could recover with time by other researchers before (Beck & Brown, 1972; Quillfeldt, 2006). As food provisioning rate and amount often vary stochastically in this species, the use of facultative hypothermia may enable chicks to survive longer fasting periods, when energy reserves to keep body temperature at a normothermic level are decreasing. Additionally, regular feedings of storm-petrels seem to provide more food for the chick than needed on a daily basis (Ricklefs & Schew, 1994). This leads to accumulated fat reserves that can peak at masses up to 50 % higher than adult's body mass, and hereby increase the fledging success in case of unexpected periods of low feeding rates (Quillfeldt & Peter, 2000; Ricklefs & Schew, 1994). This mass peak seems close to the end of structural growth (Mauck & Ricklefs, 2005; Ricklefs & Schew, 1994), in Wilson's Storm-petrels at an age of 45 to 50 days (Obst & Nagy, 1993, own observation). To be able to fly chicks have to decrease this weight again during their last days at the nest. Among procellariiform birds different ways of mass loss have been reported: lipid consumption (Obst & Nagy, 1993; Ricklefs & Schew, 1994) or water loss (Phillips & Hamer, 1999) are among possible explanations for this process. During this period of mass reduction chicks only occasionally receive food from parents (Mauck & Ricklefs, 2005; Ricklefs & Schew, 1994).

1.2.3 The Hatchling - Growth and Immune System

During the 50 to 60 day long hatchling period the semi-precocial hatchlings of the Wilson's Storm-petrels have to grow and mature: skeletal structures grow, flight feathers develop, and muscles strengthen for flight. Several skeletal structures, like the culmen or tarsus, show steep growth starting almost immediately after hatching. Others, like the wings, show a later start of rapid growth (Cape Petrel (*Daption capense*): Weidinger, 1997; Wandering Albatross (*Diomedea exulans*): Lequette & Weimerskirch, 1990; Bulwer's Petrel (*Bulweria bulwerii*): Nunes & Vicente, 1998; Least Storm-petrel (*Oceanodroma microsoma*): Bedolla-Guzmán, Masello, Aguirre-Muñoz, Lavaniegos, & Quillfeldt, 2017).

Another crucial step for hatchlings is the development and maturation of a well-functioning immune response. Most knowledge of the development and function of the avian immune system is based on detailed studies on domestic chicken (*Gallus gallus domesticus*) or other domestic fowl (Adelman, Ardia, & Schat, 2012; Davison, 2012).

However, studies investigating ontogeny of the immune system in free living species suggest similarities to the concepts establish using domestic fowl (Adelman et al., 2012). Hatching chicken have an innate, basal immune system that includes macrophages and heterophiles (Kaspers & Kaiser, 2012). In addition to protection through phagocytosis of pathogens or infected cells, hatchlings of many species received antibodies by their mother that were deposited to the yolk during egg formation (Apanius, 1998; Härtle, Magor, Göbel, Davison, & Kaspers, 2012; Kaspers & Kaiser, 2012). Depending on the species, their functioning will decrease after a few days or weeks, and the chick's own immune defense needs to mature for an efficient immune response (Garnier et al., 2012; Gharaibeh & Mahmoud, 2013). Within a week after hatching phagocytic and bactericidal activities of heterophils in chicken increased significantly, indicating that maturation of innate cells plays an important role in immune defense of young birds (Wells, Lowry, DeLoach, & Kogut, 1998). A second important step for a fast and efficient immune response is a functional adapted immune system. Two important structures for the adaptive immune system, the bursa of Fabricius and the Thymus, develop during embryogenesis (Apanius, 1998). Both these organs are colonized by precursor cells in the embryo, where they differentiate. Naive B cells emigrate to secondary lymphoid organs and wait for their activation, T cells migrate majorly to the spleen, intestine, or remain in the thymus (Apanius, 1998; Oláh, Nagy, & Vervelde, 2012). Antigens of bacteria, viruses or pathogens stimulate B cells, which proliferate and produce antibodies during the primary immune response (Apanius, 1998). Clones of memory B cells are generated at the same time to produce a faster secondary response in a future repeated encounter with that antigen (Apanius, 1998). T cells differentiate either into activating T helper cells or regulatory cytotoxic T cells. T helper cells, when activated through antigen presentation by endogenous cells like macrophages, amplify response of granulocytes, B cells and also regulatory T cells (Apanius, 1998; Kaufmann, 2025). These cells are important for ending an immune reaction before it can cause unwanted cell damages (Kaufmann, 2025). With age and each encounter of pathogens the immunologic memory grows and the immune system matures, making the immune response more functional and efficient.

1.2.4 The Fledgling

After fledging, the young Wilson's Storm-petrel relies on itself for food and protection. Until they are at least three years old Wilson's Storm-petrels do not breed (Roberts, 1941). Their whereabouts during this time are still not determined. Reports of Wilson's Storm-petrels in tropic waters during breeding season and mist net catches of pre-breeders at breeding colonies suggest that they discover close and distant parts of the oceans before they start breeding themselves (Quillfeldt & Peter, 2000; Roberts, 1941). The quality of bones, organs, and physiology acquired during development are crucial for adult life. Body size or growth symmetry can be important for efficient flight or mate choice (Brown & Bomberger Brown, 1998; Richner, 1989; Swaddle, 1997), the immune system protects the bird from diseases. In an environment that is increasingly affected by anthropogenic influence, seabirds face additional risks and threats during development and life. One example is pollution and the persistence of toxic substances in the remotest areas. Their impact on seabirds in the Antarctic will be described in the following.

1.3 Pollutants, Global Distillation Effect and Antarctica

Decades ago, Rachel Carson's book "The silent spring" raised attention to the toxic consequences of pesticides. Soon it was found that pollution did not only affect areas of use or production. Several toxins, among them legacy halogenated hydrocarbon and the heavy metal mercury (Hg) are highly volatile, spread by aerial transport over long distances and enter aquatic system through deposition (Sanganyado & Kajau, 2022; Wania & Mackay, 1993; Wania & Mackay, 1996). In warm latitudes pollutants evaporate and migrate through the atmosphere to cooler areas like mountain chains or the poles, where they cold condensate and enter the terrestrial or marine systems again, a process called global distillation (Goldberg, 1975; Wania & Mackay, 1993; Wania & Mackay, 1996). This sets especially cold, aquatic and marine environments at high risks and even in the remotest and most pristine environments high loads of persistent organic pollutants (POPs) were discovered (Wania & Mackay, 1996). Eventually, twelve substances or groups of substances of persistent organic pollutants were forbidden for use, production, im- and export with only limited exceptions by the Stockholm convention in the signing countries in 2004 (United Nations Environment Programme [UNEP], 2018). Among them is the group of industrially used polychlorinated biphenyls (PCBs) and the insecticide dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD, together DDX). Ever since, additional substances are evaluated regularly, if they should be included in this list. Today, the list of environmentally persistent pollutants is long and includes among others pesticides, industrial chemicals or production by-products (UNEP, 2018). However, existing substances are hard to eliminate in the environment and their distribution and re-introduction especially into the aquatic ecosystems is ongoing, through landfill leachate, terrestrial run-offs, or melting ice of glaciers (Bogdal et al., 2009; Sanganyado & Kajau, 2022). Once POPs or heavy metals like mercury enter the food web, they accumulate in organisms and biomagnify along trophic levels, reaching highest concentrations in top predators (Fisk et al., 2001; Jarman et al., 1996; Langis, Langlois, & Morneau, 1999; Morel, Kraepiel, & Amyot, 1998; Quillfeldt et al., 2023). Wilson's Storm-petrels prey on Antarctic krill, squid, small fish, and amphipods (Quillfeldt, 2002), species that were reported to accumulate pollutants through bioconcentration and foraging (Chiuchiolo, Dickhut, Cochran, & Ducklow, 2004; Corsolini & Sarà, 2017). During egg production storm-petrels will deposit pollutants contained in nutrients from their pre-laying exodus to the egg (Barron, Galbraith, & Beltman, 1995; Beck & Brown, 1972; Verreault, Villa, Gabrielsen, Skaare, & Letcher, 2006). These contaminants build a certain burden for the embryo from its first moments of development on. Among often reported impacts of POPs on development in several bird species are reduced hatchability, mass and growth, but also alterations of hard-tissue like feet or bills, soft-tissue like liver or heart as well as immunosuppression were found (Harris & Elliott, 2011; Yamashita et al., 1993). Chick survival is further impaired by reduced parental activities, especially nest attendance, of adults with high POP concentrations (Harris & Elliott, 2011). High concentrations of mercury can lead to feather loss in juveniles, reduced hatching success, chronic diseases or death (Wolfe, Schwarzbach, & Sulaiman, 1998). Many studies concentrated on water and seabirds or birds of prey, but also passerine species were affected (Harris & Elliott, 2011; Wolfe et al., 1998). In Antarctic birds so far beak deformations have been reported from Antarctic Cormorants (*Phalacrocorax bransfieldensis*; Casaux, 2004) and Giant Petrels (*Macronectes giganteus*; Marti, Bellagamba, & Coria, 2008), as well as beak and spinal deformations from Emperor Penguins (*Aptenodytes forsteri*; Golubev, 2020; Pütz & Plötz, 1991; Splettstoesser & Todd, 1998). These deformations occurred in such small numbers that they cannot be linked to pollution and various causes could be the origin. We should bear two things in mind: First, in a changing environment, several stressors might be increasing, such as food availability, diseases, or availability of breeding habitats. The combination of threats may be more harmful than each on its own (Trathan et al., 2014). Second, potentially not only the concentration of pollutants is important but also the moment of exposure. A look at pollutant burden at the very beginning of life is important to understand implications later during ontogeny and adulthood.

2 Aims and Structure of Thesis

2.1 Objectives

This thesis focused on Wilson's Storm-petrel development and the challenges chicks face growing up in Antarctica. The severe climate with its unpredictable weather events at the breeding sites of this species results frequently in stochastic feeding patterns of the parents and spontaneous fasting periods for the chicks. For a hatchling, sufficient resources for efficient and successful development are crucial. Without, maintaining high rates of development and constant body temperatures at the same time might not be possible. Using facultative hypothermia has been described as an elegant option to escape substantial resource loss, which is especially important in regions where the temperature difference of ambient and body temperature is as high as in Antarctica. My interest was to determine patterns for the use of heterothermy, and to understand the importance of environmental and parental influences. Second, I was interested in potential physiological trade-offs between ensuring survival using facultative hypothermia and effective development of the chicks, as the influence of heterothermy on development in wild birds is poorly understood. Lastly, I wanted to analyzed the anthropogenic impact in form of pollution on Wilson's Storm-petrels. In the formerly pristine Antarctica pollution became omnipresent throughout the food webs decades ago. Many organisms are exposed to high concentrations from the first moment on. I was interested in pollutant loads of embryos that establish starting concentrations for hatchlings.

In summary, what are challenges for developing storm-petrels in the current environment and how are they coping with them?

To answer this, the main objectives of this thesis are:

- Evaluating environmental and parental influences on body temperatures and causes for hypothermia during chick development
- Examining consequences of hypothermia during chick development
- Assessing pollutant profiles of Wilson's storm-petrel embryos in past and present samples

2.2 Thesis Structure

The study was conducted during three consecutive years at a breeding colony of Wilson's Storm-petrels close to the Argentinean Base Carlini (later named CA breeding colony) around the Tres Hermanos hill on King George in the South Shetland Islands, Antarctica (Fig. 2.1 & 2.2). For assessment of pollutant concentrations additional samples from the years 1998, 2001 and 2003 collected at the same colony were used.

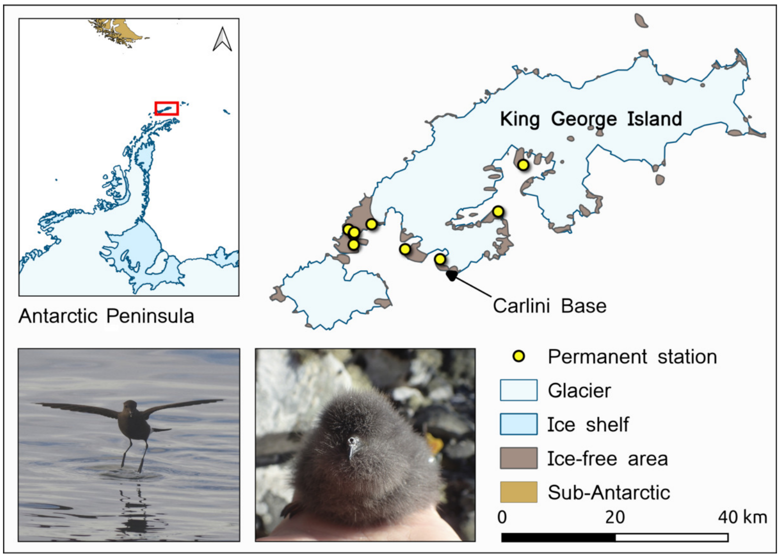

Figure 2.1: Location of study site on King George Island, South Shetland Islands and study species, adult and chick [Chapter 3].

Figure 2.2: Snow covered scree slope of Tres Hermanos.

Chapter 1 *Facultative hypothermia as a survival strategy during snowstorm induced food shortages in Antarctic storm-petrel chicks*

→ Determination of factors influencing body temperature in nestlings

→ Describing hypothermia occurrence, beginning and ending in storm-petrel chicks

→ Discussing parameters causing hypothermia in chicks

Chapter 2 *Consequences of heterothermy during development on the physiology and growth of Antarctic storm-petrel chicks*

→ Analyzing influence of body temperature and occurrence of facultative hypothermia on

- growth
- nutritional physiology
- immunology

in Wilson's Storm-petrel chicks

Chapter 3 *Persistent organic pollutants and mercury in a colony of Antarctic seabirds: higher concentrations in 1998, 2001, and 2003 compared to 2014 to 2016*

→ Determination of current and past pollutant concentrations of PCBs, DDX and Hg

→ Transfer patterns of POPs during embryogenesis

3 Chapter Outline

The following studies represent the objective of this thesis (Fig. 3.1). Three main directions of interest are split in different chapters.

Chapter 1:

Facultative hypothermia as a survival strategy during snowstorm induced food shortages in Antarctic storm-petrel chicks [published]

Outline:

This first publication looked at the interplay of environment and parental care during the development of Wilson's Storm-petrel chicks, and their impacts on the chick's thermoregulation. Various storm-petrels from around the world are known to use heterothermy occasionally. To investigate how body temperatures are linked to provisioning, chick condition and environmental conditions a total of 50 chicks were monitored during three consecutive breeding seasons at the CA breeding colony. Daily measurements of body mass allowed calculations of feeding rates and determination of days without provisioning. At the same time body temperature was measured in the cloaca. First, the occurrence of facultative hypothermia in contrast to passive cooling as a consequence of not being able to maintain stable body temperatures could be established: chicks were observed with body temperatures below 36 °C, which represents the lowest ten percent of measured body temperatures that could raise these body temperatures actively to normothermic values without receiving food and hence, energy and warmth by their parents. Daily body temperatures were positively influenced by age and feeding, while minimal nocturnal temperatures had a negative effect. Body temperature was not affected by body condition in chicks that received food in the previous night. If chicks did not receive food during the previous night, a positive correlation between body temperature and body conditions was observed. During one day fasting intervals most chicks stayed normothermic, other's body temperatures dropped by up to 21 °C. Facultative hypothermia was less used from chicks with good body conditions. During longer fasting intervals after snow storms blocking the nest sites for parents, most chicks became hypothermic. This way almost all chicks survived periods of no or only unreliable provisioning of up to eleven days, before being fed regularly again and re-establish their body mass and temperature again. This outcome shows the importance of facultative hypothermia to escape starvation. However, it also raises the question if frequent use of hypothermia might have negative effects during chick development. Further research is needed to evaluate where trade-offs might be and analyze how well adapted Wilson's Storm-petrel chicks are to periods of insufficient provisioning in a severe environment like Antarctica.

Contributions:

Lead author; data and sample collection in the field (nest search, monitoring and measuring nestlings); data preparation (calculation of feeding rates) and analyses.

Chapter 2:

Consequences of heterothermy during development on the physiology and growth of Antarctic storm-petrel nestlings [published]

Outline:

The concluding publication of this thesis aimed to shed light on possible trade-offs of facultative hypothermia for Wilson's storm-petrel chicks. Influence of body temperature in general and consequences of hypothermic events in particular were analyzed with regard to certain developmental parameters. The main interests were growth, nutritional and immunological physiology. For this, growth and body temperatures were monitored daily throughout three breeding seasons in the CA breeding colony. Parameters for different physiological aspects were analyzed from weekly taken blood samples. The study could show that lower body temperature influenced growth, the combination of adapted and innate immunity, and fat physiology negatively. Growth data suggests that quick recovery from hypothermia after feeding and rewarming is possible. For other aspects more data is needed to gain insight into recovery rates after facultative hypothermia.

In general, this publication implicates the benefits of heterothermy for Wilson's storm-petrel chicks, suggesting a good adaptation to the current climate conditions and feeding situation. Future studies analyzing if any aspects found here could have implications for adults later in life would be valuable.

Contributions:

Lead author; data and sample collection as well as experiments in the field (nest search, monitoring, measuring, and sampling nestlings, immune challenge); partial laboratory work (sex determination; blood smear preparation and counting, triglyceride, hematocrit); data preparation (calculation growth curves) and statistical analyses.

Chapter 3:

Persistent organic pollutants and mercury in a colony of Antarctic seabirds: higher concentrations in 1998, 2001, and 2003 compared to 2014 to 2016 [published]

Outline:

The last publication focused on anthropogenic threats during the development of Wilson's Storm-petrels that occur at the very beginning, but may affect the bird for its entire life. Using abandoned storm-petrel eggs collected at the CA breeding colony, I analyzed which organic pollutants were detectable and at which developmental stage they were transferred to the embryo. Eight PCBs, including the dioxin-like (dl) congeners PCB 105 and 118 ($\sum$ PCBs: 59-3403 ng g^{-1} wet weight (ww)) as well as 4,4'-DDE, and 4,4'-DDD $\sum$ DDX: 19-1035 ng g^{-1} ww) were detected in the embryos. Embryos in their last third of development showed highest pollutant load per g body weight. This is analogue to fat metabolism from egg yolk during embryogenesis. Abandoned eggs provide a non-invasive tool of monitoring pollutants. Results presented in this study emphasis the importance of comparing similar age classes or whole egg content to address the non-linear transfer of organic pollutant from yolk to embryo. Additionally, this publication gives insight how pollutant load differs from more recent years compared to samples over a decade older. Organic pollutant concentrations from 2001 and 2003 in embryos as well as Hg concentrations in egg membranes from 1998 and 2003 were compared to organic pollutants and Hg concentrations from 2014 to 2016. While for DDX no difference was found between time intervals, PCB and Hg concentrations were higher in older samples. Though more investigations are needed to establish a time trend for these findings, it could hint to a slow recovery of the pelagic Antarctic environment from PCBs after their global ban came into force in the year 2004. If the pattern found in this study will continue also for Hg is questionable, as its use is at best stable, in some regions even increasing, in the Southern Hemisphere. Summarizing, seabird chicks from the Antarctic may face less contamination of some pollutants in the future, but as others may remain for longer bio-available at high concentrations, regulations and monitoring is needed to conserve this fragile environment.

Contributions:

Lead author; partial sample collection in the field (2015 - 2017); sample preparation; pollutant extraction; data and statistical analyses.

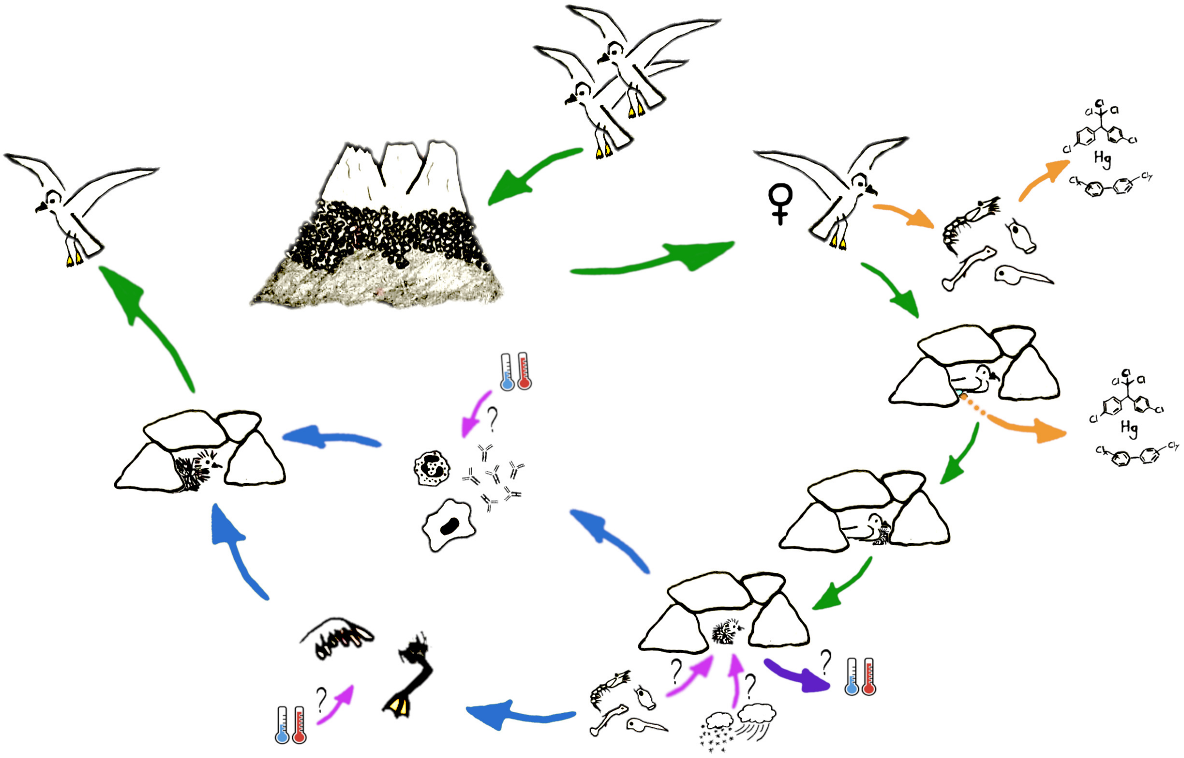

Figure 3.1: Illustration of breeding period of Wilson's Storm-petrels at the CA breeding colony. → indicate the chronological steps during breeding. → indicate uptake of food and pollutants, as well as contained pollutants in the egg. → indicate developmental processes, → indicate in this thesis analyzed influences, → indicates the analyzed reaction of influences. Blue and red thermometers present cold and warm body temperatures.

4 General Conclusion and Outlook

All chapters together describe current challenges of developing Wilson's Storm-petrels from egg to fledging on the South Shetland Islands. The chicks were found to cope with the challenges of the stochastic feeding behavior of their parents, which is pronounced after severe weather incidences, by using heterothermy [Chapter 1]. Lowest observed body temperature was 18 °C. Chicks were able to rewarm actively from these again, without receiving food or interacting with an adult bird [Chapter 1]. This confirms the occurrence of facultative hypothermia in Wilson's Storm-petrel chicks, in contrast to passive hypothermia. The occurrence of most pronounced facultative hypothermia could be linked to feeding behavior of adults after snowstorms [Chapter 1]. While many chicks were normothermic directly after a snowstorm and several chicks received food, almost all chicks experienced higher rates of irregular feeding and fasting shortly after [Chapter 1]. During storms parental birds may be unable to forage or to return to the colony (Watson, 2014). These absences of their parents, most likely due to self-maintenance, left chicks fasting for up to eleven days after snowstorms [Chapter 1]. In general, receiving food was linked to higher body temperatures than fasting for at least one night. This effect was even stronger in chicks with lower body conditions [Chapter 1]. Lower body conditions suggest a recent or ongoing energy shortage, which makes the preserving of reserves by reducing body temperatures to decrease mass loss even more important.

With the positive effects of surviving times of energy shortages, low body temperatures and facultative hypothermia have trade-offs of decreasing structural growth, circulating leukocytes, and triglyceride concentrations [Chapter 2]. The effect of mean short term body temperature on the combined immune response of innate and adapted immune system was close to significance in chicks close to fledging [Chapter 2]. It is possible that the same challenge for chicks of an age with higher use of hypothermia would reveal a significant association to body temperatures. More data from differently aged chicks would complete the picture. The response of the innate immune system was not negatively influenced by low body temperatures [Chapter 2]. For the tarsus, a single event of hypothermia did not yet slow growth rates [Chapter 2]. However, an accumulation of hypothermia or slightly reduced body temperatures resulted in slower growth. Wings were already affected after a single event of hypothermia and growth rates were reduced [Chapter 2]. Only a few days later wing growth rates recovered to rates similar than before hypothermia. All studied chicks successfully fledged, indicating that found effects are not preventing them from starting into the adult life [Chapter 2].

Lastly, this thesis identified high concentrations of persistent organic pollutants and mercury that Wilson's Storm-petrel chicks are exposed to from the very beginning [Chapter 3]. The concentrations of the eight detected PCBs were higher than in other Antarctic species with similar diets like Adélie Penguins (*Pygoscelis adeliae*; Cipro, Taniguchi, & Montone, 2010; Corsolini, Borghesi, Ademollo, & Focardi, 2011). Concentrations in earlier years were higher than in more recent years, indicating a decrease of these legacy pollutants in the pelagic system [Chapter 3], most likely due to the global ban after 2004 by the Stockholm Convention (UNEP, 2018). Also, concentrations of Hg found in egg membranes were higher in earlier than in recent years [Chapter 3]. As processes using Hg like gold mining in the southern hemisphere are ongoing (Eagles-Smith et al., 2018), it is yet to observe if the here found decreasing trend will continue or if concentrations will rise again, as shown in sub-Antarctic regions (Carravieri, Cherel, Jaeger, Churlaud, & Bustamante, 2016; Mills et al., 2020). Concentrations of two detected DDT metabolites, 4,4'-DDE and 4,4'-DDD, did not differ significantly between earlier and more recent years [Chapter 3], potentially reflecting the ongoing use in malaria defense, especially in the southern hemisphere. The death of analyzed embryos was linked to snowstorms lasting for several days during incubation. Hence, I assume that doses of pollutant were below lethal and death was related to abandonment of the eggs [Chapter 3]. In general, no harmful effects of POPs or Hg, like alterations of feet or bill (Harris & Elliott, 2011; Yamashita et al., 1993), strikingly slower growth rates (Harris & Elliott, 2011), or feather loss (Wolfe et al., 1998) were found in embryos or hatchlings of this breeding colony. Internal effects, like immunosuppression, alterations of the heart (Harris & Elliott, 2011) or chronic diseases (Wolfe et al., 1998) may not be aparent at the nest, but could have a high impact on the fledged bird. This thesis concludes that currently Wilson's Storm-petrels are well adapted to survive periods of infrequent provisioning during hatchling time [Chapter 1], and seem to recover from temporary reduction of development due to hypothermia well enough to fledge [Chapter 2]. While the analyzed concentrations of pollutants in embryos are high [Chapter 3], they do not seem to pose a lethal threat to the developing bird at the moment.

The importance of understanding chick development, their challenges and coping strategies becomes aware when looking at the worldwide decline of seabirds (Dias et al., 2019). A recent study analyzing population dynamics of Wilson's Storm-petrels breeding on King George Island, showed a 90 % decline in population size for the CA breeding colony between 1996-1999 and 20001-2020 (Ausems et al., 2023a). Laying success in this population decreased significantly over time (Ausems et al., 2023a). However, hatching rate from these eggs was highly variable among years (41.0 % ± 26.8 %), not indicating increased non-viability or mortality of embryos at any specific time (Ausems et al., 2023b). Fledging success even increased over the years (Ausems et al., 2023a), which is in accordance with our findings from Chapter 2 and 3 that investigated challenges during development do not seem to effect chick survival at the moment. It seems likely that reasons for population decline lay outside the breeding season (Ausems et al., 2023a). The ratio between breeding and non-breeding birds observed at the CA breeding colony remained similar, suggesting that recruitment into the colony still takes place (Ausems et al., 2023a), but also that both, numbers of established breeders and pre-breeders declined. Little is known about Wilson's Storm-petrels between the end of chick development and the start of breeding. During this period factors from chick development could still impact the young birds. Chicks using facultative hypothermia regularly during their development could have shorter wings as adults, resulting from temporarily reduced growth rates [Chapter 2]. This will result in higher energetic demands during long distance flights (Hahn et al., 2015) and affect survival (Morrison, Hipfner, Gjerdrum, & Green, 2009). Lower circulating leukocytes [Chapter 2] may protect a bird less well in case of disease or parasitic infection. Lower triglyceride concentrations [Chapter 2] could lead to less reserves when fledging, and hence a higher risk of not surviving the first weeks of independent foraging at sea (Hamer et al., 2001). Additionally, measured concentration of pollutants represent only the natal starting load of toxic substances a bird is exposed to during life. A study using geolocator-immersion loggers (GLS) tracked Wilson's Storm-petrels from the CA breeding colony during non-breeding between 2023 and 2025 (Schumm et al., 2026). Migration routes and wintering sites reveal stop-overs at areas of cumulated ecological risk by hexachlorocyclohexanes (HCHs; Coast of Canada and Brazil, south of Greenland), PCBs (Coast of USA and Brazil), or DDX (south of Greenland) (Fig. 4 Schumm et al., 2026; Zhang et al., 2024). Additionally, all four groups of organic legacy pollutants HCHs, DDX, organochlorine pesticides (OCPs), PCBs) analyzed by Zhang et al., 2024 show medium to high ecological risks for the Antarctic Peninsula. High concentrations of pollutants in adult seabirds can have both, impacts on own condition as well as on reproductive success. In Thick-billed Murres (*Uria lomvia*) Hg concentrations could have a decreasing effect on diving and hence, successful foraging behavior, by decreasing the hormone triiodothyronine, which is involved in oxygen consumption rate (Esparza et al., 2022). The hatching probability of eggs from Bermuda Petrel (*Pterodroma cahow*) females with higher POP loads was lower (Campioni et al., 2024). In general, nest attendance during incubation and chick rearing is impaired in many seabird species bearing high concentrations of Hg or POPs (Harris & Elliott, 2011; Wolfe et al., 1998). Studies in Glaucous Gulls (*Larus hyperboreus*) show associations of POPs with nematode intensity in breeding season, which may imply suppression of the immune system of these pollutants, when birds face additional energetic demands of chick rearing (Sagerup, Henriksen, Skorping, Skaare, & Gabrielsen, 2000). A similar association could not be found after breeding, when adults could recover and showed good body conditions again (Sagerup et al., 2009). In Great Black-backed Gulls (*Larus marinus*) nesting success where negatively impacted by organochlorine contaminates at sites or in years with bad compared to good environmental conditions (Bustnes, Fauchald, Tveraa, Helberg, & Skaare, 2008). Similarly, negative influence of POPs on reproductive performance in Great Skuas (*Stercorarius skua*) could be eliminated for the first hatched chick in a colony with poor foraging conditions by supplementary feedings, improving foraging conditions for the parents (Bustnes et al., 2015). These studies emphasize how varying environmental or health conditions result in differing effects of pollutants and imply that during adverse conditions even low concentrations of pollutants may have severe effects on stressed birds (Bustnes et al., 2015; Bustnes et al., 2008; Sagerup et al., 2000; Sagerup et al., 2009). The CA breeding colony experienced differing environmental conditions like prey abundance, nest availability and entombment of nests during incubation or chick rearing during the early years considered for pollutant analyses (Büßer et al., 2004, Chapter 3). In general, years with low krill abundance still showed average hatching success, implying birds could cope with this challenge alone (Büßer et al., 2004). However, another year (1998) with potentially low prey abundance due to unfavorable winds showed hatching success less than half as high than in years with low prey abundance (Büßer et al., 2004). Egg membranes collected from hatched chicks from that year showed the highest Hg concentrations during analyzed years [Chapter 3]. If also parents were exposed to high Hg concentrations that year, their breeding behavior could be affected as found in other seabirds (Wolfe et al., 1998), resulting in lower nesting success. While it will not be possible to analyze impacts of pollutants in retrospective, these results may still suggest that combined effects of poor environmental conditions and pollutant concentrations in Wilson's Storm-petrels have a higher impact than the sum of each effect alone.

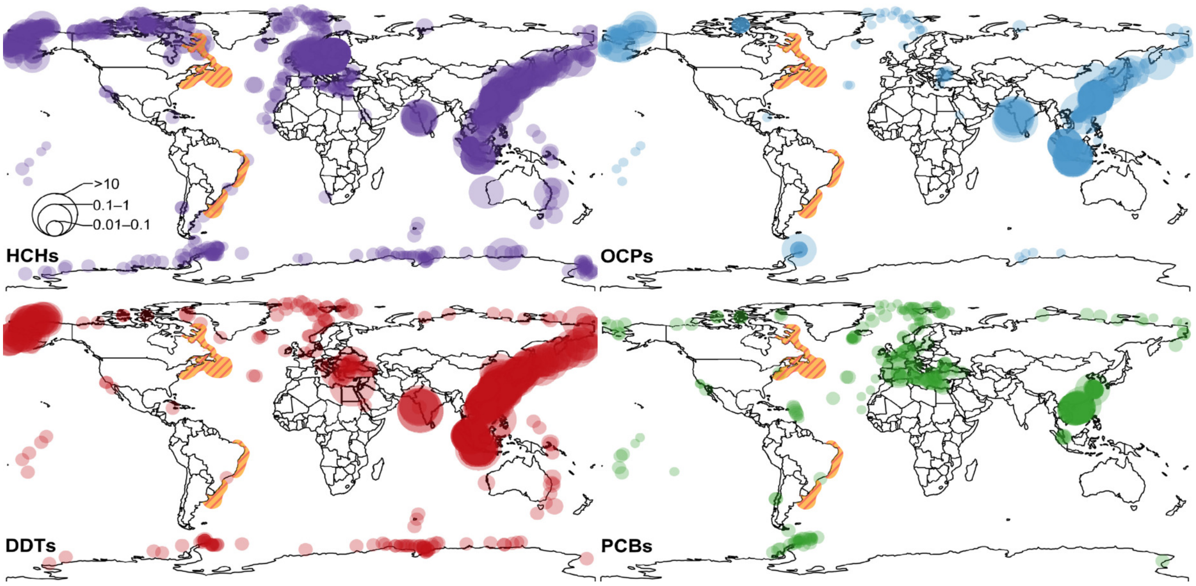

Figure 4.1: Ecological risk areas of POPs from Zhang et al. (2024) and wintering habitats of Wilson's Storm-petrels (dashed outlines in magenta) by Schumm et al. (2026).

Antarctica, and especially the Antarctic Peninsula, is predicted to experience severe climatic changes during the next years and decades, some of which are already ongoing (Bozkurt, Bromwich, Carrasco, & Rondanelli, 2021; Clarke et al., 2007; Ducklow et al., 2007; Znój et al., 2017). Among those changes several will be unfavorable for breeding Storm-petrels. At the start of the breeding season nests might often be still blocked from winter and spring storms and snow fall, as precipitation is predicted to raise in this area (Bozkurt et al., 2021; Marshall, Thompson, & van den Broeke, 2017). Consequently, later breeding may result in a miss-match between times of highest energy demand and prey availability as shown e.g. in the Arctic seabird Cassin's Auklet (*Ptychoramphus aleuticus*; Hipfner, 2008). This, together with lower stocks of Krill in years of limited sea ice cover during winter (Clarke et al., 2007; Loeb et al., 1997) could pose insufficient prey availability during incubation and chick rearing, and could cause malnutrition or starvation of adults and chicks. Another risk during incubation and chick rearing is caused again through increased precipitation, as frequent blockage of nests might end or elongate breeding duration further, causing starvation and facultative hypothermia in chicks (Büßer et al., 2004; Marshall et al., 2017; Quillfeldt, 2001, Chapter 1). With higher use of hypothermia, negative effects shown on a small scale in this thesis [Chapter 2] may sum up and form fundamental risks for juvenile or fledgling survival. In the combination with a later season, chick development might shift so much that parents are forced to leave the chicks before they are ready to fledge, leaving them to starve at the nest. Additionally, the question arises, if regular fasting times during elongated foraging trips of the parents in order to find more scarcely distributed prey or prior to hypothermia, will cause re-mobilization of pollutants that were so far stored in the adipose tissues of a chicks. It is yet to show if this way concentrations may reach amounts that are toxic at sensitive times during development. While in general pollutants in the pelagic system may decrease further (D. S. Ellis, Cipro, Ogletree, Smith, & Aronson, 2018; van den Brink, Riddle, van den Heuvel-Greve, & van Franeker, 2011), even low concentrations might have harmful effects for chicks and adults if several conditions during breeding season are poor (Bustnes et al., 2015; Bustnes et al., 2008). Not all unfavorable conditions may occur during the same season. However, chances are that most years may experience at least one, making adult birds more susceptible to impacts of pollutants with the outcome of e.g. reduced nest attendance and hence, breeding success. Last, but not least, PCBs, DDX and Hg are by far not the only harmful substances in the worldwide oceans. Regularly, new pollutants are added to the list of the Stockholm Convention or registered in the European REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation (European Commission, 2018; UNEP, 2018). Among substances that are known to have reached Antarctic waters and to have entered trophic webs is the group of perfluoroalkyl substances (PFAS), which's restriction process of single and all uses and is ongoing (European Chemicals Agency [ECHA], 2023), and the chemicals of emerging concern methylparabens, which is classified as toxic to aquatic life with long lasting effects, and oxfendazole, which is classified very toxic to aquatic life with long lasting effects (Alygizakis et al., 2025; Bargagli & Rota, 2024; ECHA, 2018, 2025). The processes of reducing their use and production can be lengthy, and in the meanwhile more pollutants are entering the markets without thorough knowledge about their toxicity. Additionally, pollutants can interact, meaning that the combined effect of several substances might be more toxic than the cumulative effect alone (Chen & Bunce, 2004; Zhang et al., 2024).

This thesis concentrated on different steps of development of Wilson's Storm-petrels breeding on an Antarctic island. It established an order of magnitude for embryo contamination [Chapter 3], showed the thermoregulatory capabilities of chicks to use and avoid facultative hypothermia [Chapter 1], and finally had a first look at possible consequences the current and extended use of facultative hypothermia has on chick development [Chapter 2]. Both, pollutant load and fasting likely pose stress for developing chicks. The link that is observed in adult birds that poor health or environmental conditions worsen impacts of pollutants (Bustnes et al., 2015; Bustnes et al., 2008; Sagerup et al., 2000; Sagerup et al., 2009), is likely also true for chicks during their energetically demanding development. To analyze this and monitor emerging and ongoing threats and challenges it would be great to also link pollutant load directly to physiological processes:

- First of all, are, and if, how are pollutant loads changing during chick development? Are concentrations diluted by less contaminated food from different foraging areas, or is the opposite the case, as foraging area during chick rearing may contain similar pollutant concentrations?
- How is the Hg concentration in chicks compared to their egg membranes? Similarly, to organic pollutants, will concentration changes during development occur? Especially, will there be a reduction of concentrations with the start of feather growth, as Hg might be implemented to reduce body load?
- Will there be re-mobilization of organic pollutants in fasting chicks? If so, can this process be slowed by lower body temperatures or facultative hypothermia?
- Are impacts of pollutants detectable in chick development? If so, are there interactions of these impacts with environmental and health conditions like described for adults?
- Are pollutant concentrations affecting use of facultative hypothermia in any direction?
- Can excretion pathways during chick development be identified, e.g. through feces, dunes or feathers?

With advancing technology making constant monitoring of body temperature or metabolic rate possible even in the field, as well as analytical procedures needing minimal amount of sample, some of these questions might be possible to be addressed in the near future. Marine birds are often described at sentinels of the sea, as they are long-lived, feed on a high trophic level, and have only few if any predators on top of them. Hence, their well-being is closely linked to their trophic and ecological niche (Hazen et al., 2019; Tabor & Aguirre, 2004; Thibault, Houlbrèque, Lorrain, & Vidal, 2019; Velarde, Anderson, & Ezcurra, 2019). Population declines as observed in Wilson's Storm-petrels are alarming, and the reasons for their decline most likely will reveal threats also for other species of the same ecosystem. This thesis emphases risks and challenges Wilson's Storm-petrels had to face during ontogeny observed throughout three breeding seasons. However, until additional studies link these findings to their health, fitness and breeding success as adults no conclusions can be made on a population level. To put the findings of this thesis together with future approaches into a larger context, the continuation of long-term studies is severely needed and findings should be compared to other breeding colonies in Antarctica and the Sub-Antarctic. Especially with the ongoing changes of the Antarctic climatic conditions it is necessary to discriminate between local, regional, or trans-regional conditions and effects, as well as seasonal differences in weather in contrast to climatic changes (Sauser, Delord, & Barbraud, 2021). Completing the picture will give us valuable clues where and how conservation action would be effective for Wilson's Storm-petrels, which would improve conditions for ecologically similar or even prey species at the same time.

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Chapters

Chapter 1

Facultative hypothermia as a survival strategy during snowstorm induced food shortages in Antarctic storm-petrel chicks

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Facultative hypothermia as a survival strategy during snowstorm induced food shortages in Antarctic storm-petrel chicks

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Storm-petrel

ABSTRACT

Wilson's storm-petrels (*Oceanites oceanicus*) are the smallest marine birds breeding in Antarctica, where events like snowstorms often prevent parents from providing food daily for their offspring. To minimize energy expenses, Wilson's storm-petrel chicks can reduce their metabolism and body temperature by entering hypothermia. Hypothermia is reported to impact development, hence we hypothesized that hypothermia will be majorly used after long fasting periods. Chick development in a breeding colony of Wilson's storm-petrels on the South Shetland Islands was monitored daily during three consecutive summers by recording chicks' body mass and temperature, as well as environmental parameters. Provisioning, and body conditions were highest in 2017, and chicks became hypothermic most frequently in 2016. Body temperature was influenced by age, mass, body condition, and minimal nocturnal temperatures. While most chicks were able to maintain stable body temperatures when not fed for one day, some chicks' body temperatures decreased by up to 21 °C. Age did not differ between those two groups, but chicks maintaining their active body temperatures had higher body conditions. Snowstorms were typically followed by several days of unreliable food provisioning and continuous days of fasting. Most chicks were hypothermic during this time, and were hence able to survive periods of food shortages, reverse their low body temperatures after the next feeding event, and regain body mass. We conclude that hypothermia is a strong survival strategy to endure times of fasting, which might be necessary for Antarctic storm-petrel chicks to reach adulthood. However, in future scenarios, which may include more frequent snowstorms due to climate change, malnourishment could lead to more frequent use of hypothermia, which could affect chicks' development.

1. Introduction

Many bird and small mammal species around the world can reduce body temperature to minimize energetic costs during resting (Geiser, 1998; Hwang et al., 2007; Schleucher and Withers, 2002). This facultative hypothermia and its energetic benefits range on a continuous scale from normotherm body temperatures to deep torpor, when animals appear lifeless and cold (McKechnie and Lovegrove, 2002). It can occur on a daily basis for some hours, as found in hummingbirds or several bat species (Geiser, 1998; Krüger et al., 1982). Hamsters, bats or storm-petrels can reduce their body temperatures during food shortages or cold temperatures for several days, when energy intake cannot cover the expenses for maintaining a constant body temperature (Boersma, 1986; Ruf et al., 1993; Wojciechowski et al., 2007). On the other hand it can be used to prepare migrating or hibernating animals for an energy demanding time to come (Geiser and Brigham, 2012). Next to these benefits also costs are associated with hypothermia. The lethargic state of torpid animals hinders immediate reactions when predators approach (Eichhorn et al., 2011; Hainsworth et al., 1977) and can result in predation, as shown for fork-tailed storm-petrel chicks predated by fungus beetles when torpid (Wheelwright and Boersma, 1979). Temperature controlled biological functions like enzyme-catalysed reactions can be slowed (Heldmaier et al., 2013; Hochachka and Somero, 2002). Furthermore, body temperatures affect the molecular structure of lipids and proteins, for example rendering biological membranes more viscous (Cossins and Prosser, 1978; Somero, 1995). Such biochemical changes influence many physiological functions and potential costs include metabolic imbalance (Jensen and Bech, 1992). During the reproductive cycle this not only affects adults' survival chances, but also their reproductive success. Depending on the phase of reproduction hypothermia can be beneficial or, on the contrary, harmful. In several bat species the sperm storage during winter seems to be benefitted by

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facultative hypothermia, as fertilization can be delayed for several months until the climate is favourable (Geiser and Brigham, 2012). During pregnancy, however, hypothermia can prolong gestation and hence is assumed to extending the development period of the offspring in bats (Audet and Fenton, 1988; Racey and Swift, 1981). Comparably embryos in storm-petrels are known to tolerate periodic chilling by several days of egg-neglect, but it will increase the total incubation time (Boersma and Wheelwright, 1979; Vleck and Kenagy, 1980). This delay of prenatal development can be risky, as it can put survival chances during the next winter or migration at risk (Ransome, 1989).

Hypothermic responses are not only found in adult animals, but also in juveniles. However, to use and profit from torpor or facultative hypothermia birds and mammals first have to establish the capability of active thermoregulation to maintain a stable body temperature in absence of food or a heat source (Geiser and Brigham, 2012). Altricial young show thermogenic responses in the early prenatal period, however they are not yet able to maintain a stable body temperature out of the thermo-neutral zone (Blumberg and Sokoloff, 1998). In precocial birds first endotherm reactions occur even before hatching and the effectors for thermoregulation are functional (Nichelmann et al., 1998; Nichelmann and Tzschentke, 2002). However, the efficiency is low (Nichelmann and Tzschentke, 2002). Hence, ability of active torpor or hypothermia use is unlikely. In between altricial and precocial animals range many seabirds that are considered semi-precocial: they hatch down-covered and show differing degrees of mobility, but depend on their parents' food delivery (Hamer et al., 2001). The efficiency of the endotherm system increases in species-specific times during the prenatal period (Nichelmann and Tzschentke, 2002). Some semi-precocial albatross and shearwater species show thermogenic responses within hours after hatching (Whittow, 2001). Others, like chicks of Wilson's storm-petrels are known to actively regulate their body temperature after five days (Gębczyński, 1995): in experiments chicks could maintain their body temperature at 5 °C ambient temperature for 30 min, afterwards one and three day old chicks' body temperatures and resting metabolic rates decreased, while five day old ones could maintain their resting metabolic rate and body temperatures. After thermoregulation is established hypothermia and torpor are used by juveniles in various situations. For example, juvenile Siberian hamsters used spontaneous torpor, potentially in preparation for winter, after weaning but before reaching maturation (Bae et al., 2003). In absence of food they were torpid at an even younger age. Likewise, nestling storm-petrels were reported to enter hypothermia in times of food shortages (Gębczyński, 1995). Once they are thermally independent, use of facultative hypothermia can be a powerful survival strategy. However, its negative impacts may weigh more for juveniles than for adults: depressed physiological functions during reduced body temperature may result in a reduction in growth and development (McAllan and Geiser, 2014; Racey, 1981). Thus, there is a trade-off between reducing energetic costs and investing in body functions such as growth and investment into immunology. Consequently, facultative hypothermia should be avoided as long as the nutritional state allows staying normothermic. In temperate areas hypothermia is often related to periods of cold or severe weather that make foraging impossible or decrease prey abundance (Doucette et al., 2012; Kunz, 1988). In regions like Antarctica prey abundance and therefore foraging success is probably usually not depressed by low temperatures (Quillfeldt, 2001), but weather extremes like strong winds or snowstorms, may lead to hypothermia due to fasting (Watson, 2013).

In the present study we examine hypothermia in a colony of the Antarctic seabird, the Wilson's storm-petrel (*Oceanites oceanicus*). Nestlings of Antarctic storm-petrels are semi-precocial and they are subjected to the combined stress of hunger (long intervals between feedings), cold, and long rest phases, making it potentially difficult to achieve a positive energy balance (Beck et al., 1972; Hamer et al., 2001). The regular use of hypothermia in petrels has been confirmed for one species breeding in temperate climate (Fork-tailed storm-petrels, Boersma, 1986) and one species in the Antarctic (Wilson's storm-petrels, Gębczyński, 1995). Wilson's storm-petrel chicks younger than five days entered hypothermia in times of food shortages (Gębczyński, 1995). Similarly, Fork-tailed storm-petrel chicks had reduced body temperatures while fasting (Boersma, 1986). Body temperature of smaller, younger chicks was found to be lower than body temperature of larger, older chicks, probably due to better surface-area volume ratios and feather insulation of the older ones. The more food the chicks gained the higher body temperatures could be observed. While food load or body temperature did not correlate directly with growth rates, higher variability in food loads that included large and small meals resulted in higher growth rates than more regular, but smaller food amounts (Boersma, 1986).

In this study we analyzed data along the temperature continuum between normothermic and hypothermic chicks. The term torpor was only used to describe animals that appeared completely lifeless. In particular, we tested the following hypotheses:

(1) Poor body conditions increase facultative hypothermia in Wilson's storm-petrel chicks. (2) Older chicks, with better insulation and better thermoregulatory capabilities, are less likely to enter hypothermia, and (3) facultative hypothermia will be more frequent after snowstorms.

2. Material and methods

2.1. Species and study site

Wilson's storm-petrels are marine birds that after fledging spend most of their lives offshore, but come to land for breeding (Quillfeldt, 2006). They are the smallest endotherms breeding in Antarctica, weighing on average 38 g and with a wing length of 15 cm (Beck et al., 1972; Quillfeldt, 2006). Their breeding colonies can be found in Antarctic and subantarctic ice-free areas, where the petrels most often nest in cavities below rocks (Quillfeldt, 2006; Roberts, 1941). They raise a single chick per year maximally and both parents participate in provisioning of the chick (Quillfeldt, 2006; Roberts, 1941). Depending on environmental conditions like snow cover, and parents' experiences chicks hatch between beginning of January and mid-March (Büßer et al., 2004; Quillfeldt, 2006; own observation). Like most petrels, Wilson's storm-petrels leave their young alone in the nest during the day at an early age, and majorly visit them during the night (Quillfeldt, 2006; Roberts, 1941). Adults often undertake foraging trips that last several days (Gladbach et al., 2009). During and after snowstorms nest sites might be blocked for several days and prevent adults to access their chicks (Quillfeldt, 2001). The study took place at a colony of Wilson's storm-petrels breeding in the scree slopes of the “Three brothers” hill about 1.5 km from Carlini Research Station on King George Island (Isla 25 de Mayo), South Shetland Islands (62° 14′ S, 58° 40′W). Monitored nests were all underneath big rocks and varied in depth from 20 cm to 60 cm. The monitoring period started each year a few days after the first chick hatched and lasted until the last ship left the station at the end of March. In 2015 the first chick hatched on Julian day 51 (median: 61), in 2016 on day 37 (median: 55), and in 2017 on day 18 (median: 30). Only in 2017 it was possible to observe nine of eleven chicks fledging. In previous years and for the other chicks we assumed successful fledging, if we found no dead chick in the nest the following season (2015: 6 of 6, 2016: 17 of 20, 2017: 22 of 24).

2.2. Monitoring and measuring of chicks

During the incubation period of three consecutive years we searched for active nests and measured eggs (length, width, mass). With these measurements we estimated their hatching dates according to Quillfeldt (2001) to avoid disturbing the parents during the incubation period by frequent nest checks. We looked for hatched chicks five days after this estimated date and afterwards monitored the first 20 chicks that hatched daily until fledging/the end of the season while parents were foraging. When a chick died or was no more accessible during our observations we included a later hatched chick into the daily monitoring. Only chicks living past their 10th day were included into this data set. Analyses are based on data from 50 chicks monitored during three years (2015: 6, 2016: 20, 2017: 24). If adult birds were present at a nest, they were not disturbed and we checked the nest again the following day. We measured chicks' body mass to 0.1 g with a digital scale, and chicks' temperatures with a digital thermometer (Omega, HH506A, resolution 0.1 °C) and a connected thermocouple probe (Omega, Model HYP-2) in the cloaca, hereafter referred to as body temperature. We recorded breath rates by counting visible breathing for 10 s. Every three days we measured chicks' growth parameters: tarsus length to 0.1 mm using callipers and wing length to 1 mm using a stopped rule. The first measurements of each chick were used to determine its age following a logistic regression calculated from growth data from 1996 (accuracy for tarsus length 20 mm: 12.0 ± 0.3 days; Quillfeldt and Peter, 2000). With daily chick masses we calculated feeding rates and frequencies (i.e. food loads provided by parents, compare Gladbach et al., 2009), taking into account daily mass differences and the estimated metabolic loss during the day. If chicks appeared completely lifeless, we weighted them, and put them back into their nest immediately.

We analyzed body temperatures along the continuous gradient between 17 °C and 42 °C. The term torpor refers to chicks that appeared completely lifeless. We did not measure body temperatures in these birds.

2.3. Other

Wind speed [knt], and air temperature [°C] were collected at the local weather station located at the Argentinean Carlini station, and managed by their meteorologists eight times a day every 3 h, starting at midnight.

2.4. Data analyses

Statistical and graphical analyses were conducted in R (R Core Team, 2018; Version 3.4.4). Boxes of boxplots show data of the upper and lower quartile, their whiskers represent the 1.5 fold interquartile range from the box. The age independent parameter "body condition" describes the residual mass to the population mean mass of chicks of the same age, using data from ten breeding seasons (compare Quillfeldt, 2002a). Time of day is the time when we measured a chick's body temperature. Minimal nocturnal temperature was obtained from the weather station and was the minimal value between 1800 h the day before to 0600 h on that day. Accordingly, maximal wind speed was the maximal value during the same time.

We used a linear mixed effect model (R packages "lme4"; Bates et al., 2015, and "car"; Fox and Weisberg, 2011) to describe the influences of environmental, age, time of day, and provisioning parameters on chicks' body temperatures. The data were nested in chick identity and year as random effects to control for daily measurements of the same chicks during the study period and a possible family effect of chicks from the same nest in different years. To improve model fit body temperature data were ranked. Age, body condition, time of day, minimal temperature during the night, maximal wind speed during the night (all continuous), year (factorial) and fed (factorial, indicating if chick was fed in the previous night) were included as fixed effects, as well as an interaction between body condition and fed. We included variables and the interaction was based on biological reasons. No collinearity between fixed effects was found.

We tested the relation between chicks' body temperatures and the numbers of days without food with a Spearman's rank correlation for all data together as well as for medians per chick. With Kruskal-Wallis Chi-squared tests we analyzed differences of median feeding rate per chick, and median body condition per chick among years. For between year analyses we used Wilcoxon's rank sum test with continuity correction.

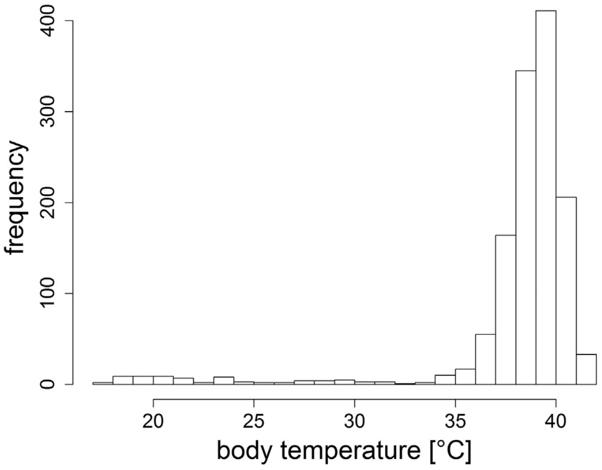

Fig. 1. Distribution of body temperatures (°C) for Wilson's storm petrel nestlings. > 90% of body temperatures from 50 monitored chicks ranged between 36 and 42 °C with a median of 39 °C.

3. Results

3.1. Body temperature variability and hypothermia

Over the three observed years body temperatures of monitored 50 chicks ranged between 17.7 °C and 42.0 °C (Fig. 1). The median of all measurements was 39.0 °C, 75% of chicks'measured cloaca temperatures were 38 °C or higher, with < 10% of chicks being observed with body temperatures below 36.0 °C. One chick appeared cold and lifeless for 11 days. Five chicks maintained their body temperatures during three days of fasting (Table 1). Five chicks increased their body temperature by at least 4 °C without being fed (Table 2). The time of measurement did not significantly affect body temperatures, and older chicks had higher body temperatures than the young ones (Fig. 2; Table 3).

3.2. Influence of feeding and body condition

On average chicks were fed 5 g each night (Table 4). The amount of food that was provided to the chicks by their parents did not differ significantly among years (Kruskal-Wallis-Test: $X^2 = 5.2$, d.f. = 2, $p = .073$; Fig. 4), but chicks in 2017 received significantly more food than chicks in 2016 (Wilcoxon's rank sum test: W = 145, $p = .025$; Table 4). Body condition was highest in 2017 and lowest in 2016 (Kruskal-Wallis-Test: $X^2 = 23.0$, d.f. = 2, $p < .001$, N_{chicks} 2015: 6,

Table 1
Occurrences of fasting chicks for three days without drop of body temperatures. Table shows observed years, IDs, ages, the mass of the chicks before fasting, their mass loss during three days of fasting, the averaged mass loss per day for the three fasting days (± SD) as well as the average body temperature during those three days of fasting (± SD). Each line represents data from one chick.

Year	ID	Age [days]	Mass [g]	Mass loss [g]	Mass loss per day [g]	Mean body temperature [°C]
2015	1	25	53.15	15.91	5.30 ± 5.42	39.2 ± 0.2
2016	4	29	52.15	10.99	3.66 ± 3.87	40.0 ± 0.5
2017	5	32	46.88	12.95	4.32 ± 0.61	36.6 ± 0.9
2017	6	32	53.81	13.15	4.38 ± 2.68	40.0 ± 0.3
2017	7	32	56.62	16.84	5.61 ± 3.21	39.3 ± 0.2

Table 2
Occurrences of reheating after temperatures below the 90% range of body temperatures of 36 °C without feeding events. Table shows observed years, IDs, ages, the mass of the chicks before fasting, their mass loss during fasting, body temperatures before and after fasting, temperature differences and the time span during which this reheating was observed. Each line represents data from one chick.

Year	ID	Age [days]	Mass [g]	Mass loss [g]	Body temperature before fasting [°C]	Time difference [h]	Body temperature after fasting [°C]	Temperature difference [°C]
2015	2	8	25.88	−3.36	31.4	11	38.8	7.4
2015	2	10	31.20	−3.44	35.6	10	40.3	4.7
2015	3	18	54.01	−8.08	35.1	24	38.6	3.5
2016	4	12	32.19	−6.75	34.3	24	38.1	3.8
2017	8	7	25.97	−3.9	34.1	14	38.8	4.7
2017	8	51	69.82	−5.26	29.0	24	40.8	11.8
2017	9	28	62.13	−4.58	35.9	24	39.8	3.9

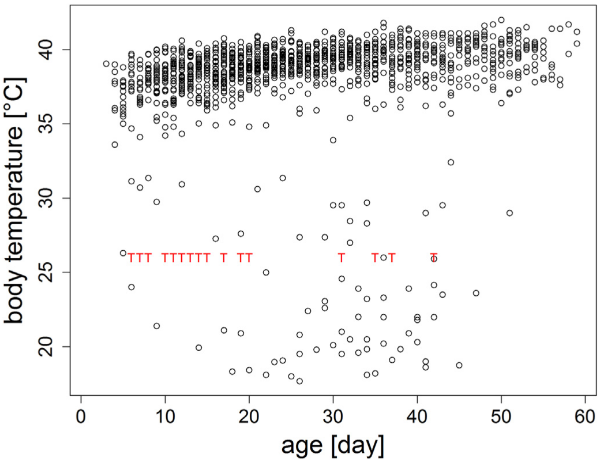

Fig. 2. Change of body temperatures of Wilson's storm petrel nestlings with age. Body temperature increases significantly with age (Table 3). Red "T"s represent torpor occurrences without temperature measurements (defined by visual judgment). Data come from 50 monitored chicks.

Table 3
Statistical values for linear mixed effects model of body temperature (dependent variable) in relation to body condition, age (days), the factor variable fed, indicating if chicks were fed or not during the previous night, time of measurement, minimal nocturnal ambient temperature (°C), maximal nocturnal wind speed (knt), the factorial variable year, and the interaction between fed and body condition. The model was nested for individual chicks (50) and years.

	F-value	d.f.	d.f. resid.	*p*-value	relation
Intercept	14.86	1	124.05	< 0.001***	positive
Body condition	63.45	1	1083.26	< 0.001***	positive
Age	117.94	1	1037.77	< 0.001***	positive
fed/unfed	3.89	1	1069.09	0.049*	positive
Time of measurement	1.14	1	1021.85	0.287	
Min. nocturnal temperature	4.71	1	1072.91	0.030*	negative
Max. nocturnal wind speed	0.06	1	1058.18	0.809	
Year	1.66	2	32.74	0.206	
Body condition: fed	51.03	1	1059.66	< 0.001***	

Table 4
Parameters for chick-provisioning (mean ± SD) for 2015–2017 for the monitored 50 chicks. Meal size shows the amount of food provided by one parent per night (g), feeding frequency shows the mean number of meals provided by both parents per night (meals/night), feeding rate describes the mean total amount of food one chick received per night including single meals, double meals and nights without feeding (g/night), and frequency of double feeding shows how often a chick received two meals when fed during the night.

	2015	2016	2017	Total
Meal size	4.46 ± 1.38	4.56 ± 0.84	5.52 ± 0.82	5.01 ± 1.02
Feeding frequency	0.78 ± 0.17	0.62 ± 0.13	0.78 + 0.11	0.71 + 0.15
Feeding rate	5.92 ± 1.79	5.35 ± 1.10	6.67 ± 1.06	6.05 ± 1.31
Frequency of double feedings	0.14 ± 0.07	0.18 ± 0.14	0.19 ± 0.09	0.18 ± 0.11
Maximal feeding rate	17.10 ± 4.77	16.50 ± 3.23	20.32 ± 2.84	18.41 ± 3.69

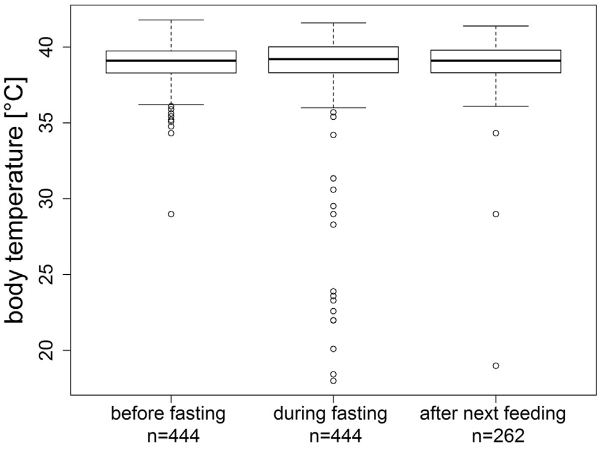

Fig. 3. Change of body temperatures of Wilson's storm petrel nestlings between fed and unfed chicks. Most chicks maintained stable, normothermic body temperatures during one day of fasting. However, some chicks decreased their body temperatures already after one day of fasting (middle column, 49 chicks) compared to the body temperatures when fed last (49 chicks). After receiving their next feeding most of chicks raised their body temperatures again (third column, 46 chicks).

2016: 20, 2017: 24). Chicks with positive body conditions showed significantly higher body temperatures (Table 3). Chicks that received food in the night before had significantly higher body temperatures than unfed chicks (Table 3). After not being fed for at least one day body temperatures decreased with decreasing body conditions (Fig. 5, Table 3). Body condition did not affect body temperature for fed chicks (Fig. 5, Table 3). The highest temperature decrease was 21 °C and in 10% of observations chicks had temperatures below 36 °C (lower range of boxplot whiskers; Fig. 3), while in 57% of the observations from 47 out of 50 chicks they had a maximal temperature decrease of 1 °C (Fig. 3). After up to six days without feeding chicks' body temperatures increased after the following feeding event up to 19.8 °C (Fig. 3). In a total of 92% of observations we still measured temperatures above 36 °C before that next feeding (data within boxplot range; Fig. 3). If chicks were not fed for several continuous days their body temperatures declined significantly with increasing numbers of days without food

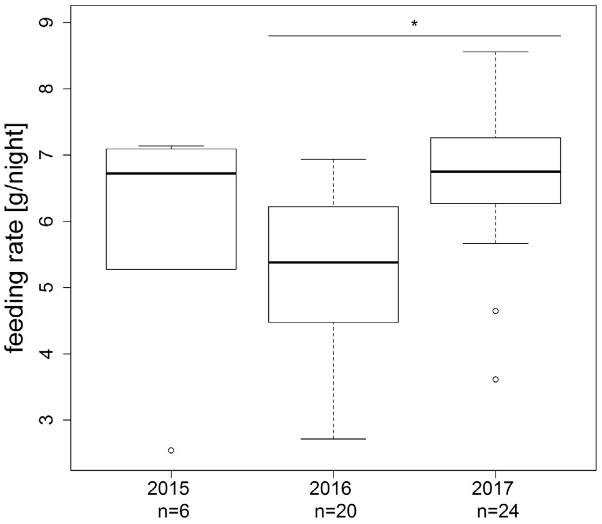

Fig. 4. Differences of provisioning in Wilson's storm petrels among years. Feeding rates (g/night) were significantly higher in 2017 (24 chicks) than in 2016 (20 chicks), but were not different from 2015 (6 chicks; compare Table 4). Significance is indicated by asterisk.

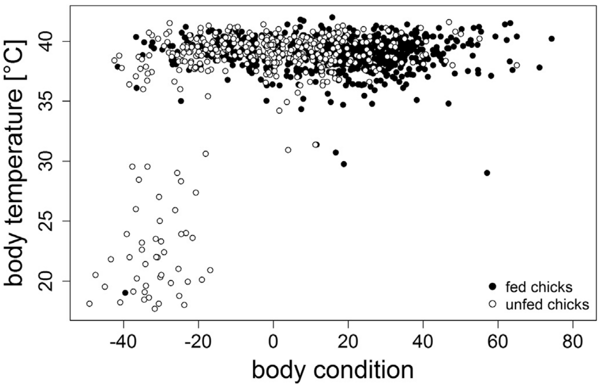

Fig. 5. Relationship between body temperatures and body conditions in Wilson's storm petrel chicks vary between fed and fasted chicks. Unfed chicks decreased their body temperature with decreasing body condition, while fed chicks maintained a stable body temperature (N_{chicks} = 50).

(for all data: Fig. 6; Spearman's rank correlation, rho = −0.067, S = 239,911,810, p = .026; for medians for each chick: Fig. 7: Spearman's rank correlation, rho = −0.190, S = 957,580, p = .013).

3.3. Influence of environmental conditions

Minimal nocturnal temperatures ranged between −6.4 °C and 4.5 °C, with a median temperature of 1.0 °C. Maximal nocturnal wind speeds had a median of 13 knt and measured between 0 and 65 knt. Chicks' body temperatures were significantly negatively affected by minimal nocturnal temperatures, but not affected by maximal wind speed during the former night (Table 3). Additionally, the later we measure a chick's temperature during the day, the warmer it was.

After two snowstorms within three days during the breeding season 2017 at the end of February we measured chicks again daily as soon as the nests had been excavated and accessible. During the following 10 days chicks experienced both, days of fasting and days of provisioning (Fig. 8). During these days after the snowstorms, 14 of 20 chicks decreased their body temperature by 10 °C (compared to body temperatures before the snowstorms) or more for at least one day, four chicks had body temperatures between 19 °C and 24 °C for three or more days. One chick died after several days without provisioning. All other chicks increased their body masses continuously again on average six days after the second snowstorm.

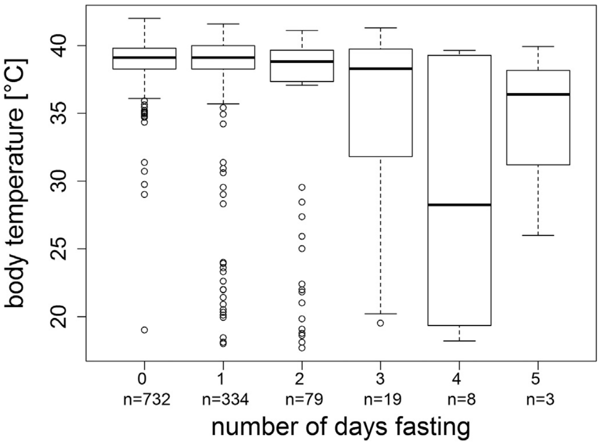

Fig. 6. Decline of body temperatures of Wilson's storm petrel nestlings during several days of fasting. Body temperatures significantly decreased when duration of fasting increased. N_{chicks} for 1st column: 50, N_{chicks} for 2nd column: 50, N_{chicks} for 3rd column: 40, N_{chicks} for 4th column: 18, N_{chicks} for 5th column: 8, N_{chicks} for 6th column: 3.

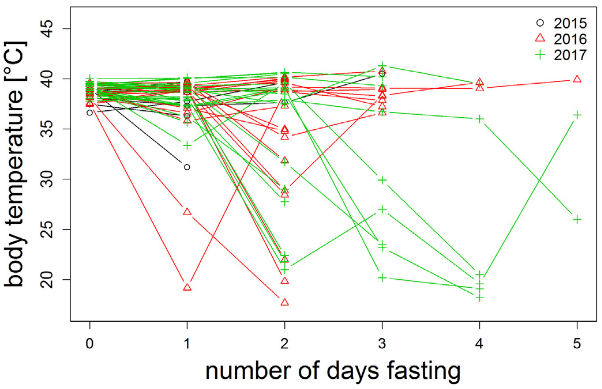

Fig. 7. Decline of body temperatures per chick during several days of fasting. Mean body temperatures of each chick were calculated for six groups: when fed during the night before (50 chicks), and after fasting for one day (50 chicks) until fasting continuously for five days (3 chicks). Different years are presented by different colours: black: 2015, red: 2016, and green: 2017. N_{chicks} for 3rd column: 40, N_{chicks} for 4th column: 18, N_{chicks} for 5th column: 8.

4. Discussion

The aim of the present study was to describe the variability of body temperatures in Wilson's storm-petrel chicks, in relation to provisioning, age, and environmental parameters such as air temperature, wind speed or snowstorms. We wanted to investigate when and why chicks were hypothermic and what characteristics this behaviour would show.

4.1. Body temperature variability and hypothermia

We found that Wilson's storm-petrel chicks growing up in Antarctica have highly variable body temperatures, ranging between 17 and 42 °C,

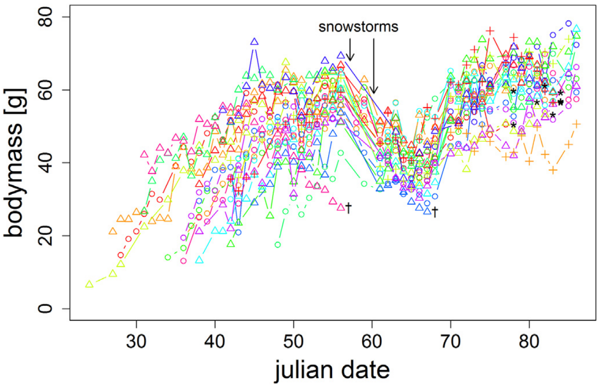

Fig. 8. Body mass development of Wilson's storm petrel nestlings during the breeding season 2017. The 24 observed chicks increased their body masses with age during the breeding season 2017. Two snowstorm incidents caused massive weight loss in all chicks. All but one regained their mass until fledging.*Indicate fledged chicks, †indicate chicks that died during the study. Arrows show snowstorm incidents. Each chick is presented by one colour – symbol combination.

the majority of measurements lay between 38 and 42 °C. According to Prinzinger et al. (1991) these values would cluster in the range of birds in the active (41.02 °C ± 1.29 °C) and resting (38.54 °C ± 0.96 °C) phase of the day and well within the range of body temperatures reported for other small petrels (Warham, 1973). The chicks of this study were only measured in the nest, during their development before fledging. On several occasions we found hypotherm chicks with temperatures as low as 17 °C, which is slightly lower than minimal temperatures for birds in hypothermia found in other studies (Krüger et al., 1982; Prinzinger et al., 1991), or than the 22.5 °C calculated by Geiser and Ruf in their review for birds weighing about 40 g (minimal body temperature during torpor for 40 g bird = 30.2 + 5.53 $\log_{10}(0.04) = 22.5$; Ruf and Geiser, 2015; Ruf, pers. comm.). But it is higher than the minimum of 10.6 °C reported for fork-tailed storm-petrels (Boersma, 1986). We found hypotherm chicks mainly until they were 45 days old, and hardly during their last two weeks of development, and their general body temperature raised with age (Fig. 2; Table 3). During their development many Wilson's storm-petrel nestlings reached weights exceeding that of their parents by far, without being fully grown (Fig. 8). This, and a following mass loss before fledging is a common pattern in Procellariiformes nestlings (Hamer et al., 2001; Mauck and Ricklefs, 2005; Obst and Nagy, 1993; Phillips and Hamer, 1999). Studies on northern fulmars have shown that Procellariiformes chicks accumulated fat until reaching peak mass, but retain this fat even during mass loss prior to fledging (Phillips and Hamer, 1999). Mass loss for northern fulmars seems to be caused by less frequent feedings by their parents during those last days at the nest (Phillips and Hamer, 1999). In Wilson's storm-petrels studies on energy budget of nestlings suggested a need for energy from lipid reserves (Obst and Nagy, 1993). However, different energetic demands may not be surprising compared to this 20-fold bigger bird. Additionally we found no indication that chicks were starved before leaving the nests. On the contrary, regular nest attendance and feedings until the last recorded day were frequent (unpublished data). Hence, mass loss during this phase is rather interpreted by high energy demands because wing and feather growth, and pre-fledging exercises that are common in burrow-nesting seabirds (Miskelly et al., 2009). Higher body temperatures would go hand in hand with this increased activity pattern, and improved insulation and surface-area volume ratios, and is also known from other seabirds (Ricklefs and Roby, 1983; Weathers et al., 2000). While especially late- born juvenile garden dormice use hypothermia to fatten up before hibernation (Mahlert et al., 2018), Wilson's storm-petrel chicks seem to need their time at the nest to terminate growth, and prepare fledging. Hypothermia would be unfavorable in this situation.

4.2. Influence of feeding and body condition

Provisioning of Wilson's storm-petrel chicks occurred on average on three out of four days and parents provided their chicks with about 6 g of food per night for the observed period of time (Table 4). This is slightly higher than what was calculated for Wilson's storm-petrel chicks at South Georgia (Croxall et al., 1988), but lower than observed at the same colony between 1996 and 2002 (Büßer et al., 2004). If chicks were fed during the previous night their body temperature was higher (Table 3), and chicks with higher body conditions had higher body temperatures. We found that after a fasting for at least one day, chicks' body temperatures decreased with decreasing body conditions (Fig. 5). Many studies showed the opposite trend: animals with better body conditions used hypothermia more frequently (e.g. Rojas et al., 2014; Stawski and Geiser, 2010). The reason for that discrepancy is based on the analysis of adults during non-reproductive times. Thus, adults did not face the trade-off between conserving energy versus growth and development of themselves or their young. Rather feeding imposed the potential of being predated, and fasting an opportunity to prevent predation. Hence, only animals in good conditions are able to abstain from foraging and this way decrease the risk of predation, while animals in bad conditions will need to search and find food in order to survive. However, Chicks on the other hand cannot influence whether they will receive food or not, and for them the most important is to survive the hatchling time until fledging. Only when survival is threatened by starvation it would be advantageous to lower their metabolic rate and to conserve their remaining energy resources. Studies on other storm-petrels came to similar conclusions, hypothermia was reported to occur after fasting or weather events that had presumably negative impact on provisioning (Boersma, 1986; Watson, 2013). This is additionally supported by the fact that after only one or two days of fasting a majority of chicks were still normotherm, while after three or more days hypothermia became more frequent and only few chicks maintained active body temperatures (Figs. 6, 7, Table 1). If structural development is completed and the main aim is to gain fat reserves, hypothermia was shown to successfully bridge times of scarce food, and allow intermittently fastened juvenile garden dormice to gain weight at similar rates than those fed ad libitum (Giroud et al., 2014). For Wilson's storm-petrel chicks structural growth does not finish until fledging (unpublished data). Together with the strong seasonality of the Antarctic it might be dangerous for chicks to be hypothermic too frequently: food abundance seems to be an important factor for growth rates in Wilson's storm-petrel chicks, and is decreasing towards autumn. After rapid growth of late-hatched chicks development becomes slower towards the end of the season when resources are probably declining (Quillfeldt and Peter, 2000). A delay in growth might result in a lower degree of development at fledging or adults leaving the breeding colony before chicks are fledged. Compared to data from 1996 to 2003 breeding seasons in especially 2015 and 2016 were late (Büßer et al., 2004). While nests are blocked with ice and snow petrels cannot start breeding. Studies on garden dormice showed that even though late-born juveniles showed enhanced growth rates, they could not completely catch up with their earlier born conspecifics (Stumpfel et al., 2017). This restriction is most likely also true for Antarctic storm-petrels. Hence, with an already delayed start uninterrupted development becomes even more important. For the future climatologists predict higher snow fall for western Antarctica and the peninsula, caused by warmer air temperatures and higher water evaporation (Turner et al., 2014). Snow fall in spring and shortly before the breeding season starts could delay egg-laying and cause an unfavorable initial situation for chick survival.

Literature of naturally occurring irregular feeding and its influence on hyothermia is scarce. However, Superina and Jahn (2013) found that armadillos feeding on low quality food, spent prolonged times hypothermic compared to those individuals fed with high quality food. This nutritional challenge might be comparable to that of chicks facing irregular feeding intervals, as both will lead to a worse body condition. We could not control for different food qualities among chicks in this study, but former studies investigating diet compositions of Wilson's storm-petrel chicks and adults reported a general pattern of preferred food items at different times during the breeding cycle instead of individual differences (Quillfeldt, 2002b). Hypothermia thus postpones and by that possibly reduces the risk of starvation. After the extended resting time at very low energy expenditures armadillos might find food of higher quality, while petrel chicks might be fed by their returning parents again. Like in fork-tailed storm-petrels facultative hypothermia in Wilson's storm-petrels could be an adaption to the frequently found gorge-and-fast provisioning pattern, as a combination of hypothermia during fasting to avoid maintenance costs for endothermy, and rapid growth after large meals could make up for development delay during hypothermia (Boersma, 1986).

4.3. *Influence of environmental conditions*

During severe environmental conditions, like low ambient temperature or high wind speed, other authors reported enhanced hypothermic behavior (Dietz and Kalko, 2006; Geiser, 2004, 1988; Watson, 2013). In our study we could not find such an influence of maximal nocturnal wind speed on Wilson's storm-petrel chicks' body temperature, but minimal nocturnal temperatures influenced their body temperatures negatively. As they grow up in the extreme environment of Antarctica Wilson's storm-petrel chicks experience low temperatures on a daily basis. Temperature extremes during the night differed over the years by only about 3 °C above and below the mean minimal temperature. This difference is potentially even smaller inside the cavities where the chicks are raised. For other burrow-nesting petrels breeding in sub-Antarctic regions outside temperatures did not or only slightly influence metabolic rates of birds (Adams and Brown, 1984). This could be true for Wilson's storm-petrel nestlings as well. Possibly chicks start moving more when temperatures drop slightly to find a better spot within the nest, and produce this way more heat in general. While it influences body temperature in general, it is less likely to be one of the main drivers for hypothermia for this species.

Long fasting periods occurred at all times, but especially after snowstorms. With an predicted increase of snow-fall in western Antarctic areas and the peninsula, snow storms are assumed to occur more frequently due to climate change (Turner et al., 2014). For the present data of 2017 nests were often accessible again after one to three days. Most chicks were fed already soon after such an event, but many chicks experienced a time of irregular feedings for the following days with long fasting times that in one case led to starvation of a chick in 2017. Especially young chicks often die during this entombment and the following fasting (Büßer et al., 2004; own observation). Surviving chicks became often hypothermic within few days after the storm, while they were often observed to be normothermic directly after the storm. Hypothermic responses can therefore not be linked directly to food shortages due to blockage of nests. However, as this pattern was shown for the majority of the breeding colony it still seems to be a valuable explanation that the poor provisioning's main factor was the snowstorm. As it is common for long lived animals, it is also known for storm-petrels to first care for themselves when in bad conditions, before provisioning their chicks again (Quillfeldt and Möstl, 2003). After snowstorms adults hence might be in a poor nutritional state themselves and may need to ensure their own survival before being able to regularly provide for their offspring again. This pattern was also found for another petrel species, the Antarctic petrel (*Thalassoica Antarctica*, Tveraa et al., 1998). It was shown that Antarctic petrel adults in good conditions fed larger meals to their chicks. Their chicks had better conditions themselves, and were more likely to survive than those of birds starting the experiment in worse body conditions. Within the next ten days after the snowstorm all chicks except one were fed regularly again, could increase body mass, and fledge. However, several snowstorms during one season could undermine chicks' fat reserves, and frequent hypothermic responses could delay development and put chicks at risk for successful fledging.

5. Conclusions

Body and torpor temperatures of Wilson's storm-petrel chicks fit well into the range of other animals of comparative size and activity. Hypothermic responses mirrors very closely the challenges Wilson's storm-petrel chicks face during their development: if provisioning is regular chicks maintain high body temperatures. However, the more unpredictable provisioning is, the more frequent hypothermia is observed in the chicks. The possibility of this strategy enables the chicks to survive dangerous times of limited food supply for example due to snowstorms. Thus this behavior may be especially important in years to come as climate change is predicted to increase snow fall in their breeding area, the Antarctic peninsula, and hence increase chances of blocked nests during the breeding season (Turner et al., 2014). As torpor usage enabled most chicks to survive extended times of unpredicted provisioning a next step will be to investigate if using torpor more frequently will have negative impacts for their future lives, for example because a decreased time for development forces them to fledge in a poorer body condition (compare Quillfeldt and Peter, 2000).

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Chapter 2

Consequences of heterothermy during development on physiology and growth for Antarctic storm-petrel nestlings

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Consequences of heterothermy during development on the physiology and growth of Antarctic storm-petrel chicks

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ABSTRACT

The ability to endure a wide range of body temperatures, called heterothermy, offers many species worldwide the possibility to survive times of malnourishment by lowering metabolic rate and body temperature. During development, facultative hypothermia and therefore lowered body temperatures might have disadvantages. Our study aimed at identifying potential trade-offs between the use of facultative hypothermia and investment into growth, immunity, and nutritional physiology in chicks of an Antarctic seabird, the Wilson's Storm-petrel (*Oceanites oceanicus*). To answer these questions, we used the irregular occurrence of facultative hypothermia of chicks from a free-living colony breeding on the South Shetland Islands, Antarctica. Our study showed that chicks experiencing lower body temperatures or snowstorms had slower growth, fewer circulating leukocytes, and lower triglyceride concentrations. These findings suggest reduced investment into physiology and growth during events of facultative hypothermia. Patterns of wing growth following facultative hypothermia suggest that chicks may recover within little time after feeding and rewarming. For other parameters, more data of intervals with facultative hypothermic body temperatures followed by normothermy are needed to allow similar conclusions. Our results suggest that while heterothermy seems a beneficial strategy for chick survival in this species, it incurs partially reversible trade-offs with other physiological traits.

1. Introduction

Post-hatching development is crucial for chick survival. Precocial birds need to acquire thermogenesis, attain fledging size and grow flight feathers, as well as reaching organic maturity and gaining a functional immune system before they will be ready for their independent adult lives. Insufficient development like stunted growth or a weak immune response will limit both the bird's short- and long-term survival (Christe et al., 1998; Hõrak et al., 1999; Lindström, 1999). In long-lived birds, parental survival often has a higher priority than offspring survival. In several seabird species, for example, a clutch consists of a single chick, which contributes only little to the parents' fitness (Clark and Ydenberg, 1990). Delayed maturation and late fledging may cause parents to abandon their chick too early, as remaining at the breeding site could have a negative impact on their own survival (Clark and Ydenberg, 1990). Hence, avoiding a prolonged time of development, growth and maturation is important for chicks' survival.

Different parameters have been identified as being important pillars of chick development. Growth of extremities and organs is crucial for fledging and thereby assuring survival (Oyan and Anker-Nilssen, 1996). Some structures seem to be universally important for chicks, like the nervous system (Oyan and Anker-Nilssen, 1996). Others differ in importance among bird species according to their ecology: Body mass increase, wing, leg or skull growth might be prioritized during development depending on foraging, escape or other survival strategies. While Atlantic Puffin (*Fratercula arctica*) juveniles are independent of their parents at fledging and most likely benefit from well-developed wings and brains for successful foraging (Oyan and Anker-Nilssen, 1996), chicks of *Uria* or *Alca* species are fed even after fledging and strong legs for paddling while swimming might be more important for foraging than fully grown wings or feathers (Croxall and Gaston, 1988). Generally, bigger individuals often face better reproductive and survival chances (Saraux et al., 2011). It is therefore crucial for chicks to invest into maturation and structural growth in order to fledge before their further rearing becomes too costly for their parents.

A good physical state enables a hatchling to cope with less ideal

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environmental and feeding conditions during post-hatching development. Several physiological parameters can be used to evaluate the physical state. For example, low blood levels of fatty acids like triglycerides show a bird is rather in a fasting, than in a resorptive state where it can build up reserves (Jenni-Eiermann et al., 2002). High haematocrit (i.e. the percentage of erythrocytes in the blood) indicates high blood oxygen-carrying capacity of an individual. During development, chicks increase their haematocrit values through erythropoiesis (Fair et al., 2007; Quillfeldt et al., 2004). Different parameters like dehydration, parasitism, or nutrition cause additional variation in haematocrit (Fair et al., 2007). The variation and change of both parameters, triglycerides and haematocrit, can give insights into a hatchlings physical condition.

At hatching, chicks are protected from parasites and pathogens by their innate immune system and antibodies that were deposited to the yolk by their mothers during egg production (Garnier et al., 2012). However, functionality of maternal antibodies decreases within days or weeks and some innate immune functions like induced local and systemic inflammation are energetically costly (Garnier et al., 2012; Gharaibeh and Mahmoud, 2013). Especially during energy demanding times it is advantageous to be able to use less costly immune effectors (Lee, 2006). Moreover, development of the adaptive immune system which provides specific responses due to the immunological memory, is slow and is more advantageous in later life (e.g. repeated exposure to the same pathogen) (Lee, 2006).

Procellariiform species are long lived and both parents are involved in raising one semi-precocial chick per season (Hamer et al., 2001). Until the down-covered chick achieves homeothermy, it has to be guarded and brooded and only one parent can forage to provision the chick at a time (Ricklefs and Roby, 1983). Active thermoregulation establishes spontaneously after hatching in some procellariiform species (e.g, Laysan Albatross *Phoebastria immutabilis*; Black-footed Albatross *Phoebastria nigripes*), but can take three to five days (e.g, Wilson's Storm-petrel *Oceanites oceanicus*), or even more than a week in others (e.g., Antarctic Petrel *Thalassoica antarctica*) (Dawson and Whittow, 1994; Bech et al., 1991; Gębczyński, 1995). When both parents leave for foraging at the same time, long absence or severe weather conditions can cause discontinuous and unpredictable food provisioning for their offspring, leading to energetically challenging times. Being endothermic, adult birds usually maintain a constant body temperature. In 31 petrel species an average body temperature for adult birds of 38.8 ± 0.2 °C has been determined (Warham, 1971). Chicks of several species show the ability to adjust their body temperature and metabolic rate according to energetic demands to survive e.g. times of limited food. In such cases, the metabolic rate is reduced and body temperature lowered, sometimes until a chick appears completely lifeless. This flexibility is known as heterothermy and has been described in species from several avian orders and from different parts of the world, among which are several seabirds (Boersma, 1986; Geiser, 1998; Krüger et al., 1982; Kuepper et al., 2018; Watson, 2013). Different terms are in use to clarify, if cold or hypothermic body temperatures result from a passive process of the inability of maintaining a constant normothermic temperature, or an active, regulated process that can be reversed at any time. To identify deep or shallow torpor, which describe regulated processes, Geiser et al. (2014) showed the need of assessing both, the metabolic rate and body temperature at the same time. Without measurements of the metabolic rate, the authors advise cautious use of the term torpor. Other authors solved this issue by describing the pattern of low body temperatures and adding the observed, regulated mechanism, by using the term “facultative hypothermia” (McKechnie and Lovegrove, 2002). To the best of our knowledge there are no studies investigating short and long-term consequences of heterothermy and facultative hypothermia during development in birds. Chicks have high nutritional demands during development and may face energy trade-offs that could challenge survival (Schew and Ricklefs, 1998). Insufficient nutrients or energy for the chick can reduce growth and delay maturation (Schew and Ricklefs, 1998). Wilson's Storm-petrels' semi-precocial chicks, that grow up in ice-free areas of the Antarctic and sub-Antarctic, have been shown to establish homeothermy until an age of five days and use heterothermy especially during elongated times of food scarcity, for example after snowstorms blocked nest entries for feeding parents for several days (Kuepper et al., 2018; Quillfeldt, 2001; Gębczyński, 1995; Roberts, 1941). The facultative mechanism of the observed hypothermia has been described by chicks that ended their hypothermia without receiving food and hence, additional energy supply to up regulate and maintain normothermic body temperatures again (Kuepper et al., 2018). In this study, we hence use the term “facultative hypothermia” when presenting consequences of heterothermy on growth, nutritional status, and function and development of the immune system (McKechnie and Lovegrove, 2002).

We predicted that growth will be slowed down during facultative hypothermic events, but the next feeding event might allow accelerated growth (compare Schew, 1995; Turner and Lilburn, 1992). We expect triglyceride concentrations in the blood to be low after fasting or facultative hypothermic events, indicating energy extraction from fat reserves (Jenni-Eiermann and Jenni, 2012; Quillfeldt et al., 2004). We further predict that haematocrit will increase with age, but during facultative hypothermic events, this increase should be slowed down (Quillfeldt et al., 2004). The immune response should be higher in individuals maintaining a more constant body temperature, which suffer less from malnutrition and are able to consistently invest into development of various immune branches (Ibañez et al., 2018; Kulaszewicz et al., 2017).

2. Methods

2.1. Study site and study species

At a body weight of 38 g and a wing span of 15 cm Wilson's Storm-petrels are the smallest nativebirds breeding in Antarctica, where their breeding colonies can be found in ice-free areas of the Antarctic and Sub-Antarctic islands (Beck and Brown, 1972; Quillfeldt, 2006). They have single-egg clutches maximally once a year and often nest in cavities below rocks (Quillfeldt, 2006; Roberts, 1941). Environmental conditions like snow cover, and parents' experiences determine the time of egg laying and hatching of chicks, which can range between beginning of January and mid-March (Büßer et al., 2004; Kuepper et al., 2018; Quillfeldt, 2006). The egg is incubated during a period of 38 to 54 days and parental care during incubation and for the chick is shared by both parents until they fledge at an age between 50 and 70 days (Beck and Brown, 1972; Roberts, 1941).After establishing active thermoregulation at around five days of age, chicks are left alone during the day and are mostly visited during night time for provisioning by their parents to avoid predation (Quillfeldt, 2006; Gębczyński, 1995; Roberts, 1941). During elongated times of fasting, e.g. after snowstorms blocked nest sites, and prevented adults to access their chicks (Ausems et al., 2023; Quillfeldt, 2001), chicks are capable of heterothermic responses (Kuepper et al., 2018; Quillfeldt, 2001). Here we studied a colony of Wilson's Storm-petrels breeding in the scree slopes of the “Three brothers” hill about 1.5 km from Carlini Research Station on King George Island (Isla 25 de Mayo), South Shetland Islands (62° 14′ S, 58° 40′W). Accessible nests were in scree slopes underneath big rocks and in depth from 20 cm to 60 cm.

2.2. Monitoring, sampling, and sample storage

During the reproductive seasons of three consecutive years (February–March 2015, 2016 and 2017) a maximum of 20 chicks per year were monitored daily from approximate age of five days to fledging / end of the season at the end of March, when the last ship left the station (hatching date estimation followed Kuepper et al., 2018; Quillfeldt, 2001). When a chick died or was no longer accessible, we included its data if it was older than ten days at this moment. However, to reach a total of 20 chicks to monitor until fledging, we included the next hatching chick into the daily monitoring. Two chicks were included at an age > 20 days. Data of these were only included into analyses using short- or mid-term mean body temperatures and not to those needing a full history of measurements. Only chicks living past their 10th day were included into this data set. Analyses are based on data from 50 chicks monitored during three years (2015: 6, 2016: 20, 2017: 24). If adult birds were present at a nest, they were not disturbed and we checked the nest again the following day. We measured daily both, chicks' body mass to 0.1 g with a digital scale, and chicks' cloacal body temperatures with a digital thermometer (Omega, HH506A, resolution 0.1 °C). We measured chicks' growth parameters every three days: tarsus length to 0.1 mm using calipers and wing length to 1 mm using a stopped rule. The first measurements of each chick were used to determine its age following a logistic regression calculated from growth data from 1996 (accuracy for tarsus length 20 mm: 12.0 ± 0.3 days; Quillfeldt and Peter, 2000). Normothermic temperatures were defined as an interval of similar distance above and below the median of all observed body temperatures (39 °C), with the upper border being the maximum value of all measured body temperatures (42 °C) (Kuepper et al., 2018). If chicks appeared completely lifeless, we reduced the protocol to weighing and put them back into their nest immediately after. For further analyses, a temperature of 27.5 °C, the median of all measured body temperatures below normothermic temperatures (36 °C – 42 °C), was assigned to those observations. If data could not be recorded due to guarding parents or severe weather, a missing data point (NA) was recorded for it.

Weekly blood samples of maximum 70 μl were taken from the wing vein using 24 G single use hypodermic needles (Sterican© Braun), and disposable heparinized micro-haematocrit capillaries (Hirschmann®) from chicks when at least nine days old and at least 25 g heavy. Capillaries were sealed with haematocrit tube sealing wax (Hirschmann®), and transported back to the laboratory after the monitoring round. Temperatures during the transport where never above 2 °C. In the field lab, blood samples were centrifuged for 5 min at 2376 *g* using a haematocrit centrifuge. Plasma, red blood cells and total blood volume were measured to the closest 0.5 mm using a wing ruler. Haematocrit was calculated from blood cell and plasma length measurements in the capillaries: $\text{length}_{\text{red blood cells}} / \text{length}_{\text{whole blood}} = \text{haematocrit}$.

Plasma and red blood cells were separately stored at −20 °C until further analyses that were conducted after the field season. For immune measurements we used only samples without interrupted freezing cycles.

The sex of chicks was determined by molecular analysis based on blood samples dried on FTA ® (Whatman™) classic cards (Griffiths et al., 1998).

2.3. Triglycerides

Plasma triglyceride concentrations were determined using standard spectrophotometric test combinations following the kit instructions (Triglyceride liquid REF 17624H, and ClinChem Control1 REF 16150, Sentinel diagnostics, Milan, Italy), and modified for small amounts of plasma (1 μl of sample in duplicates). Concentrations were expressed as mg dL^{-1}.

2.4. Immune measurements

In order to cover both innate and adaptive immune branches, their cellular and humoral effectors and processes with different costs we selected four measurements regularly used in avian eco-immunological studies.

2.4.1. Total and differential white blood cell counts

Blood smears were made directly in the field using 1–2 drops of whole blood before sealing capillaries. Back at the field station blood smears were fixed in methanol (99.8%, Sigma-Aldrich) for 30 s, air dried and stored at room temperature. After the return from the field site smears were stained in a 1:5 Giemsa: buffer solution pH 7.00 (Giemsa stock solution, T862.1, Roth) for 25–30 min, and then rinsed with desalted water, and air-dried. Blood smears were examined under an optical microscope (Zeiss Primo Star) using immersion oil and a 1000-fold magnification. Microscopical slides were scanned and heterophiles, basophiles, eosinophiles, monocytes, and lymphocytes were counted according to Hawkey and Dennett (1989) until their cumulative total reached 100 leukocytes. Heterophiles, basophiles, eosinophiles and monocytes are innate immune cells with various roles in defences, while lymphocytes are the cellular effectors of the adaptive immune system (Pap et al., 2010b). Erythrocytes were counted in five representative microscopic fields, and multiplied with the number of analyzed microscopic fields. The total erythrocyte number was used to calculate the number of leukocytes per 10,000 erythrocytes (Lobato et al., 2005). The ratio between heterophils, the most frequent innate immune cells, and lymphocytes of the same sample (H-L ratio) represents a measure for long-term exposure to a stressor (Davis and Maney, 2018). All cell counts were conducted by the same observer. Monocytes occurred only rarely in blood smears (< 1%) and were not considered for further analyses.

2.4.2. Bacterial killing assay

The bacterial killing assay (BKA) against *Escherichia coli* (ATCC No 8739) was used to characterize the functional activity of a bird's constitutive innate immune system (Vincze et al., 2022). We used a version of the assay previously described in Brust et al., 2022. Briefly, in duplicate 12 μl, 1:3.5 PBS-diluted sample was pipetted in 96-well plate and mixed with 4 μl of ~1.5 × 10^5 colony-forming units (CFU)/ml. Positive (not containing any plasma) and negative controls (not containing any bacteria or plasma) were run on each plate. After incubation for 30 min at 37 °C, 83 μl of tryptic soy broth (#X938.1, Carl Roth GmbH) was added to each well. Absorbance at 300 nm was measured with a spectrophotometer (Biotek; μQuant Microplate Spectrophotometer) to determine background absorbance and again after the plates had been incubated for 12 h at 37 °C. The BKA (%) was quantified as the bacteria growth in plasma after 12 h subtracted by the background absorption, in relation to the positive control (Brust et al., 2022; Vincze et al., 2022).

2.4.3. Phytohemagglutinin assay

Challenge with phytohemagglutinin is one of the most common assays used in avian eco-immunology. Although originally was interpreted as a proxy for the T-cell mediated immunity (Pap et al., 2010a), recent studies showed that elicits inflammatory immune response, as part of the induce innate immune system (Martin et al., 2006; Santiago-Quesada et al., 2015). For phytohaemagglutinin (PHA) tests 5 mg PHA-P (L8754, Sigma-Aldrich) was diluted with 2.5 ml phosphate-buffered saline (PBS). Tests were conducted in chicks aged between 48 and 52 d, and 10 μl were injected in the wing web of one wing. The other wing web was injected with the same amount of phosphate buffered saline (PBS) as a control. Wing webs were measured to the closest 0.05 mm before injection, and 24 h later on both wings using a thickness gauge. During the PHA challenge chicks were not disturbed by daily weighing or temperature measurements.

2.4.4. Total immunoglobulin Y (IgY) concentration

Immunoglobulin Y (IgY) is the main type of antibody in birds and is the effector of the humoral adaptive immune system. We measured the total IgY concentration in duplicate using an ELISA with commercial anti-chicken antibodies (Brust et al., 2022; Berardi et al., 2026). ELISA plates (82.1581.200, 96 wells, Sarstedt) were coated with 100 μl of 1:2000 diluted plasma sample. After several incubations and washing cycles, 100 μl of polyclonal rabbit anti-chicken IgY conjugated with peroxidase (A-9046, Sigma) at 1:250 (*v*/v) and later 100 μl of revealing solution [peroxide diluted 1:1000 in ABTS (2,20-azino-bis- (3-ethylbenzthiazoline-6-sul phonic acid))] was added. The final absorbance was measured at 405 nm using a photometric microplate reader (µQuant Microplate Spectrophotometer, Biotek) and the optical density (OD) was subsequently defined as total serum IgY levels (Brust et al., 2022; Prüter et al., 2020).

2.5. *Statistical analysis*

All calculations and statistical analyses were conducted in R, if not otherwise stated functions from the package base were used (R Core Team, 2022). For non-parametric comparisons of more than two groups (residuals of tarsus and wing growth) a Kruskal-Wallis rank sum test was used. If the result was significant, groups were compared using the posthoc Dunn's test from rstatix package (Kassambara, 2021). For analyses with repeated measurements per individual general linear mixed effects models were calculated by package lme4 (Bates et al., 2015) with the chicks' ID as a random effect. Parameters for models were chosen based on biological relevance: As several studies found differences between the sexes in immune function and hence investment into immunity (Vincze et al., 2022), we considered it likely that remaining resources for chicks to be invested into e.g. growth might differ between males and females. We therefore included sex in all analyses. Using a type 3 ANOVA F-Tests and *p*-values were calculated general linear mixed effects models. Model fit was tested by residual diagnostics reported via the R package DHARMa (Hartig, 2022). Figures were created using functions from R base and ggplot2 packages (density distribution plots; Wickham, 2016). The parameter "body condition" used in Fig. 1 is age independent and describes the residual mass to the population mean mass of chicks of the same age, using data from ten breeding seasons (compare Quillfeldt, 2002; Kuepper et al., 2018).

2.5.1. *Growth*

Based on all available chick tarsus and wing data for the considered time years 2015 to 2017 a standard growth curve for Wilson's Storm-petrel chicks was calculated by four parameter logistic regression using the FlexParamCurve p. ckage in R (Oswald et al., 2012):

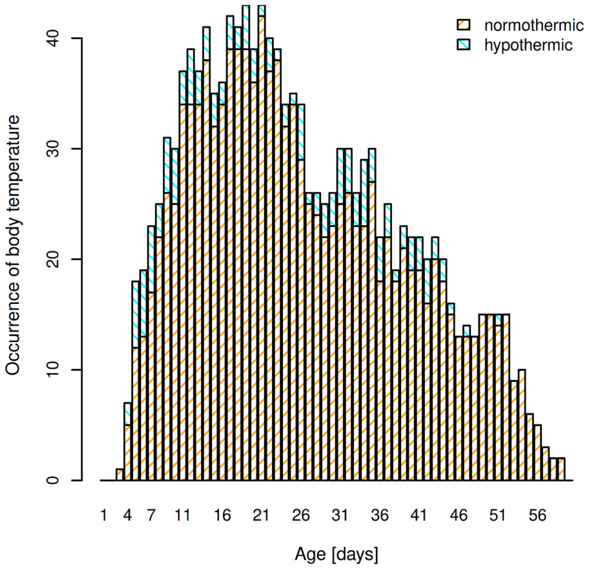

Fig. 1. Occurrence of normothermic (orange) and hypothermic (blue) body temperatures in Wilson's Storm-petrel chicks for each age. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$tarsus(t) = A + \frac{B}{1 + e^{-(b + k^{*}t)}}$$

with *t* expressing age in days of chick, *A* being the lower asymptote, $B + A$ being the upper asymptote, $b = -\ln(B)$, and *k* the growth constant. Relative tarsus and wing growth for the period of steep, almost linear growth (tarsus: age 10–24 d, wing: age 18–44 d) was calculated as differences between measurements and this standard curve.

A Mann-Whitney-*U* test with Dunn's post-hoc test was used to determine differences between relative tarsus or wing length at the last measurement before, and the two next measurements after facultative hypothermia. Temperature influence during this interval was analyzed with a general linear mixed effect model with mean body temperature during the interval as a parameter for short-term influence, and sex as fixed effects and ID of the chick as a random effect. Relative tarsus length had to be transformed to ranks instead of absolute numbers for tarsus growth, and relative wing length was transformed by $(x_{max} + 1)/x$ to avoid the right skewness of the response and meet the model assumptions.

Individual growth curves of tarsus and wing were built for chicks with at least five measurements, using four parameter logistic regressions (s. above). The resulting graphs ($n_{tarsus} = 28$, $n_{wing} = 19$) are provided in the supplementary material Figs. F1 and F2. Maximal tarsus growth rates were calculated from the slope at the inflection point, adjusted for four parameter regression after Richner (1989): $dy/dT = (k^*B/2)(1\text{-}B/2B)$ reduced to $g\_max = k^*B/4$ (see also Banach et al., 2021). Furthermore, the age at 90% of maximal tarsus length is given as an indicator for the end of steep growth (Gebhardt-Henrich and Richner, 1998), and maximal tarsus lengths were calculated from these growth curves. For wing growth maximal wing growth rate (slope at inflection point), and age at 20% of maximal wing growth indicating the onset of steep growth were calculated. For onset of tarsus growth and end of wing growth insufficient data were available to obtain meaningful estimates. Hence, these parameters were excluded from further analyses. For each calculated parameter, the influence of body temperature was analyzed by general linear models with the parameter as the response and fixed effects as given in Table 1.

2.5.2. *Physiological parameters*

To examine the influence of body temperature on immune and physiological parameters, general linear models (PHA swelling; blood cell counts of lymphocytes, heterophils, eosinophils, and basophils per 10,000 erythrocytes as well as the ratio of heterophils to lymphocytes

Table 1
Parameters used as fixed effects in general linear models analyzing influence of body temperature on growth. Parameters were determined by logistic regressions.

Explanatory variable	Fixed effect
Maximal tarsus growth rate	Age at maximal growth rate sex mean temperature until age at maximal growth rate
Age at end of steep tarsus growth	Sex mean temperature until age at end of steep growth
Maximal tarsus length	Age at maximum tarsus length sex mean temperature until age at maximum tarsus length
Maximal wing growth rate	Age at maximal growth rate sex mean temperature until age at maximal growth rate
Age at onset of steep wing growth	Sex mean temperature until age at onset of steep growth

(H-L ratio)), or general linear mixed effects models with chick ID as a random effect, if more than one sample per individual was analyzed, were used (haematocrit; triglycerides; BKA; IgY). Considered fixed effects were sex, and mean body temperature (PHA; blood cell counts), as well as age in mixed effects models (haematocrit; triglycerides; BKA; IgY). Individuals without sex determination were excluded from these models. For blood cell counts we used one blood smear of each individual at age 40 ± 2 days (median = 40 days). Additionally we analyzed cell counts relative to age before and after snowstorm events using a Wilcoxon signed rank test with continuity correction. Parameters relative to age were calculated by using residuals of general linear models of cell counts with the fixed effect age as the explanatory variable of linear models. Only meaningful H-L ratios (< 1.0) were considered for statistical analyses and six outliers had to be removed. Cell count for monocytes (0.3% of leukocytes) were in 47 out of 61 samples zero, hence no regression models were applied due to low sample size. We determined

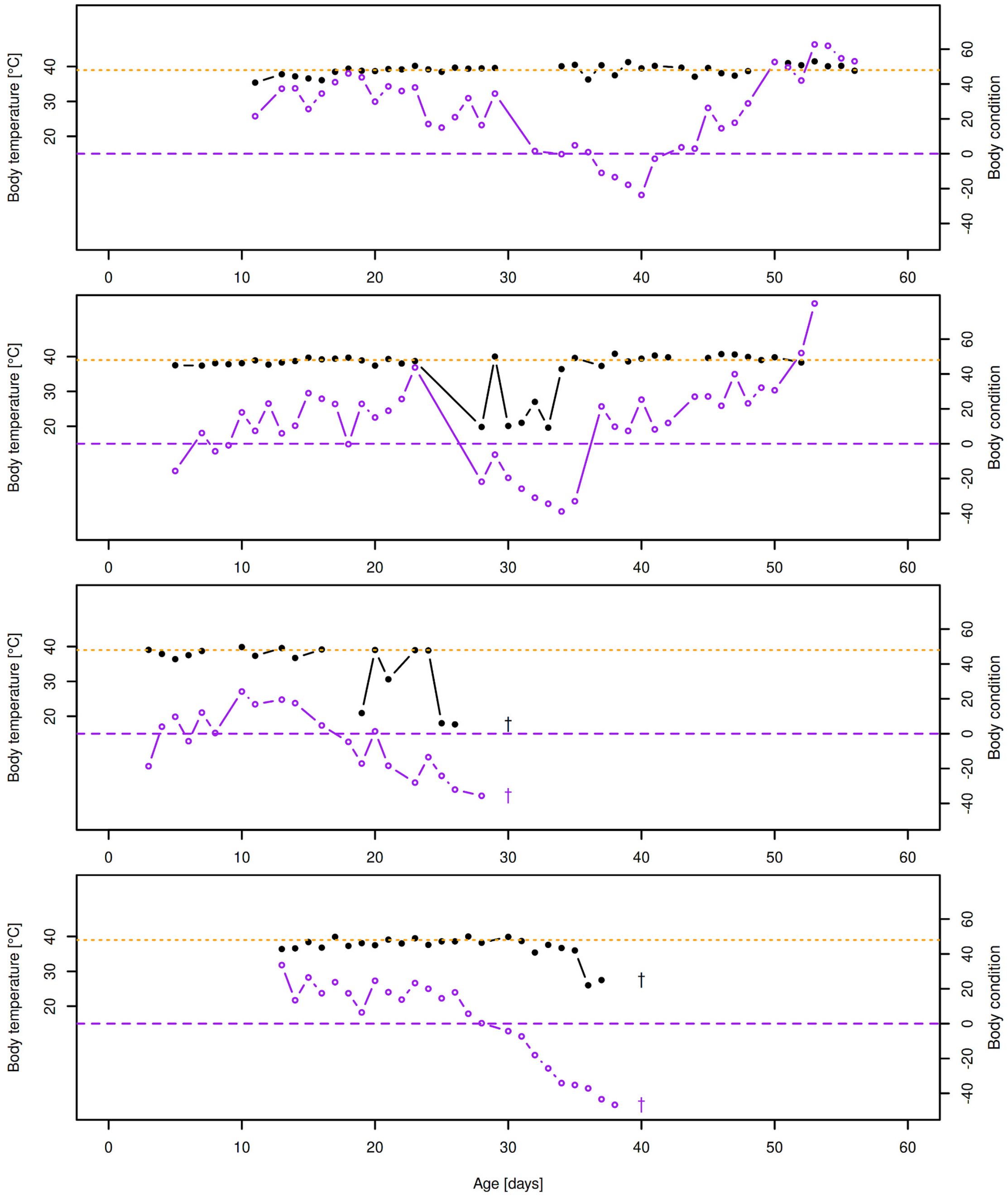

Fig. 2. Body temperatures (black dots) and body conditions (purple circles) for four of the observed chicks during the observation period. Dotted red line represents 39 °C, dashed purple represents the average body condition of chicks. Small letter t indicates the end of monitoring due to death of the chick. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

long-, mid-, and short-term effect of body temperature with models using mean body temperature until measurement (hereafter mean body temperature), mean body temperature of previous ten days (body temperature 10d), and mean body temperature of previous five days (body temperature 5d) as a fixed effect, respectively. Consequently, several models were run using in parts similar parameters. Results are hence only to be considered as hints for effects and further research, and should be treated as such.

Data distribution of IgY levels caused too high Eigenvalue of models to conduct reasonable statistics. Hence, data set was separated into two similarly sized sets. One set included all measurements of individuals with IgY maximum concentrations below 0.055, the other consisted of all individuals with maximum concentrations higher than 0.055. To assure other parameters of interest were equally distributed between these subsets, age and mean sum body temperature were tested using a Wilcoxon test. Both data sets were analyzed with the same models as described above.

The differences of PHA induced swellings between control and test wing after 24 h, as well as blood cell counts before and after snowstorm events, were tested with Wilcoxon singed rank tests with continuity correction. Wing web differences of control group between measurement directly after injection and 24 h later were as well tested with Wilcoxon singed rank tests with continuity correction.

3. Results

3.1. Body temperature and survival

Detailed descriptions on Wilson's Storm-petrel chicks' body temperatures are presented in Kuepper et al. (2018). Summarizing, body temperatures ranged from 17.7 °C to 42 °C, with a median of 39 °C (Kuepper et al., 2018). Less than 10% of all measured body temperatures were below 36 °C and hence, measured during an event of facultative hypothermia. Chicks used facultative hypothermia between the age of four days until the age of 51 days (Fig. 1). Older chicks were not observed to have body temperatures below 36 °C anymore. Out of 50 monitored chicks, 44 used facultative hypothermia at least once and only six chicks stayed normothermic during the whole observation period. Chicks could maintain normothermic body temperatures for up to three days of fasting and increase their body temperature from events of facultative hypothermia by more than 4 °C without receiving food (Kuepper et al., 2018). The longest bout of facultative hypothermia was found in one chick that appeared lifeless for eleven days before showing normothermic body temperatures again (Kuepper et al., 2018). Four of the 50 chicks monitored for this study died. The body temperatures against time of two of these are presented in Fig. 2, together with those of two chicks that survived until fledging.

3.1.1. Growth

3.1.1.1. Tarsus length.

Residuals of chicks' tarsus growth showed values above (positive) or below (negative) average tarsus length relative to a chick's age. These residuals were not different between the last measurement before an event of facultative hypothermia, the closest next measurement after facultative hypothermia or the next completely normothermic measuring interval (Kruskal-Wallis rank sum test, $\chi^2 = 4.36$, $p = 0.113$; Fig. 3). A general linear mixed effect model showed a significant increase of tarsus residual with increasing mean body temperature during the measuring interval (Table 2).

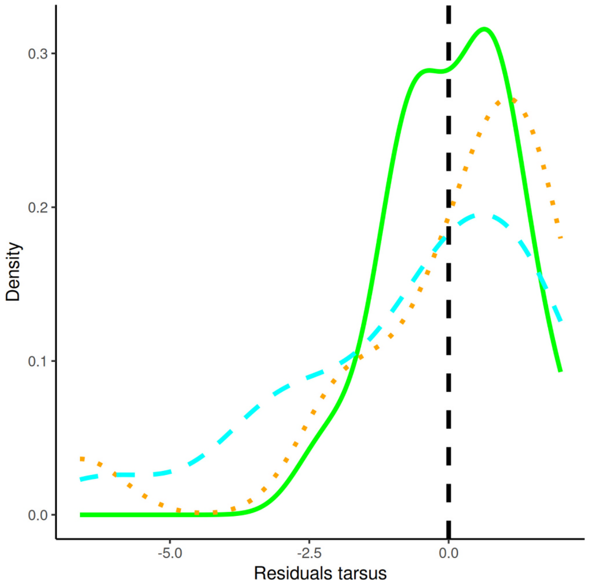

Fig. 3. Deviation of residuals of tarsus length relative to age before (green, solid) and after (lightblue, dashed) an event of facultative hypothermia, as well as the deviation of tarsus length residuals relative to age for the following measurement interval (orange, dotted), during which chicks maintained normothermic body temperatures. Median of residuals was 0.17 before facultative hypothermy, 0.01 after facultative hypothermia, and 0.54 for the following normothermic interval. Dashed black line shows values of standard growth curve, where residuals are zero. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Influence of body temperature on the tarsus growth: Estimates and test statistic for fixed effects of general linear mixed effects model for tarsus residuals for mean body temperature during interval.

	Estimate	Std. Error	F	*p*	
Intercept	34.58	34.40	1.01	0.317	
Mean body temperature	1.62	0.74	4.78	0.031	*
Sex	−2.8	22.59	0.15	0.858	

178 Observations in 50 groups by ID; marginal R^2: 0.01; conditional R^2: 0.80.

Neither maximal growth rate nor age at 90% maximal tarsus length of a chick was significantly influenced by mean sum body temperature until considered age (Supplementary Material T1 & T2). Maximal tarsus length was significantly, slightly positively, influenced by mean body temperature (Table 3).

Table 3
Influence of body temperature on the final tarsus length: Estimates and test statistic for general linear model for maximal length of tarsus for mean body temperature until asymptote, age at asymptote, and sex.

	Estimate	Std. Error	F	*p*	
Intercept	23.02	6.54			
Mean body temperature	0.34	0.16	4.55	0.043	*
Age	−0.01	0.04	0.01	0.937	
Sex (male)	−0.15	0.37	0.15	0.701	

Residual standard error: 0.96 on 24 df, multiple R^2: 0.16, adjusted R^2: 0.06, $F_{3, 24}$: 1.57, p: 0.223.

3.1.2. *Wing length*

Wing length residuals showed values above (positive) or below (negative) average wing length relative to a chick's age. These residuals were significantly different between the last measurement before an event of facultative hypothermia, the closest next measurement of wing length after facultative hypothermia or the next completely normothermic measuring interval (Kruskal-Wallis rank sum test, $\chi^2 = 8.50$, df $= 2$, $p = 0.014$; Fig. 4). The posthoc Dunn's Test showed that wing growth during the interval including the facultative hypothermic event was significantly slower than in the next completely normothermic interval, leading to negative residual differences in the first, and positive residual differences in the second interval (Dunn's test: statistic $= 2.70$, $p = 0.007$, adjusted statistic $r = 0.478$, adjusted $p = 0.021$). The differences of residuals of the other measured intervals did not differ significantly among each other (Dunn's test between interval with facultative hypothermia and interval of last measurement before facultative hypothermia and measurement after fully normothermic interval: statistic $= 0.40$, $p = 0.686$, adjusted statistic $r = 0.07$, adjusted $p = 1$; test between interval of last measurement before facultative hypothermia and measurement after fully normothermic interval and fully normothermic interval: statistic $= 2.30$, $p = 0.022$, adjusted statistic $r = 0.406$, adjusted $p = 0.065$). A general linear mixed effect model showed a small significant decrease of wing residual with increasing mean body temperature during the measuring interval (Table 4).

The maximal growth rate was not influenced by the mean body temperatures, sex, or age (at inflection point, supplementary material T3). The age at 20% of maximal wing growth was positively influenced by mean body temperature (Table 5).

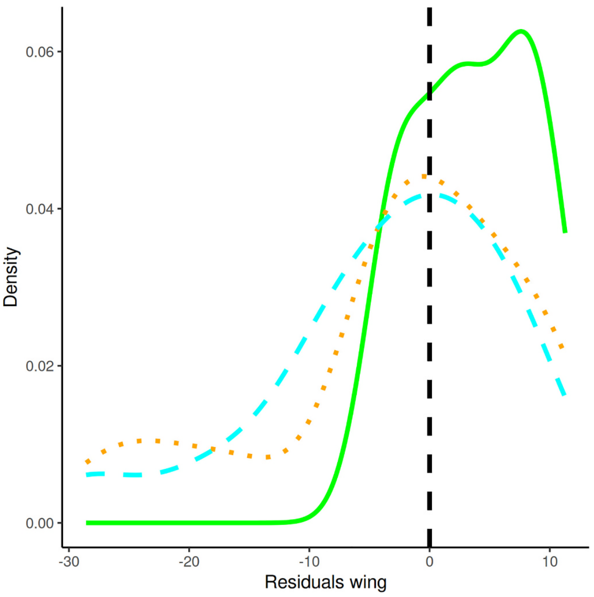

Fig. 4. Deviation of residuals of wing length relative to age before (green, solid) and after (lightblue, dashed) an event of facultative hypothermia, as well as the deviation of wing length residuals relative to age for the following measurement interval (orange, dotted), during which chicks maintained normothermic body temperatures. Median of residuals was 3.05 before facultative hypothermy, −1.51 after facultative hypothermia, and − 0.57 for the following normothermic interval. Dashed black line shows values of standard growth curve, where residuals are zero. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4

Influence of body temperature on the residual wing growth: Estimates and test statistic for fixed effects of general linear mixed effects model for wing residuals for mean body temperature during interval.

	Estimate	Std. Error	F	*p*	
Intercept	59.8	7.4	65.42	< 0.001	***
Mean body temperature	−0.4	0.2	5.96	0.015	*
Sex (females)	−1.0	5.7	0.41	0.663	

238 Observations in 49 groups by ID: marginal R^2: 0.03; conditional R^2: 0.57.

Table 5

Influence of body temperature on the onset of wing growth in Wilson's Storm-petrel chicks: Estimates and statistics of general linear model for age at 20% above lower wing growth asymptote with sex, and mean body temperature until this age.

	Estimate	Std. Error	F	*p*	
Intercept	−13.05	17.98			
Mean body temperature	0.75	0.49	4.8	0.046	*
Sex (male)	3.17	1.50	4.4	0.055	.

Residual standard error: 2.87 on 13 df; multiple R^2: 0.42, adjusted R^2: 0.33; $F_{2,13}$: 4.65, *p*: 0.030.

3.1.3. *Nutritional physiology*

3.1.3.1. *Haematocrit.*

Haematocrit is increasing significantly with age (Fig. 5), mean body temperature had no significant effect on haematocrit, neither for the whole duration (Table 6) nor for the last five or ten days (supplementary material T4 & T5).

3.1.3.2. *Blood plasma triglyceride levels.*

Triglyceride plasma levels were lower in chicks with lower mean body temperatures (supplementary material T6) and lower mean body temperatures for the past five days, with a stronger effect for recent body temperatures (Table 7, Fig. 6). Age or sex did not did not affect triglyceride levels. Mean body temperatures of the last ten days did not influence triglyceride levels (supplementary material T7).

3.1.4. *Immune system*

3.1.4.1. *Functionality of the humoral innate immunity measured by bacterial killing assay.*

The ability of a chick's blood plasma to kill bacteria was not significantly influenced by its age, sex, mean body temperature, or mean body temperature of the last five days (supplementary material T8 & T9). Models with mean body temperature of the last ten days showed

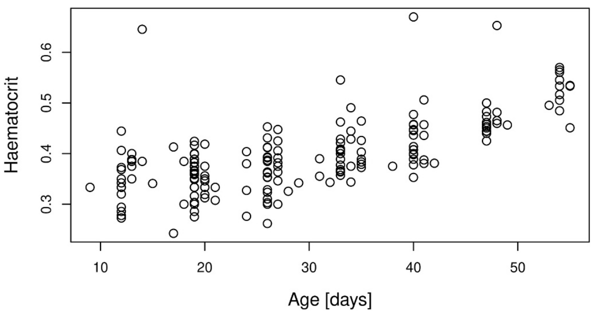

Fig. 5. Haematocrit in relation to age in Wilson's Storm-petrel chicks.

Table 6
Influence of body temperature on the haematocrit of chicks: Estimates and test statistic for fixed effects of general linear mixed effects model for haematocrit for age, mean body temperature, and sex.

	Estimate	Std. Error	F	p	
Intercept	0.319	0.089	12.43	< 0.001	***
Age	0.004	< 0.001	86.43	< 0.001	***
Mean body temperature	−0.001	0.002	0.19	0.667	
Sex	0.004	0.011	0.15	0.700	

168 observations in 45 groups; marginal R^2: 0.41; conditional R^2: 0.49.

Table 7
Influence of body temperature on the triglyceride concentrations in chicks: Estimates and test statistic for fixed effects of general linear mixed effects model for of triglycerides for mean body temperature 5d, age, and sex.

	Estimate	Std. Error	F	p	
Intercept	−27.1	74.1	0.13	0.720	
Mean body temperature (last five days)	4.2	1.9	4.58	0.035	*
Age	0.1	0.3	0.04	0.842	
Sex (male)	−7.9	9.4	0.69	0.411	

111 observations in 45 groups by ID; marginal R^2: 0.06; conditional R^2: 0.23.

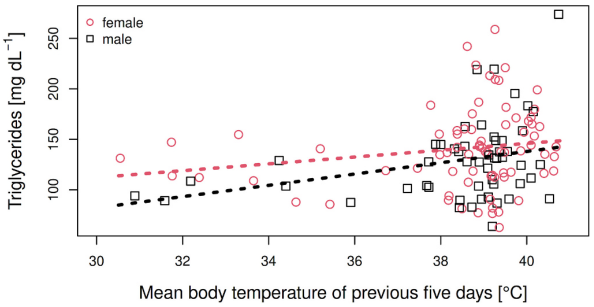

Fig. 6. Influence of mean body temperature of previous five days on plasma triglyceride concentration in Wilson's Storm-petrel chicks. Red line and circles represent females, black line and boxes represent males. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly higher BKA scores in female than in male chicks (Table 8, Fig. 7).

3.1.4.2. *IgY concentration.*

Individuals that had maximal IgY levels below 0.055 were neither different in age, or in mean body temperature until measurement compared to those with maximal levels higher than 0.055 (Wilcoxon rank sum test with continuity correction, mean body temperature: W = 1020, p = 0.515; mean body temperature (five days): W = 883.5, p = 0.834; mean body temperature (ten days): W = 655, p = 0.937; age: W = 955.5, p = 0.950). With increasing age both groups showed significantly higher concentrations (Table 9, Table 10, Fig. 8). Among Individuals with maximal IgY levels higher than 0.055, females had higher IgY concentration than males (Table 10). Tests with mean body temperature of the last five or ten days before measurements showed similar results in both, models with low and with high maximal IgY levels, and no influence of body temperature on IgY concentration (supplementary material T10 - T13).

Table 8
Influence of body temperature on the bacterial killing activity of Wilson's Storm-petrel chick blood plasma: Estimates and test statistic for fixed effects of general linear mixed effects model for of BKA for mean body temperature of past ten days, age, and sex.

	Estimate	Std. Error	F	p	
Intercept	9.31	13.48	0.42	0.521	
Mean body temperature (last ten days)	0.03	0.34	0.01	0.926	
Age	0.05	0.07	0.49	0.490	
Sex (male)	−3.90	1.69	5.11	0.038	*

41 observations in 19 groups by ID; marginal / conditional R^2: *NA* because of singularities.

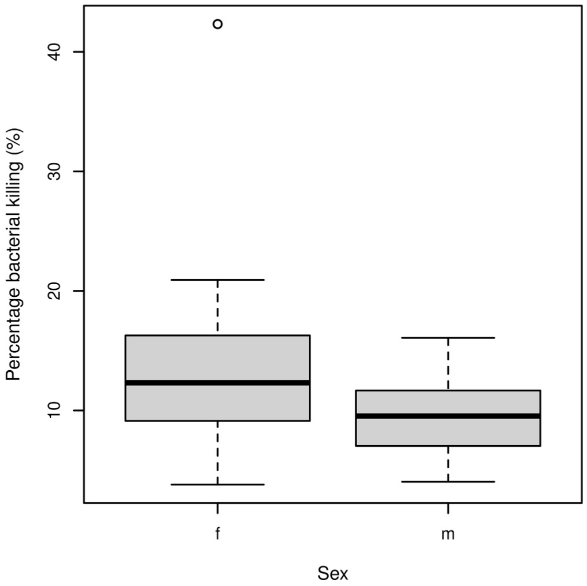

Fig. 7. Differences of BKA values in male and female Wilson's Storm-petrel chicks.

3.1.4.3. *Induced inflammatory immune response.*

Wing web reaction swellings induced by phytohaemagglutinin injections were significantly higher than swellings of the control wing after 24 h (Wilcoxon signed rank test with continuity correction, V = 120; $p < 0.001$). Wing web swellings of control wings did not differ significantly after 24 h compared to directly after injection (Wilcoxon signed rank test with continuity correction, V = 80.5; p = 0.256). Swellings were not significantly influenced by mean body temperature until day of injection (supplementary material T14), nor by mean body temperature of the previous five (Fig. 9, Table 11) or ten days (supplementary material T15). Males showed significantly greater swellings than females in models with mean body

Table 9
Influence of body temperature on low IgY concentration in Wilson's Storm-petrel chick blood: Estimates and test statistic for fixed effects of general linear mixed effects model for IgY for mean body temperature, age, and sex for individuals with IgY levels up to 0.055.

	Estimate	Std. Error	F	p	
Intercept	−0.125	0.116	1.01	0.323	
Mean body temperature	0.003	0.003	0.93	0.342	
Sex	0.009	0.005	3.44	0.113	
Age	0.001	< 0.001	20.64	< 0.001	***

41 observations in 9 groups by ID; marginal R^2: 0.40; conditional R^2: 0.48.

Table 10

Influence of body temperature on high IgY concentration in Wilson's Storm-petrel chick blood: Estimates and test statistic for fixed effects of general linear mixed effects model for IgY for mean body temperature, age, and sex for individuals with IgY levels above 0.055.

	Estimate	Std. Error	F	*p*	
Intercept	−0.239	0.298	0.57	0.456	
Mean body temperature	0.008	0.008	0.86	0.362	
Age	−0.061	0.019	10.36	< 0.01	**
Sex	0.002	0.001	10.70	0.002	**

45 observations in 13 groups by ID; marginal R^2: 0.38; conditional R^2: 0.42.

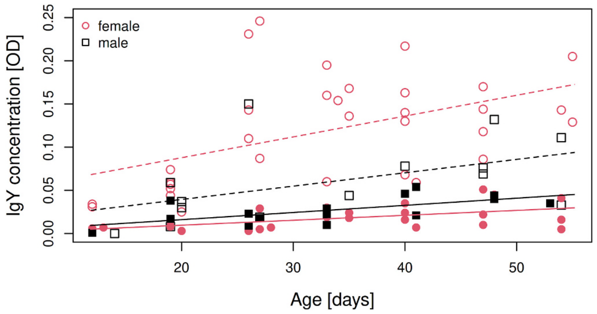

Fig. 8. Influence of age on IgY concentrations for female and male Wilson's Storm-petrel chicks with maximal IgY concentrations below 0.055 (solid symbols, solid lines) and above 0.055 (open symbols, dashed lines). Red line and circles represent females, black line and boxes represent males. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

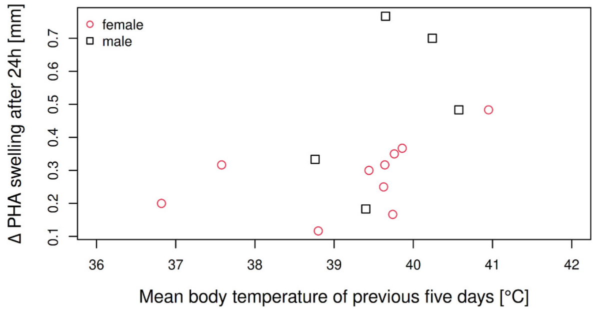

Fig. 9. Response to immune challenge of females and males in Wilson's Storm-petrel chicks: PHA swelling (difference of wing depths 24 h after an immune challenge with PHA), in relation to the mean body temperature of previous five days. Red line and circles represent females, black line and boxes represent males. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 11

Immune response of Wilson's Storm-petrel chicks to a challenge with PHA: Estimates and test statistic for fixed effects of general linear mixed effects model for PHA swelling for the mean body temperature during the last five days, and sex.

	Estimate	Std. Error	F	*p*	
Intercept	−2.14	1.56			
Mean body temperature	0.06	0.04	4.305	0.060	.
Sex	0.18	0.09	4.091	0.066	.

Residual standard error: 0.15 on 12 df, multiple R^2: 0.41, adjusted R^2: 0.31, $F_{2,12}$: 4.20, *p*: 0.041.

temperatures until PHA tests and with mean body temperatures for the previous ten days (Fig. 10, supplementary material T15).

3.1.4.4. Circulating immune cells.

Blood cell counts of blood smears (lymphocytes (49.9%), heterophils (19.3%), eosinophils (12.1%), and basophils (18.4%)) of chicks were not significantly influenced by body temperature or mean body temperature 5d or 10d (supplementary material T16 – T27). The *p*-value of the H-L ratio was below 0.1 with lower values at higher mean body temperature during the past five days (Table 12). For heterophils and eosinophils males showed higher values than females in models using long- or short-term mean body temperatures (supplementary material T19, T20, T22, T23).

Absolute numbers of lymphocytes were lower after snowstorms (Fig. 11). The *p*-value for eosinophiles was <0.1, with lower counts after compared to before snowstorms. Other cells counts or H-L ratio were not affected by snowstorm events (Table 13).

4. Discussion

4.1. Body temperature and survival

Little is known about direct effects of facultative hypothermia during development, and even less studies aimed to understand its short- or long-term consequences for wild birds. To the best of our knowledge, this study is the first to analyze effects of heterothermy on developing wild birds using the field observations of facultative hypothermia. Normothermia was observed earliest in a three-day old Wilson's Storm-petrel chick in this study and heterothermy was used by chicks from age four to 51 days. Only in the oldest chicks that were close to fledging were not observed using facultative hypothermia anymore (Fig. 1). Almost all chicks used heterothermy at least once during their development and only four of the monitored chicks died during the study. As presented in Fig. 2, some chicks maintained normothermic body temperatures even during times of low body conditions, while others used facultative hypothermia at similar body conditions. This was also true for the four chicks that died. Hence, the low number of non-surviving chicks with their individual death causes did not allow any conclusions about survival chances based on the use of facultative hypothermia. We can only

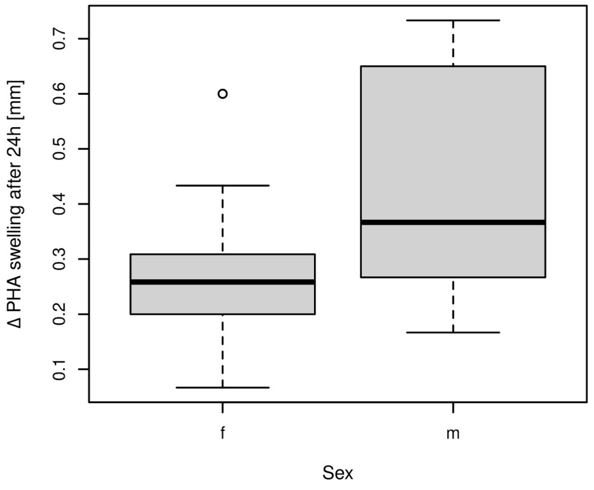

Fig. 10. Sex differences in the immune response of Wilson's Storm-petrel chicks to a challenge with PHA (swelling measured after 24 h).

Table 12

Influence of the body temperature of Wilson's Storm-petrel chicks on their H-L ratio: Estimates and test statistic for fixed effects of general linear mixed effects model for H-L ratio for mean body temperature 5d, and sex.

	Estimate	Std. Error	F	*p*	
Intercept	1.0	0.40			
Mean body temperature	−0.02	0.01	3.7	0.086	.
Sex	0.10	0.06	2.4	0.138	

Residual standard error: 0.13 on 16 df, multiple R^2: 0.28, adjusted R^2: 0.19, $F_{2, 16}$: 3.09, *p*: 0.074.

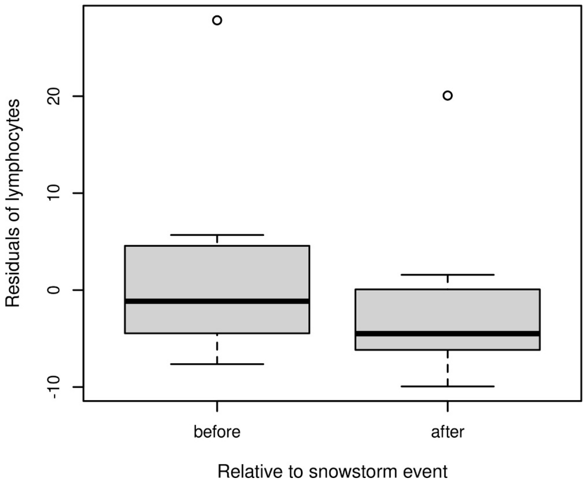

Fig. 11. Influence of snow storms on the lymphocyte numbers of Wilson's Storm-petrel chicks.

Table 13

Difference leukocyte counts in Wilson's Storm-petrel chick blood before and after snowstorms: Statistical parameters for different blood cell counts.

Cell type	V	*p*	
H-L ratio	27	0.119	
Lymphocytes	131	0.048	*
Heterophils	85	1	
Eosinophils	129	0.060	.
Basophils	96	0.671	

assume that chicks in bad conditions profit from facultative hypothermia as a majority of the chicks using facultative hypothermia survived until fledging. Our main aim for this study was to find out if Wilson's Storm-petrel chicks under-going frequent events of facultative hypothermia face trade-offs that might influence their performance and fitness later in live. Other studies used adult birds to learn more about the use of facultative hypothermia or torpor during mass gain phases prior to migration, or to survive daily fasting during sleep (Carpenter and Hixon, 1988; Geiser and Brigham, 2012; Krüger et al., 1982). As facultative hypothermia in Wilson's Storm-petrel chicks occurs most often after fasting periods (Kuepper et al., 2018), it is difficult to distinguish effects caused by facultative hypothermia from those caused by fasting. In the following we will discuss the direct and indirect implications of heterothermy on growth, nutrition, and immunity in detail.

4.2. Growth

Our study revealed effects of mean body temperature and facultative hypothermia on growth. While the first reflects a long-term effect of heterothermy, with an accumulation of facultative hypothermic events leading to lower average body temperatures, the second reflects the direct effect of an recent event of facultative hypothermia. Interestingly, wing and tarsus growth were affected differently by long-term and direct effects of facultative hypothermia. Tarsus growth was not directly affected by facultative hypothermia, however, higher mean body temperatures enhanced tarsus growth. Wing growth was slowed by facultative hypothermia with a small recovery within a few days, but mean body temperature had a small, negative effect on its growth. However, this negative effect might be a result from several events of reduced growth during facultative hypothermia: while body temperatures were normothermic again, wing growth was yet slightly delayed and only reached average rates after some days. In general, limitations of growth in the literature are more commonly linked to food availability and malnutrition. Use of facultative hypothermia is known to be caused by elongated durations of food depletion in Wilson's Storm-petrel chicks (Kuepper et al., 2018), and hence, indirectly effects of facultative hypothermia also reflect effects of malnutrition. Intervals of only one or few days of fasting may easily be buffered by reserves (Schew, 1995), and only longer fasting intervals that lead to facultative hypothermia might lead to decreased investment into growth to save energy (Boersma, 1986; Moe, 2004; Ricklefs, 1987). Slowed growth during food restriction could result in retarded, parallel or accelerated growth following realimentation, or in this case reheating. In different bird species parallel growth after realimentation has been shown (body weight and tibia width in turkeys (*Meleagris gallopavo*), Turner and Lilburn, 1992; skull, tarsus and wing length in European Shags (*Phalacrocorax aristotelis*), Moe, 2004). Parallel growth in Wilson's Storm-petrel chicks could cause the developing period becoming too short for them to fledge in time before fall and winter storms would turn the breeding grounds into harsh or even dangerous habitats for the adults. Therefore, the chicks' lives could be at risk, when adults would have to leave their chicks before they are ready to survive by themselves. Our findings suggest slightly imposed growth in wings after slowed growth during facultative hypothermia, but no comparable effect for tarsus growth. Hence, energy saving during heterothermy compared to malnutrition under normothermy might allow chicks to use this energy for catch up growth after they rewarm and receive their next meal in at least some tissues. In studies comparing growth rates of European Starlings (*Sturnus vulgaris*) and Japanese Quails (*Coturnix japonica*) decrease of growth in different tissues was observed (Schew, 1995). The two species were coping with different strategies during food restriction or malnutrition: starling chicks' rates of structural growth only decreased slowly and seemed to be related to the consumption of reserves (Schew, 1995). Quail chicks' structural growth rates decreased to low levels immediately, before reserves could be used up (Schew, 1995). After re-alimentation their growth rates returned to normal values within 24 h. Analogue, in a second study quail chicks showed drops in body temperature and metabolic rate after the first day of food restriction, but both parameters increased quickly after re-alimentation (Schew, 1995). Facultative hypothermia might allow individuals to pause maturation at the same time than growth. This way enhanced growth after facultative hypothermia might enable chicks to fledge at a similar time and size than without fasting induced facultative hypothermia. In some mammals and birds accelerated growth was found (tibia length in turkeys, Turner and Lilburn, 1992; body mass in cockerels, Osbourn and Wilson, 1960; body weight and growth in Meerkats (*Suricata suricatta*), Huchard et al., 2016; body mass and spine length in children, Martorell et al., 1979). This implies that normal growth rates are optimized at a non-maximal level (Schew and Ricklefs, 1998), which might allow juveniles to accel their growth rates after facing reduction because of food restriction or heterothermy. Structural growth may not represent an adequate measure for all species as depending on most important survival factors growth rate reduction might be adapted and differentiated between tissues and life history strategies (Gebhardt-Henrich and Richner, 1998). Also other explanations for the differences between tarsus growth, which was not directly affected by facultative hypothermia, and wing growth, come to mind. First, tarsus growth happens earlier in live than wing growth. Tarsus growth might occur at a time when structural growth is the most important developmental step for the chick and hence, is prioritized by energy allocation (Moe et al., 2004; Reid et al., 2008). Second, tarsus and wing growth may be affected differently by the growth rate – functional maturity trade-off - which implies that for some structures maturity is more important than pure growth, and facing limited resources investment should be aimed at maturity instead of size (Ricklefs et al., 1998). Here, this trade-off might be stronger in wings than in legs, for a bird that spend the majority of its live on sea. To get closer to these questions it would be interesting to consider more parameters of growth that have their peaks at different ages, to see, if there a positive effect of heterothermy during times of food restriction can be found like in our and former studies.

4.3. *Nutritional physiology*

The physiological state of chicks was directly affected by facultative hypothermia, as shown by lower plasma triglyceride levels after frequent events of facultative hypothermia, but was not affected for longer, as haematocrit values revealed, as well as a missing effect of mean body temperatures of several days on triglyceride levels. Using heterothermy allows chicks to lower metabolic rate and hence, lose less body mass during fasting than they would when normothermic. As chicks often experience a phase of fasting before using facultative hypothermia, lower body conditions are expected before they enter facultative hypothermia (Kuepper et al., 2018). Our results suggest that effects of facultative hypothermia on chicks' physiology point in the same direction, and do not go beyond effects of fasting and the resulting lower body condition. Haematocrit was also in an earlier study in the same species not affected by body condition (Quillfeldt et al., 2004). Increasing haematocrit values with age were shown in several other studies before (Atwal et al., 1964; García et al., 2019; Kostelecka-Myrcha et al., 1973; Prinzinger et al., 2012; Puerta et al., 1989), High triglyceride levels are linked to recent feeding and body mass increase (Jenni-Eiermann et al., 2002; Jenni-Eiermann and Jenni, 1994; Quillfeldt et al., 2004). But triglyceride levels were not related to body mass changes over five to seven days in the Red Knot *Calidris canutus islandica* (Jenni-Eiermann et al., 2002), similar to our results. Hence, chicks seem to be able to recover quickly from fasting and start building up reserves again. In general, Wilson's Storm-petrel chicks in this study showed higher triglyceride levels than adult birds, and only the lowest levels of chicks were similar to average Wilson's Storm-petrel adults' levels (94 mg dl^{-1}; Quillfeldt et al., 2004).

4.4. *Immunity*

Body temperatures of Wilson's Storm-petrel chicks had no significant effect on their immunity. However, chicks with higher mean short-term body temperatures tended to elicit a stronger inflammatory response, which consists of a combination of innate and adaptive immunity, and tended to have, in comparison, higher amounts of adaptive immune cells relative to innate immune cells. Separating humoral parameters of the innate and adaptive immune answer, we neither found influences of mean body temperature on the innate, nor the adapted immunity alone. In our study, well-nourished chicks will be less often in need for facultative hypothermia to preserve energy reserves, and can hence, maintain higher mean body temperatures. Most parameters analyzed here are not considered to be directly affected by malnutrition. Even though one study argues that higher costs of thermoregulation during molt could lead to lower immunoglobulin concentrations in Chinstrap Penguins (*Pygoscelis antarcticus*, Palacios et al., 2018), in general energy demand is not expected to be a limiting factor for immunity in long lived birds like the Wilson's Storm-petrel (Apanius and Nisbet, 2006). An exception is the H-L ratio, which we used to present a combined response of innate and adaptive cellular immunity and which is known to be linked to long-term exposure of stressors (Davis and Maney, 2018). Our result that lower mean body temperatures, which are associated with fasting (Kuepper et al., 2018), came with higher H-L ratios, are in line with findings from Lobato et al. (2005): Healthy, well-nourished chicks of Pied Flycatchers (*Ficedula hypoleuca*) showed low H-L ratios, which were majorly mediated by abundance of heterophils. In a different setting, where we compared leukocyte levels before and after snowstorms, we found decreased numbers of lymphocytes, the counterpart to heterophils in the H-L ratio after snowstorms. That lymphocyte numbers were affected by snowstorms that block parents from feeding and hence, result in fasting and often facultative hypothermia in chicks, could hint that here body temperature is more important than or adds up to the influence of nutritional intake alone: While a malnourished chick might still have sufficient energy to maintain its adapted immunity, low body temperatures might cause a limited maintenance or even down regulation.

Another indication that recent facultative hypothermia affect immunity is associated with the results of the immune challenge: higher short-term mean body temperatures tended to correlate with a higher inflammatory response. The challenge was performed at a chicks' age of 50 ± 2 days and the observed mean body temperatures of individuals ranged within only 4 °C (measured body temperatures during interval: 23.6 °C – 42.0 °C). Out of 15 experimental chicks 13 experienced fasting during the five day period before the challenge and only one reduced its body temperature below 36 °C. For chicks at this age, short-term fasting might not be severe enough to cause heterothermy. Additional analyses with chicks at younger ages or exposed to more pronounced recent fasting and heterothermy could lead to a more accurate picture also for combined responses of innate and adapted immunity.

4.5. *Sex differences*

Our study showed for almost all parameters differences between female and male chicks: Onset of rapid wing growth was earlier in females than in males, and a slight female bias in the innate and adaptive immunity could be observed. It is interesting that sex differences during growth occur, even though males and females of Wilson's Storm-petrels do not have a size dimorphism (Roberts, 1941). Unfortunately, as we could not monitor all individuals until fledging, we have no knowledge if or when males could catch up or if size differences continued when the young birds left the nest. Hence both, advantages for different timings or different investment into wing growth could explain our results. Next to growth, females also seemed to invest slightly more into their adaptive immune system. This is in line to a cross-species analysis on sexual dimorphism during breeding season among 97 bird species (Vincze et al., 2022). Other studies reported differently biased immune response depending on season (female bias during breeding, male bias during non-breeding season (Valdebenito et al., 2021); male bias during pre-laying (Kulaszewicz et al., 2017); male bias during breeding (Palacios et al., 2018)). A study on house sparrows (*Passer domesticus*) suggests that sex differences in adapted immunity are related to season (Pap et al., 2010a). The different energy demands during e.g. breeding season between males and females could be an explanation. On contrary to other parameters measured in this study, PHA caused stronger reactions in males than in females and cells from the innate immune system circulated at higher frequencies in the males' blood. Due to the combined response to phytohemagglutinin of the innate and adapted immune system, it is difficult to distinguish the reasons for this bias. Results from the blood cell counts could hint that here the innate immunity was responsible for stronger reactions in males. All above-mentioned studies were conducted in adult birds, where investment into self-care and reproduction are opposing each other (Kulaszewicz et al., 2017). Differences apparent at chick age may reflect differences in development between males and females and not necessarily a pattern of sexual dimorphism at adulthood. While we cannot rule out that by chance or for unknown reasons females during observation time had better physical fitness, our results could also point to less available energy resources for males. For sure, our results suggest that even in chicks sex differences should be kept in mind for analyses on developmental parameters.

5. Conclusion

In summary, our data suggest that facultative hypothermia at the frequency observed in this study did affect wing growth, and triglycerides negatively. All aspects showed quick recovery, or no long-term effect, and may not form a strong impact on development of the studied individuals. This field study used irregular data of heterothermy obtained with minimally invasive methods in a wild population of *Oceanites oceanicus*. More pronounced bouts of facultative hypothermia and stronger differences among individuals might shed a better light on the impacts of facultative hypothermia for developing chicks in the wild, especially regarding their development of the immune system. Breeding grounds of Wilson's Storm-petrels, especially those on the Antarctic Peninsula, are predicted to experience climatic changes like increased precipitation in the future (Bozkurt et al., 2021; Znój et al., 2017). More snow and ice blocking nests before or during the breeding season may lead to shorter possible rearing periods and higher use of facultative hypothermia. Hence, while observed consequences of facultative hypothermia in this study do not seem to outweigh the positive effect of surviving periods of fasting, continuing monitoring is necessary to evaluate the future viability of these findings. Additionally, tracking survival and recruitment into breeding colonies of the studied individuals would be interesting for future perspectives to learn how the chick development impacts survival to adulthood and fitness of the adults.

CRediT authorship contribution statement

Nadja D. Kuepper: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gábor Árpád Czirják:** Writing – review & editing, Writing – original draft, Data curation, Resources, Methodology. **Marcela Monica Libertelli:** Writing – review & editing, Project administration, Conceptualization. **Petra Quillfeldt:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2026.111983.

Data availability

The dataset generated and analysed during this study is available in the PANGAEA database (Felden et al., 2023): https://doi.pangaea.de/10.1594/PANGAEA.987818.

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Chapter 3

Persistent organic pollutants and mercury in a colony of Antarctic seabirds: higher concentrations in 1998, 2001, and 2003 compared to 2014 to 2016

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ORIGINAL PAPER

Persistent organic pollutants and mercury in a colony of Antarctic seabirds: higher concentrations in 1998, 2001, and 2003 compared to 2014 to 2016

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Abstract
Over decades, persistent organic pollutants (POPs) and trace metals like mercury (Hg) have reached the remotest areas of the world such as Antarctica by atmospheric transport. Once deposited in polar areas, low temperatures, and limited solar radiation lead to long environmental residence times, allowing the toxic substances to accumulate in biota. We investigated the load of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDTs) and metabolites (DDEs, DDDs) in embryos from failed eggs of the smallest seabird breeding in Antarctica, the Wilson's storm-petrel (*Oceanites oceanicus*) at King George Island (Isla 25 de Mayo). We compared samples of different developmental stages collected in 2001, 2003, and 2014 to 2016 to investigate changes in pollutant concentrations over time. We detected eight PCBs including the dioxin-like (dl) congeners PCB 105 and 118 (ΣPCBs: 59-3403 ng g^{-1} ww) as well as 4,4’-DDE, and 4,4’-DDD (ΣDDX: 19-1035 ng g^{-1} ww) in the embryos. Samples from the years 2001 and 2003 showed higher concentrations of PCBs than those from 2014 to 2016. Concentrations of DDX was similar in both time intervals. Furthermore, we determined Hg concentrations in egg membranes from 1998 to 2003, and 2014 to 2016. Similar to PCBs, Hg in egg membranes were higher in 1998 than in 2003, and higher in 2003 than in the years 2014 to 2016, suggesting a slow recovery of the pelagic Antarctic environment from the detected legacy pollutants. Embryos showed an increase in pollutant concentrations within the last third of their development. This finding indicates that contaminant concentrations may differ among developmental stages, and it should be taken into account in analyses on toxic impact during embryogenesis.

Keywords Wilson’s storm-petrel · Polychlorinated biphenyls (PCBs) · DDX · Hg · Ontogeny

Introduction

Persistent organic pollutants (POPs) and trace metals like mercury (Hg) are known to affect the environment and living organisms for a long time (Jacob 2013; Eagles-Smith et al. 2018). This is especially relevant for legacy organochlorine compounds, which are still abundant in many places as a consequence of their low degradation rates, even though they have been banned in most countries for decades (UNEP 2018). Both, POPs and Hg, can travel far distances through global distillation (Wania and Mackay 1996; Choi et al. 2008; Kang et al. 2012), hydrospheric transport (Wania and Mackay 1993; Fuoco et al. 2009), and migrating animals (Braune et al. 2005; Fort et al. 2014). Thus, they can reach pristine polar environments such as Antarctica, where cold temperatures, circumpolar currents, snow, and ice capture them (Wania and Mackay 1996). Additionally, passive air sampling suggests that Arctic and Antarctic stations also emit pollutants to the environment (Choi et al. 2008).

Through food intake and bioconcentration, POPs and Hg accumulate in biota and biomagnify through the food webs (Morel et al. 1998; Langis et al. 1999; Fisk et al. 2001). Top predators are especially prone to concentrate high contaminant loads (Haraguchi et al. 2009; Krahn et al. 2009), and both mammals and birds are at risk of transferring part of their own load to the next generation (Verboven et al. 2009; van de Merwe et al. 2011). One non-invasive method of monitoring POPs in birds is to collect abandoned eggs. Within the egg, the yolk contains the highest amount of the

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highly lipophilic polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs) and the metabolites dichlordiphenyldichlorethen (DDEs) and dichlordiphenyldichlorethan (DDDs; hereafter DDX is used to represent DDTs, DDEs and DDDs together) in an egg is found in the yolk. Only negligible amounts are found in the albumen that contains < 1% of the egg lipids (Drouillard and Norstrom 2001; Russel et al. 1999). The egg yolk, the main nutrient carrier for an embryo, is eventually consumed entirely by the embryo (Noble and Cocchi 1990), and hence, the full load of the yolk's POPs will be transferred from the egg to the hatchling. On the other hand, Hg accumulates majorly in the albumen proteins of an egg (Wolfe et al. 1998; Grajewska et al. 2015). Still, Hg concentrations of yolk and egg membrane closely correlate with Hg concentrations in albumen, which makes all tissues suitable to monitor Hg concentration in eggs (Brasso et al. 2012; Peterson et al. 2017).

Pollutants like PCBs, DDX, and Hg affect bird fitness and survival. DDX became infamous more than 50 years ago when it was linked to eggshell thinning (e.g., Tucker and Haegele 1970). Resulting eggshell breakage caused severe declines of whole bird populations (WHO 2002; Elliott et al. 1988; Johnstone et al. 1996; Vasseur and Cossu-Leguille 2006). Within the group of PCBs, dioxin-like PCBs (dl-PCBs) have properties similar to dioxins, and can cause endocrine disruptions in animals (Tanabe 2002). They seem to cause mal-development of the lymphatic system and embryo deformation in some bird species (Bosveld and van den Berg 1994). In female little auks (*Alle alle*), high Hg concentrations caused smaller eggs the following breeding season (Fort et al. 2014), but in mallards also caused deformation of embryos, early mortality, and brain lesions (Wolfe et al. 1998). All these pollutants place a significant threat to wildlife (Goutte et al. 2014). They threaten the stability of bird populations due to their impact on reproductive success, especially of those at the top of the food chain (Fisk et al. 2001; Langis et al. 1999).

The Wilson's storm-petrel (*Oceanites oceanicus*) is a common predator breeding in subantarctic and Antarctic regions. Here, we investigated whether POP and Hg contamination for this species changed within the last decades around the Antarctic Peninsula. We collected eggs that were abandoned, e.g., due to prolonged adverse conditions such as snowstorms from the same colony in 2001, 2003, and from 2014 to 2016 (Quillfeldt 2001; Büßer et al. 2004), as well as membranes of hatched eggs in 1998, 2003, and 2014 to 2016. As Wilson's storm-petrels collect all nutrients needed to form the egg in one location during the pre-laying exodus (Beck et al. 1972), an egg will be influenced by the pollutant concentration of this area. To evaluate differences of pollutant levels among years, it is important to be aware of potentially different nutrient origins. Both, the foraging area and the trophic position of prey species could influence pollutant concentrations in seabird eggs (Braune et al. 2005; Carravieri et al. 2014, 2016; Corsolini and Sará 2017; Cherel et al. 2018; Mills et al. 2020). To evaluate foraging habits and areas among years, we analyzed stable isotope values for carbon and nitrogen. In the Southern Ocean, δ^{13}C values are linked to the latitudes of foraging areas (Cherel and Hobson 2007; Quillfeldt et al. 2005, 2010). Hence, we investigated whether pre-exodus areas of Wilson's storm-petrels were similar among the studied time periods, or whether a transition to more northern or more southern feeding grounds took place. Through enrichment of ^{15}N relative to ^{14}N from prey to predator, δ^{15}N indicates the trophic position of an animal (Post 2002; Weiss et al. 2009). We used this to test whether prey of Wilson's storm-petrels ranged within similar trophic positions or whether trophic positions changed among the years. Additionally to temporal differences of pollutant exposure, we investigated whether embryos incorporate pollutants at a constant rate, as timing of toxic effects may be crucial for embryonic development, and analyzed different age groups of embryos. Explicitly, we addressed the following questions:

1. What are POP concentrations in embryos, and Hg concentrations in egg membranes of Wilson's storm-petrels?
2. Do POP and Hg concentrations in embryos and egg membranes, respectively, differ depending on embryo age? And can we, hence, learn when POPs are transferred from the egg to the embryo?
3. Do POP and Hg concentrations change over the years? In addition, do stable isotope analyses indicate differences in pre-laying exodus area and trophic position that may explain differences in pollutants?

We expected to find POP concentrations in the same range as eggs of seabirds with similar foraging preferences from the same area, like Adélie penguins (*Pygoscelis adeliae*; Cipro et al. 2010; Corsolini et al. 2011). Furthermore, we expected POP transfer to follow lipid transfer from yolk to embryo, but no variation of Hg concentrations in egg membranes through the development. If no change of pre-laying exodus area or trophic feeding level could be detected (Noble and Cocchi 1990), we expected lower POP concentration in samples from more recent years due to the extensive ban of those substances (UNEP 2018), and no change in Hg concentrations among years, due to continuing input into the environment and transport to polar areas (Streets et al. 2017; Soerensen et al. 2012).

Materials and methods

Species and study site

The study took place at the "Tres Hermanos" hill about 1.5 km from Carlini Research Base (Fig. 1) on King George

Island (Isla 25 de Mayo), South Shetland Islands (62° 14′ S, 58° 40′ W), where a research project on Antarctic storm-petrel species was set up in 1995. Breeding success and number of established nests were observed for more than 20 years at a colony of Wilson's storm-petrels. Here, breeding takes place in the scree slopes underneath big rocks, where the depth of accessible nests varies from 20 to 60 cm. Wilson's storm-petrels are small marine birds weighing on average 38 g (Fig. 1; Beck et al. 1972; Quillfeldt 2006). This common predator feeds mainly on Antarctic krill (*Euphausia superba*), squid, small fish, and amphipods (Quillfeldt 2002). Wilson's storm-petrels spend their life at sea when they are not breeding, and can be found in all oceans except for Arctic waters (Quillfeldt 2006). Their breeding sites are in the Antarctic and on subantarctic islands. Breeding pairs establish their nesting burrow in cavities below rocks (Quillfeldt 2006; Roberts 1941), before the females leave again prior to egg-laying on a pre-laying exodus. This foraging trip is used to accumulate nutrients to produce a single egg that contains the recently consumed pollutants, and hence, establishes the burden of pollutants for every surviving hatchling (Beck et al. 1972; Hobson et al. 1997, 2000).

Egg and egg membrane collection

In all years, active nests (incubating adult or egg) were identified during the incubation period from mid-December to the end of January; all accessible eggs were measured once in the absence of adult birds (length, width, weight) using calipers and a digital balance. With these measurements, we estimated the chicks' hatching dates by density loss over time according to Quillfeldt (2001). We looked for hatched chicks 5 days after this estimated date and monitored nestlings regularly afterwards until they fledged, or the end of the season. Eggs that did not hatch until the estimated date were checked at most four more times, approximately every 5 days. Whenever possible nest checks were done at sight to reduce possible disturbance of incubating adults. After a chick hatched, its nest was searched for the eggshell to collect the egg membrane. Membranes were freed from shell parts and dirt by rinsing them with tap water, air dried at the field station, and stored at room temperature in 2003, and from 2014 to 2016. In 1998, eggshells were collected, transported and stored frozen (− 20 °C) until needed. Membranes were then separated from the shell, rinsed under tap water, air dried, and stored at room temperature until further analyses took place.

Snowstorms and the resulting blocking of nest entrances regularly cause failure of hatching of chicks (e.g., Büßer et al. 2004). These eggs and all other abandoned and accessible eggs were collected at the end of the season (i.e., end of March) when no further hatching could be expected. As the inaccessibility of the nests for parents and, hence, hypothermia was the main reason for embryos to die before hatching, we expect pollutant concentrations found in this study to be representative for eggs that hatched. In 2001, embryos were dissected at the research station. In 2003 and 2014 to 2016, collected eggs were preserved whole, and egg membrane and embryo samples were prepared during egg dissection after leaving the field station. All samples were stored at − 20 °C until preparation of samples.

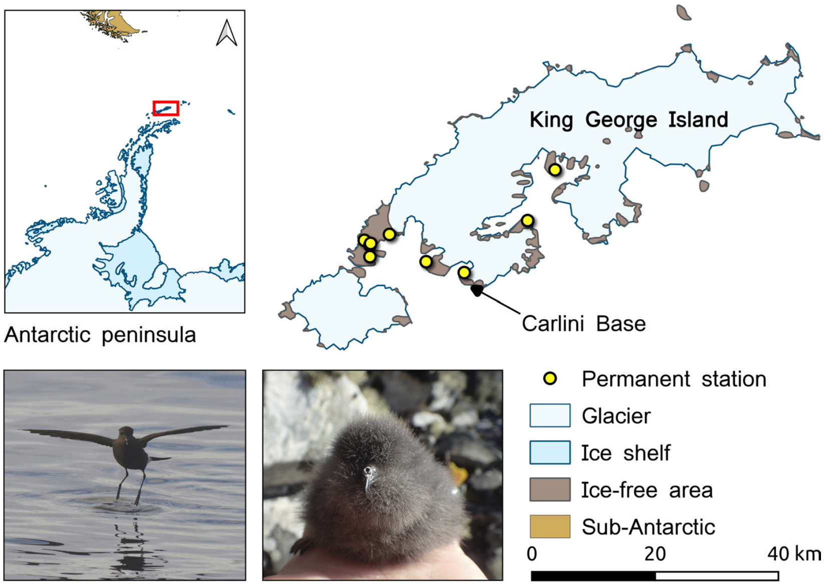

Fig. 1 Study area at Carlini Base on King George Island (map data from Quantarctica; Matsuoka et al. 2018), and adult and nestling of the study species Wilson's storm-petrel (*Oceanites oceanicus*)

Selection and preparation of samples

We used embryos to analyze POPs, and conducted stable isotope and Hg analyses on egg membranes. To compare pollutant loads from 2014 to 2016 to earlier years, we used available, stored samples from the same colony from 1998 (only egg membranes), 2001 (only embryos), and 2003 (egg membranes and embryos) (Table 1).

Eggs from 2003 and 2014 to 2016 were thawed and opened to extract the embryos. The embryos were weighed, frozen again, and then freeze-dried to mass consistency. Storage time of frozen embryos varied by up to 15 years. Resulting wet weight (ww) was not equally reliable for all samples due to water sublimation. To correct this, ww was calculated backwards from dry weight (dw) using the average percentage of mass loss during drying from the newest samples from 2016 (81.8%, $n = 6$; compare Leat et al. 2011).

Preliminary trials on other bird material with the below described method, and literature research (compare Online Resource 8) led us to the expectation that POP concentrations in individually analyzed embryos would be below the limit of detection (LOD) or limit of quantification (LOQ) for all but two samples from 2016. All embryos but those two were therefore pooled into eight groups, clustered into two time periods of sampling (2001 and 2003, and 2014 to 2016) and for each time period into four age groups, to learn more about the temporal transfer of organic pollutants to the embryo. We estimated embryo age from ww following the equation for embryonic growth of birds described by Ricklefs (2010). Detailed calculations can be found in Online Resources 1–3. We divided the available embryos as equally as possible into four similar age groups for both time intervals, with the aim of covering a wide range of developmental steps. It is biologically relevant to be able to differentiate between the first two third of development (0–33 days) and the last third of development (34–50 days), as the major amount of lipids and, hence, lipid soluble pollutants are expected to be taken up by the embryo in the last third of development (Noble and Cocchi 1990). Age group one includes embryos with an approximate age up to 25 days, age group two includes embryos aged 26–30 days, age group three includes embryos of the ages 31–33 days, and age group four includes embryos of the ages 34–40 days (Online Resource 3). Group 5 contained only two embryos approximately 1–2 days before hatching from 2016 with an embryonic age of approximately 48 days (Online Resources 3 and 2). No comparable embryos for group 5 were available from 2001 to 2003. Dried embryos were ground as fine as possible first using a stainless steel blade grinder (Severin, 140 W) for approximately 2 min, and then using an agate mortar and pestle.

POP extraction and analysis

Chemicals

All analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The indicator congeners PCB 28, 52, 101, 138, 153, and 180 were purchased as standard mix together with PCB 118 in methanol solution ("PCB Mix 3", 10 µg mL^{-1}). The PCB congeners 49, 77, 99, 105, 110, 156, 170, 183, and 187 as well as the six DDX dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE), each as 2,4'- and 4,4'-chlorinated isomers, as well as 1,2,3,4-tetrachloronaphthalene for use as internal standard, were purchased as pure substances in powder form. The PCB 132 was purchased dissolved in iso-octane. The PCB set contains four dl-PCBs, i.e., PCBs 77, 105, 118, and 156. All substances were prepared and used dissolved in methanol.

Extraction procedure

POP extraction from dried, pulverized, and pooled embryos was performed by microwave assisted extraction (MAE) adapted from Düring and Gäth (2000), followed by sulfuric acid purification adapted from Murphy (1972). Extraction was followed by concentration of extracts by rotary evaporator followed by N_2 flow, and then transfer to an aqueous solution for measurement by headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC–MS). For extraction, 400 ± 1 mg of dried and pulverized embryos were weighed to polyfluoralkoxy (PFA) MAE vessels. Then, heat transformer disks (Weflon® disks, MLS Corp., Leutkirch, Germany) and 15 mL *n*-hexane (≥ 99%, p.a. quality, Carl Roth GmbH, Karlsruhe, Germany) were added before closing the vessels. Two types of control vessels were prepared, either with 15 mL *n*-hexane or with 15 mL *n*-hexane and 2.5 µL of a 10 ng µL^{-1} PCB standard solution (PCB Mix 3, Dr. Ehrenstorfer GmbH,

Table 1 Number and type of Wilson's storm-petrel samples used in this study for each year. All samples were collected at the study site at "Tres Hermanos" hill, close to Carlini Base

	1998	2001	2003	2014	2015	2016
embryos	0	30	10	10	10	3
egg membranes	10	0	7	9	15	12

Augsburg, Germany). 1,2,3,4-tetrachloronaphthalene was added to all samples as an internal standard (4 µL of a 5 ng μL^{-1} solution). The MAE was performed at 120 °C with 250 W for 1 min followed by 1000 W for 15 min. After passive cooling and opening of the vessels, aliquots of 12 mL were removed from the supernatant and transferred to 20-mL glass vials. For purification from greasy components, aliquots were treated with 2 mL concentrated sulfuric acid (95.0–98.0%, Sigma-Aldrich, ACS reagent), and shaken by hand for 1 min. After centrifugation (20 min at 210 RCF, Hettich Rotanta 460 R), the acidic solution was carefully removed by glass Pasteur pipettes. This procedure was repeated once with 1 mL of sulfuric acid. Afterwards, 10-mL aliquots of the acid treated *n*-hexane extracts were transferred to rotary evaporator flasks followed by their careful reduction to volumes of 200 µL. Solvent reduction was accelerated by using a water bath (40 °C), rotating the flask (150 rpm), and by applying negative pressure with a vacuum pump (350–400 mbar). Remaining extracts were transferred to fresh 20-mL headspace vials. The flasks were flushed twice with 150 µL *n*-hexane each that was also transferred to the 20-mL headspace vials afterwards. The resulting *n*-hexane volume (~ 500 µL) was largely reduced by a gentle N_2 stream. Once only a few drops remained, the vials were passively air dried. The target substances were redissolved with 100 µL of solubilizing methanol (p.a. quality, Carl Roth GmbH, Karlsruhe, Germany), followed by the addition of 10 mL NaCl solution (1 g 10 mL^{-1}) for salting out of POPs to the headspace.

Instrumental and data analysis

Measurement of POPs was performed by HS-SPME coupled to GC–MS. Basics of the performed process are given in Böhm et al. (2017) and Wiltschka et al. (2020). In the present study, the following parameters were used for automated SPME: fiber with 100 µm PDMS coating (Sigma-Aldrich), heating of the fiber prior to extraction to eliminate cross contamination: 10 min, pre-equilibration before extraction: 10 min, extraction: 60 min at 90 °C while shaking the sample vial at 250 rpm. Samples were processed by a CombiPAL autosampler (CTC-Analytics, Zwingen, Switzerland) with thermodesorption of the fiber in the injector of the GC–MS for 3 min at 270 °C. Chromatographic separation and mass detection were performed with a GC-ion trap MS (Trace GC Ultra/ITQ 900, Thermo Fisher Scientific) equipped with a XLB-type capillary column (Thermo TG-XLBMS, 60 m, 0.25 mm, 0.25 µm). Semi-quantitative screening of a sample aliquot in full-scan mode of the MS (m/z 50–500) was used to identify concentration ranges of target PCB congeners as well as to identify further PCB congeners by chlorine pattern. For quantification, the MS was used in selected ion storage (SIS) mode (designated as "selected ion monitoring", SIM, in Thermo Xcalibur software) with respective segments for the analytes. Quantification was performed based on six-point calibrations for PCBs in the range of 0.01–0.75 µg L^{-1} and for DDX in the range of 0.01 to 2 µg L^{-1}. For samples that exceeded this range, an extended 12-point calibration from 0.01 to 3.25 µg L^{-1} was used. For all analytes LODs and LOQs were determined analog to IUPAC (1997), using the numerical factor $k = 3$ or $k = 10$, respectively (Online Resource 6). Processing of GC–MS data was performed with the software 'Xcalibur' (Thermo Fisher Scientific) combined with a manual verification of peak integration. Data for all analytes were corrected based on TCN as internal standard. Concentrations from SPME–GC–MS measurements were recalculated considering aliquoting and initial sample weight of embryos. Final POP concentrations in embryos are given in ng g^{-1} dw. Detailed parameters on GC–MS conditions (i.e., oven program and parameters for MS detection) are given in the Online Resources 4 and 5.

Analysis of mercury

We determined total Hg (the sum of inorganic and organic Hg, thereafter called Hg) concentrations in egg membranes from 1998, 2003, and 2014 to 2016 (Table 1). Whenever possible, analysis was conducted with membranes from the same eggs of which embryos were used for POP analysis. Additional samples were used from hatched chicks for all years. Surface contaminants were removed from egg membranes in ultrasonic baths. Each sample was individually sonicated for 3 min in a bath filled with a 2:1 volume ratio of chloroform and methanol. Subsequently, they were rinsed twice in consecutive methanol baths and dried for 48 h at 45 °C. Clean egg membranes were cut with stainless steel scissors into tiny fragments to produce a homogeneous powder. Aliquots of 2–11 mg powdered egg membrane were used to determine Hg concentrations with an Advanced Mercury Analyzer spectrophotometer Altec AMA-254. To evaporate Hg, aliquots were heated progressively until 800 °C under an oxygen atmosphere and Hg was amalgamated on a gold-net during 3 min (Bustamante et al. 2006). Then, the net was heated to liberate the collected Hg, which was finally measured by atomic absorption spectrophotometry (Bustamante et al. 2006). Measurements were repeated two or three times for each sample until having a relative standard deviation (SD) < 10%. Accuracy and reproducibility for each set of samples were tested by analytical blanks and replicate measurements of certified reference material (DOLT-5, dogfish liver, National Research Council of Canada; certified concentration: 0.44 ± 0.18 µg g^{-1} dw). Measured Hg concentrations for the certified reference material were: 0.33 ± 0.00 µg g^{-1} dw ($n = 7$). The detection limit of the AMA was 0.05 ng. Hg concentrations were expressed in µg g^{-1} dry weight (dw).

Bulk stable isotope analysis

Stable isotope analysis (SIA) was conducted using subsamples of egg membranes from the same eggs that were used for Hg analysis. An amount of 0.32–0.50 mg (mean ± sd: 0.40 mg ± 0.04 mg) of egg membrane was weighed in tin cups. Carbon and nitrogen ratios were determined with a continuous-flow mass spectrometer (Delta V Plus, Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer [Flash EA 1112 (37 samples) or Flash 2000 (16 samples), both Thermo Scientific, Milan, Italy]. Results are expressed in the δ notation as parts per thousand (‰) deviation from the international standards Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$). The used formula is $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})—1] \times 10^3$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. To assess the quality of the analytical procedures, measurements of internal laboratory standards were conducted using acetanilide (Thermo Scientific) and peptone (Sigma- Aldrich) or two types of caffeine (USGS-61 and USGS-62; Reston stable isotope laboratory, United Stated Geological Survey) and indicated a standard deviation for analyzed samples of $< \pm 0.06$ ‰ for $\delta^{13}C$ and $< \pm 0.09$ ‰ for $\delta^{15}N$.

Statistical analyses

Only POPs with concentrations above LOQ (Online Resource 6) were included in the following analyses. Pollutants above LOD but below LOQ were presented in this paper, but were not included in any statistical analyses. Pollutants below LOD are given in the Online Resource 6, but were not considered beyond this. As the small sample size did not meet necessary requirements for non-parametric testing, we used parametric tests for statistical analyses on POPs.

Concentration differences among PCB congeners were tested with a Kruskal–Wallis rank sum test followed by Dunn's test for post hoc pairwise comparisons with Bonferroni correction (Haynes 2013), concentration differences between DDT metabolites were tested using Wilcoxon rank sum test.

To test concentration differences of PCBs and DDX among age groups in embryos, the parametric Friedman rank sum test was used with data paired for individual pollutants. For post hoc analyses between single age groups, Wilcoxon signed-rank exact tests with paired samples and *p*-values corrected according to Bonferroni were used. If ties occurred Wilcoxon signed rank tests with continuity correction were used. To test if there was a correlation between Hg concentration of egg membranes and age of the embryo, we used a Spearman's rank correlation with the embryo's dw as a proxy for embryo age. To compare Hg concentrations from samples of hatched vs. unhatched eggs, we used a Wilcoxon signed rank sum test with continuity correction.

We analyzed differences of pollutant concentrations for POPs between the available years 2001 and 2003, and 2014 to 2016 using a Wilcoxon signed rank exact test. Samples were paired for pollutants, and differences between early and recent years were tested over all samples, as well as for embryos younger than 34 days or older than 33 days separately. The *p*-values for these post hoc tests were adjusted using Bonferroni corrections. In analyses using individual age groups, pollutant concentrations in the years 2014 to 2016 were averaged for age group 4 (31–33 days) and 5 (> 33 days) to allow comparisons with years 2001 and 2003 where only 4 age groups could be analyzed. Further, PCB 170 was excluded from age comparisons, as only one sample showed concentrations > LOD for this substance. To test differences of Hg concentrations among the available years 2001, 2003, and 2014 to 2016 we used a Kruskal–Wallis rank sum test followed by pairwise Wilcoxon tests for post hoc pairwise comparisons with Bonferroni correction (Haynes 2013).

The ratio of 4,4'-DDE to the total sum of DDX was calculated as an indicator for differentiating between recent or ancient input (Fries et al. 1969; Borrell and Aguilar 1987; Yogui et al. 2003). Values above 0.7 are considered to show an origin from ancient sources, while values below 0.7 show a more recent input of commercial DDX into the environment.

Stable isotope patterns were analyzed for inter-annual differences (for post hoc analysis with Bonferroni correction) for the years 1998, 2003 and 2014 to 2016 using the Wilks' Lambda test and a One-way MANOVA (Bartlett Chi^2). Trophic positions (TP) for Wilson's storm-petrels (TP_{ocea}) were calculated according to Weiss et al. (2009), using the formula.

$$TP_{ocea} = TP_{prey} + (\delta^{15}N_{ocea} - \delta^{15}N_{prey}) \times 3.4\%^{-1},$$

where TP_{prey} is 2.3, the estimated TP of the main prey, Antarctic krill (Hodum and Hobson 2000), $\delta^{15}N_{prey}$ is 5.3 ‰, the average $\delta^{15}N$ of krill in the northern Antarctic Peninsula (Seyboth et al. 2018), and 3.4 ‰ is the assumed enrichment factor as estimated by Post (2002). Samples that clearly originated from non-Antarctic waters ($\delta^{13}C > -21$ ‰; 1998: $n = 1$; 2015: $n = 3$) were excluded for the calculation of trophic positions. We tested if Hg concentrations were linked to $\delta^{13}C$ or $\delta^{15}N$ values using Pearson's product-moment correlations.

All statistical analyses and figures were conducted in R 4.0.5 (R Core Team 2021). Map of study site was composed using QGIS 3.24 (QGIS Development Team 2022) and the Quantarctica data set (Matsuoka et al. 2018).

Results

Pollutant concentrations

In Wilson's storm-petrel embryos, eight PCBs and two DDT metabolites could be quantified (Table 2; Fig. 2). Concentrations of PCBs differed significantly among congeners (Kruskal–Wallis rank sum test, $\chi^2 = 27.28$, $p < 0.001$). The highest values among the PCBs were reached for PCB 153 with more than 1400 ng g^{-1} ww. Compared to PCBs 99 and 183, concentrations of PCB 153 were significantly higher (Dunn's Post hoc test with Bonferroni correction, PCB 99 vs. PCB 153: $Z = -3.44$, $p = 0.016$; PCB 153 vs. PCB 183: $Z = 4.57$, $p < 0.001$; results of all pairwise comparisons are presented in Online Resource 7). The sum of all detected PCBs ranged from 59 to 3403 ng g^{-1} within the samples. Among quantified PCBs, two dl-PCBs (PCBs 105 and 118) were found in sum concentrations between 12 and 549 ng g^{-1} ww. Among the DDX, 4,4'-DDE reached the highest concentrations with more than 350 ng g^{-1} ww, and was in general significantly higher than 4,4'-DDD (Wilcoxon rank sum test, $W = 11$, $p = 0.008$). The ratio of the 4,4'-DDE concentration to the total concentration of all DDX was on average 0.88 ± 0.21, and only one sample had a ratio below 0.7 with 0.36.

Additionally, PCBs 49, 52, 77, 101, 156, and 187 could be qualified, but concentrations were below LOQs (Online Resource 6). Out of these, PCBs 77 and 156 are dl-PCBs. The PCBs 28, 110, and 132 could not be detected in any sample.

Concentrations of Hg in egg membranes ranged from 0.006 to 0.436 µg g^{-1} dw (Table 2).

Pollutant concentrations and embryo age

The pollutant concentration of PCBs in embryos differed among ages in the years 2001 and 2003 compared to those from 2014 to 2016 (Fig. 3; Friedman rank sum test; 2001

Table 2 Means ± standard deviation (SD) and concentration ranges of single organic pollutants and pollutant groups as well as mean ± SD and concentration range of Hg of all investigated samples

Pollutant	All samples		2001, 2003		2014–2016	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
PCB concentrations (ng g^{-1}) ww of embryo samples						
PCB 99	23 ± 43	2–136	38 ± 65	4–136	10 ± 9	2–24
PCB 105	20 ± 22	4–74	28 ± 31	8–74	14 ± 11	4–28
PCB 118	76 ± 151	8–475	135 ± 226	16–475	28 ± 25	8–65
PCB 138	88 ± 186	6–579	158 ± 281	10–579	32 ± 31	6–67
PCB 153	242 ± 456	29–1437	395 ± 695	29–1437	119 ± 104	31–267
PCB 170	< LOD	< LOD-127	< LOD	< LOD	< LOD	< LOD
PCB 180	85 ± 169	7–527	143 ± 256	9–527	39 ± 48	7–117
PCB 183	8 ± 15	1–48	14 ± 23	2–48	4 ± 3	1–7
ΣPCBs[a]	556 ± 1082	59–3403	944 ± 1640	78–3403	246 ± 227	59–565
Σdl-PCBs	96 ± 172	12–549	163 ± 257	24–549	42 ± 36	12–93
ΣPCBs (indicator)[b]	491 ± 961	52–3018	832 ± 1458	64–3018	218 ± 205	52–516
DDT concentrations (ng g^{-1}) ww of embryo samples						
4.4-DDD	80 ± 219	< LOD-663	168 ± 330	2–663	11 ± 19	< LOD-43
4.4-DDE	110 ± 108	18–372	144 ± 153	44–372	82 ± 58	18–146
ΣDDX	190 ± 321	19–1035	312 ± 482	46–1035	93 ± 72	19–182
Hg concentrations (µg g^{-1}) dw of egg membranes						
Hg	0.062 ± 0.086	0.006–0.436	**1998:** 0.165 ± 0.124 **2003:** 0.055 ± 0.030	**1998:** 0.050–0.436 **2003:** 0.019–0.104	**2014–2016:** 0.035 ± 0.056	**2014–2016:** 0.006–0.339

ΣPCB[a]: sum of 8 individual PCB congeners that are listed in the present table, Σdl-PCB: sum of PCBs 105 and 118

ΣPCB (indicator)[b]: sum of indicator PCBs, ΣDDX: sum of 4,4'-DDD and 4,4'-DDE

[a] PCBs 49 and 187, indicator PCBs 52 and 101, as well as dl-PCBs 77 and 156, were detected below LOQ and were not added to the sum of PCBs given in the present table

[b] Indicator PCBs that were detected in this study above LOQ were PCBs 138, 153, and 180

Bold years emphasis different sampling years for Hg compared to POP analyses

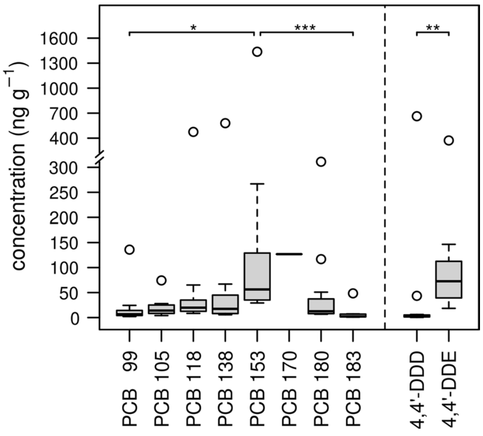

Fig. 2 Concentration (ng g^{-1} ww) for eight quantified PCBs and two DDX in embryo samples of Wilson's storm-petrels. All PCBs were found in $n = 10$ samples, except for PCB 170, that was only detected in one sample. Significant differences between congeners are indicated by brackets and asterisks: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Black bars represent median of samples, the box marks 25%, respectively, 75% quantiles. Whiskers show the point closest to but within 1.5-fold distance of interquartiles

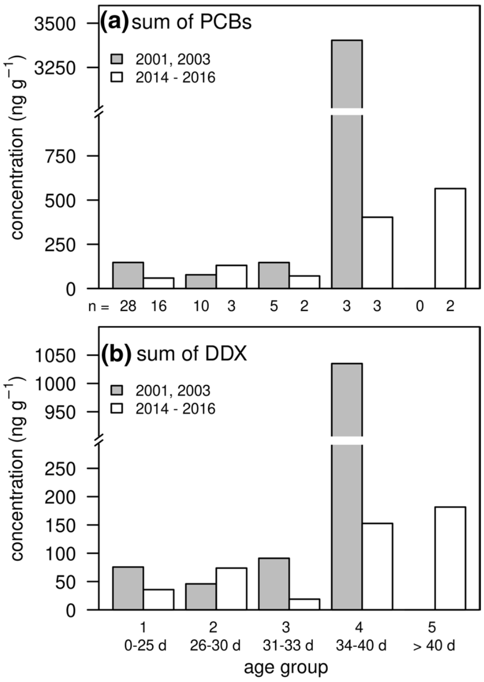

Fig. 3 Concentrations of ΣPCBs and ΣDDX from Wilson storm-petrel embryos for the years 2001 and 2003, as well as for 2014 to 2016 for each pooled age group. Embryo ages and according age groups for both figures are given below the x-axis. Each bar presents one pooled sample, n refers to number of embryos in a pooled sample, which is similar for both figures

and 2003: $\chi^2 = 20.0$; $df = 3$; $p < 0.001$; 2014–2016: $\chi^2 = 26.1$; $df = 3$; $p < 0.001$) with significantly higher concentrations in embryos aged 34 days or older compared to younger embryos (Wilcoxon signed rank exact test for eight pollutants and Bonferroni adjusted p-values; 2001 and 2003: $V = 0$; $p = 0.031$; 2014–2016: $V = 0$; $p = 0.031$; Fig. 3). No significantly different PCB concentrations could be found in embryos older than 40 days and embryos between 34 and 40 days of age in recent years (Wilcoxon signed rank exact test for eight pollutants and Bonferroni adjusted p-values; 2014–2016: $V = 5$; $p = 0.313$). No significant differences among ages could be found for DDT metabolites (Friedman rank sum test for two DDT metabolites, 2001 and 2003: $\chi^2 = 5.4$; $df = 3$; $p = 0.145$; 2014–2016: $\chi^2 = 6.7$; $df = 3$; $p = 0.107$). We found no significant correlation of higher Hg concentrations in egg membranes when embryos were older (Spearman's rank correlation, $r_s = 1232$, $p = 0.066$, $\rho = 0.39$), but Hg concentrations of membranes from hatched eggs were significantly higher than those from unhatched eggs (Wilcoxon signed-rank sum test with continuity correction; $W = 105.5$; $p < 0.001$).

Temporal differences of pollutant concentrations and foraging behavior

The overall load of PCBs was higher in 2001 and 2003 than in 2014 to 2016 (Wilcoxon signed rank exact test, $V = 50$, $p < 0.001$). For DDT metabolites, no significantly higher concentrations in 2001 and 2003 were found compared to 2014 to 2016 (Wilcoxon signed rank exact test, $V = 5$, $p = 0.078$). Also, PCB concentrations were higher in 2001 and 2003 compared to 2014 to 2016 in the group of younger ages (0–33 days; Wilcoxon signed rank exact test for eight PCBs and Bonferroni adjusted p-values, $V = 50$, $p = 0.048$) and the group of older ages (≥ 34 days; Wilcoxon signed rank exact test for eight PCBs and Bonferroni adjusted p-values, $V = 0$, $p = 0.031$) tested separately. For DDT metabolites, there were no difference in younger or older embryos from 2001 to 2003 compared to 2014 to 2016 (Wilcoxon signed rank exact test for two DDX, 0–33 days: $V = 5$, $p = 0.313$; ≥ 34 days: $V = 0$, $p = 0.5$).

Concentrations of POPs relative to each other were comparable among age groups (Fig. 4). The pollutants with the highest (PCB 153, 4,4'-DDE) or lowest concentrations (PCB 183) per sample were the same among samples. One exception was embryos aged 34–40 days from 2001 to 2003, which had a very high concentration of 4,4'-DDD. Interestingly, this value was even higher than that of 4,4'-DDE, which is dominating over 4,4'-DDD in all other samples.

In egg membranes, Hg concentrations were significantly higher in 1998 than in 2003 and in 2014 to 2016, and also higher in 2003 than in 2014 to 2016 (Kruskal

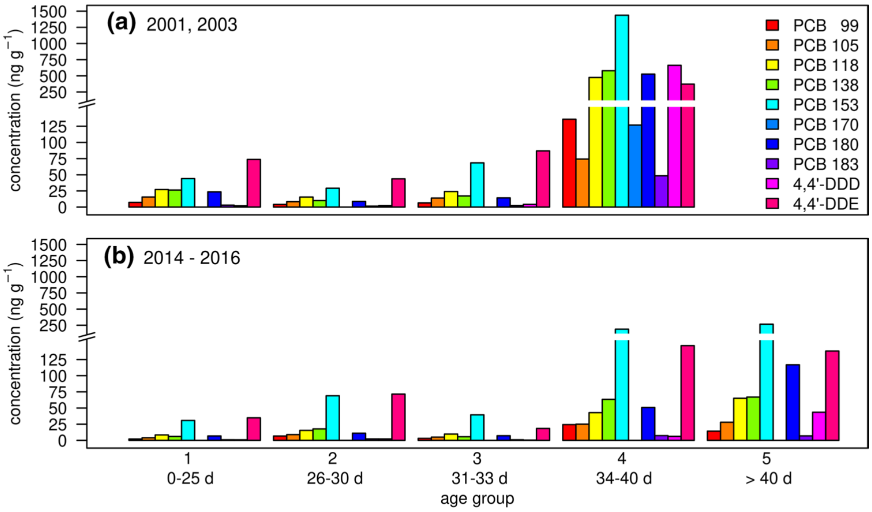

Fig. 4 Concentrations of POPs from Wilson's storm-petrel embryo samples for different age groups for **a** 2001 and 2003, and **b** among 2014 and 2016. Embryo ages and according age groups for both figures are given below the x-axis. The y-axis is scaled differently below and above the axis-break

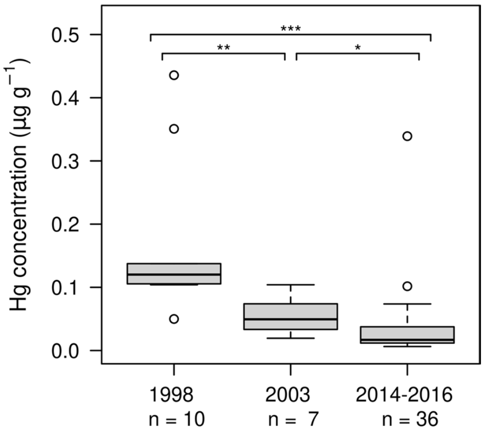

Fig. 5 Concentrations for Hg (µg g^{-1} dw) from Wilson's storm-petrel egg membrane samples for 1998, 2003, and 2014 to 2016. Significant differences between years are indicated by brackets and asterisks: *$p<0.05$; **$p<0.01$; ***$p<0.001$

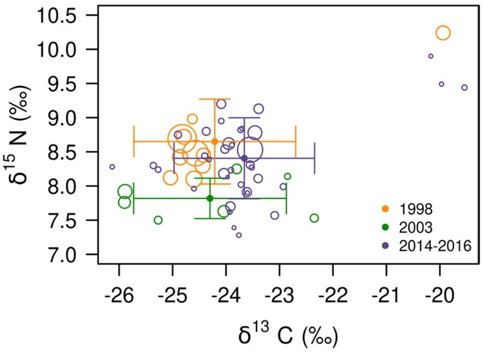

Fig. 6 Egg membrane δ^{13}C and δ^{15} N values for Wilson's storm-petrel eggs from King George Islands from 1998 to 2016. Bubble size is relative to Hg value of the same sample. Colors indicate the collection year of the sample. Solid points show means with error bars for 1998, 2003, and 2014 to 2016

Wallis rank sum test: $\chi^2=25.1$, $df=2$, $p<0.001$; Fig. 5). Between 1998 and 2003, a significant decrease of medians was observed: 59%, and between 2003 and 2014 to 2016, medians decreased by 66% (Post-hoc Analyses: Wilcoxon rank sum test with Bonferroni correction: 1998 and 2003: $W=66$, $p=0.004$; 1998 and 2014–2016: $W=349$, $p<0.001$; 2003 and 2014–2016: $W=203$, $p=0.036$; Fig. 5). Values of δ^{13}C and δ^{15}N differed significantly among 1998, 2003, and 2014 to 2016, and were significantly different between 2003 and 1998. Between 1998 and 2014 to 2016, or between 2003 and 2014 to 2016, no significant differences were found (1998 and 2003: Wilks' $\lambda=0.437$, $\chi^2=11.58$, $df=2.0$, $p=0.009$; 1998 and 2014–2016: Wilks' $\lambda=0.875$, $\chi^2=5.76$, $df=2.0$, $p=0.168$; 2003 and 2014–2016: Wilks' $\lambda=0.864$, $\chi^2=5.86$, $df=2.0$, $p=0.160$; Fig. 6). Variation within time period of trophic positions was higher than between time periods [medians (ranges) for 1998: 3.2 (3.1–3.4); 2003: 3.0 (2.9–3.2); 2014–2016: 3.2 (2.9—3.4)]. Neither values of δ^{13}C nor of δ^{15}N correlated with Hg loads (Pearson's product-moment correlation: δ^{13}C: $t=-1.269$, $df=51$, $p=0.210$, $r=-0.17$; δ^{15}N: $t=0.734$, $df=51$, $p=0.466$, $r=0.10$).

Discussion

Pollutant concentrations

The sum concentrations of all eight PCB congeners quantified in embryos in this study reached values as high as

3403 ng g^{-1} ww in 2001 and 2003, and 565 ng g^{-1} ww in 2014 to 2016. In both time periods, these sum PCB concentrations were higher than in other seabirds' eggs like penguins, gulls or petrels from the Antarctic Peninsula during the austral summer seasons 2003/2004 to 2005/2006 (Cipro et al. 2010; Corsolini et al. 2011; Online resource 8). It is worth to note that some of these studies included from eight and up to 81 PCBs (Cipro et al. 2010; Corsolini et al. 2011; Online resource 8). Similar levels were found only in skua eggs (*Stercorarius* spp.), for a total of 51 PCB congeners (Online resource 8; Cipro et al. 2013). The underlying reasons for species differences are difficult to determine. Among others, different foraging areas or trophic position of prey items are two possibilities that may explain higher concentrations when averaged for one congener (PCB concentrations/number of congeners) in Wilson's storm-petrel eggs than in skua eggs (Braune et al. 2005; Carravieri et al. 2014; Corsolini and Sará 2017). However, even compared to Adélie penguins that are similar in habitat and prey preferences, Wilson's storm-petrel embryos showed higher concentrations (Cipro et al. 2010; Corsolini et al. 2011; Online resource 8). A study on PCB concentrations in Arctic birds suggested that higher metabolic rate, and hence feeding rate, may be a reason for higher pollutant concentrations in birds (Borgå et al. 2005). Wilson's storm-petrels have an almost threefold higher basal, and more than threefold higher field metabolic rate than Adélie penguins (adjusted for body mass). Therefore, a higher food, and hence, pollutant uptake relative to their body mass could explain higher PCB concentrations (Ellis and Gabrielsen 2001).

In the present study, the highest total concentration of PCBs was 3403 ng g^{-1} ww. Adverse effects of sum concentration of PCBs in other birds' eggs occurred at 2100 ng g^{-1} ww, for example, in eggs of the Great blue heron (*Ardea herodias*), resulting in induced enzymatic reactions (Bosveld and van den Berg 1994). An increase of morphological deformations of embryos appeared in Caspian terns (*Hydroprogne caspia*) at an egg PCB concentration of 4000 ng g^{-1} ww compared to normal living embryos with egg PCB concentrations of 3600 ng g^{-1} ww (Yamashita et al. 1993). Hence, sum concentrations of PCBs in Wilson's storm-petrel embryos reaching up to 3403 ng g^{-1} could already pose a threat to the developing bird, especially during the sensitive embryonic phase. Next to 17 analyzed PCBs in the present study, up to 192 other congeners not explicitly looked for could add up to a potentially even higher PCB load. Yet, quantified sum concentrations of the years 2014 to 2016 were lower, with a maximum value of 565 ng g^{-1} ww, suggesting the toxic threat linked to PCBs was lower in more recent years. However, toxic effects clearly differ among species and some may suffer severe alterations at levels that show no effect in others. No study so far evaluated impacts of PCBs on development or fitness in Wilson's storm-petrels. While levels of PCBs detected in Wilson's storm-petrels should be observed with caution, more studies evaluating the impact on this species' health are needed.

Among PCBs, dl-PCBs are especially toxic and can bind to the aryl hydrocarbon-receptor, a ligand dependent transcription factor (Poland et al. 1976; Safe 1994). This binding can cause interference with the immune system, cell migration, or the differentiation of cells of the nervous system (Barouki et al. 2012). In this study, we detected the dl-PCBs 77, 105, 118, and 156 above LOD, of which PCBs 105 and 118 were found above LOQ. Their exact adverse potential in Wilson's storm-petrels without species-specific studies is hard to assess. The fact that in this study even the lowest concentrations of dl-PCBs 105 and 118 together were more than 500-fold higher than European limits for consumable food (eel: 0.010 ng g^{-1} ww, fish liver: 0.020 ng g^{-1} ww; European Union 2011) shows how severe those pollutant loads are in Antarctic wildlife.

The patterns of PCB congeners in Wilson's storm-petrel embryos were similar to those found in eggs of other fish- and krill-eating birds (Bosveld and van den Berg 1994; Corsolini et al. 2011; Goutte et al. 2013). Patterns of PCBs differ due to species-specific metabolization of congeners between species and their prey species (Bodin et al. 2008; Koenig et al 2012; Na et al. 2017). Certain PCB congeners with structures like meta-para vicinal H-atoms in at least one phenyl ring could be metabolized and accumulated less in fish liver and fish-eating seabirds than other congeners (Zell et al. 1978; Borlakoglu and Walker 1989). Excretion paths of PCBs for birds include preen oil, feces or maternal transfer to eggs (Barron et al. 1995; Rudolph et al. 2016; Solheim et al. 2016). While patterns of PCBs differed only to small extents in black-legged kittiwakes (*Rissa tridactyla*) between liver, feather, and preen oil samples, small differences could be seen for certain congeners (Solheim et al. 2016), which could add to species-specific PCB patterns due to differentiation in excretion paths. In this study, observed patterns are in line with those of Adélie penguins or Common terns (*Sterna hirundo*; Bosveld and van den Berg 1994; Corsolini et al. 2011; Goutte et al. 2013).

The peak level of 4,4'-DDE, a potent endocrine disruptor and androgen receptor antagonist (Xu et al. 2006; Burgos-Aceves et al. 2021) that is even more persistent in animals than its parent compound (Burgos-Aceves et al. 2021), was from a sample from 2001 to 2003 (372 ng g^{-1} ww). It reached a concentration still well below the critical concentration of reproductive failure in the sensitive brown pelican (*Pelecanus occidentalis*, 3000 ng g^{-1}; Blus 1982). Several other bird species could withstand higher DDE levels before their ability to reproduce was critically impaired such as the peregrine falcon (*Falco peregrinus)* and the osprey (*Pandion haliaetus;* Ratcliffe 1967; Johnson et al. 1975). Hence, we do not expect severe impacts on reproduction on Wilson's storm-petrels due to the comparatively lower DDE concentrations alone.

In all other samples, 4,4’-DDE concentrations were much lower, but with one exception always higher than the other detected DDX, 4,4’-DDD. Neither 2,4’-DDT nor 4,4’-DDT, the main components of commercial DDT mixes, could be detected in our samples. The ratio of 4,4’-DDE concentration to the total concentration of all DDX in those samples was higher than 0.6. DDE is only an impurity of commercial DDT production, and occurs only in low concentrations in the commercial mix. Hence, its occurrence in the wild is almost exclusively due to degradation of DDT. Both, the absence of DDT as well as the DDE/ΣDDX ratios are indicators for old input (Borrell and Aguilar 1987; Yogui et al. 2003). This result is consistent with other studies on Antarctic seabirds such as the Antarctic petrel *Thalassoica antarctica* whose 4,4’-DDT concentrations in the plasma were very low, or even below LOD (Carravieri et al. 2021). However, at a larger spatial scale, other investigations on seabirds from the Southern Ocean revealed a recent use of DDT, likely for the fight against mosquitos, which are vector of malaria (Carravieri et al. 2014). Such contrasting results reveal the importance of on-going monitoring of contamination in subantarctic and Antarctic environments to evaluate if a new increase of DDX from recent input can be observed.

In one sample, 4,4’-DDD had the highest concentrations compared to the other DDX. To the best of our knowledge, this ratio between 4,4’-DDD and 4,4’-DDE has not yet been reported in biota feeding in Antarctic waters. The reason of this high occurrence of 4,4’-DDD remains unclear. It could be a sign of an uncommon food source, as 4,4’-DDD is the main degradation product of reductive dechlorination reactions of 4,4’-DDT (Yu et al. 2011; Zhang et al. 2015). Hence, prey items like lanternfish (Myctophidae) that spend part of the day at zones of minimal oxygen and use anaerobiosis to cope with these conditions, may explain this finding (Torres et al. 2012). As we did not have stable isotope information for all eggs from this pooled sample, we could not verify whether food items for these eggs were from different trophic positions than other analyzed samples. No 2,4’-DDT, 2,4’-DDD, or 2,4’-DDE was found. This is in accordance with other studies, where these components were below detection levels or had concentrations that were orders of magnitude lower than 4,4’-DDE, which was found in highest concentrations in seabirds (Cipro et al. 2010; Corsolini et al. 2011).

Mean Hg concentrations found in egg membranes of Wilson’s storm-petrels were in the same range as those of *Pygoscelis* penguins from the South Shetland Islands (Brasso et al. 2012). It is difficult to establish thresholds for lowest observed adverse effect levels for detrimental effects of Hg in eggs, e.g., due to different thresholds among species, and threshold often refer to whole-egg Hg levels (Dietz et al. 2013; Evers et al. 2003). In bird eggs, concentrations of Hg are closely linked to different egg compartments; former studies found that mean Hg concentrations for whole-egg contents are 6.5 to 7.8 times higher than in egg membranes for Gentoo penguins (*Pygoscelis papua*) or American avocets (*Recurvirostra americana)*, respectively (Brasso et al. 2012; Peterson et al. 2017). When estimating comparable Hg concentrations for whole-eggs (dw) with these values, only one sample from 1998 (0.436 µg g^{-1} dw for egg membrane, estimate for whole-eggs: 2.8–3.4 µg g^{-1} dw) is considered to reach a concentration that poses a moderate hazard for reproduction in several bird species (0.6–1.3 µg g^{-1} ww, equals about 2.8–6.1 µg g^{-1} dw, Evers et al. 2003). Impacts of high Hg concentrations in eggs include reduced hatchability, malformations of the embryos or reduced nestling survival (Burger and Gochfeld 1997; Heinz et al. 2009; Scheuhammer et al. 2007). However, if nestlings survive long enough, birds will be able to reduce their Hg load during feather growth and molt (Bond and Diamond 2009; Falkowska et al. 2013; Albert et al. 2019). Especially very young birds can benefit from this excretion pathway as the example of common loon chicks (*Gavia immer*) shows (Fournier et al. 2002).

Pollutant concentrations and embryo age

In our samples, PCB concentrations were relatively constant for the first two thirds of the embryonic development (Fig. 3). After an embryonic age of 34 days, PCB concentrations were significantly higher. This concentration increase happened within the same time frame than in domestic chicken (*Gallus gallus domesticus*) embryos use yolk lipids for embryonic development; embryos took up only about 20% of lipids stored in the yolk during the first two thirds of their development, but more than another 65% of yolk lipids until briefly before hatching (Noble and Cocchi 1990). The investigated POPs, PCBs and DDX, have a very high affinity to lipids, and are, hence, mainly transferred to the embryos through lipids. While the domestic chicken is considered to belong to the developmental category of precocial birds, storm-petrels are considered by most researchers semi-precocial birds. However, independently of their developmental mode, birds undergo the same structurally defined developmental stages, and growth curves do not differ between precocial and altricial species, once egg size and incubation period is taken into account (Ricklefs and Starck 1998). It is, therefore, likely that also non-precocial birds show the lipid metabolism described by Noble and Cocchi (1990), which largely explains the observed POP increase during embryonic development.

We found a tendency of elevated Hg concentration in egg membranes of more developed embryos. Eggshells, known to become thinner during embryonic development

(Karlsson and Lilja 2008; Orłowski and Hałupka 2015), were observed to have decreasing Hg concentrations with increasing embryonic age (Peterson et al. 2017), but no relationship between Hg concentrations in egg membranes and embryonic age was described. As eggshells are thinning, Hg could be transported with calcium to the embryo. This way, part of Hg may not pass through the egg membrane and accumulate here. However, this could only lead to a small increase of Hg concentrations and further studies are needed to evaluate potential pathways of Hg within the egg during embryonic development.

Temporal differences of pollutant concentrations and foraging behavior

Concentrations of PCBs were higher in 2001 and 2003 than in 2014 to 2016. The global ban of many POPs by the Stockholm Convention led to a decline in worldwide, and therefore Antarctic, atmospheric contamination (Pozo et al. 2017). However, melting glaciers represent a potential source of pollutants trapped in the ice for decades (Geisz et al. 2008; Bogdal et al. 2009). Once released into the sea, their accumulation seems currently to primarily take place in benthic environments, where increases in pollutant levels can still be seen (van den Brink et al. 2009, 2011). Whether this will also have an impact on pelagic food webs is yet to be determined. Data from several studies confirm the general trend of pollutant decline in pelagic seabirds, which indicates a lower input for biota feeding in pelagic habitats (van den Brink et al. 2009; van den Brink et al. 2011). While concentrations change, no obvious change in substance relations can be observed. The reduction seen here and in several studies on pelagic seabirds is consistent with known half-life values for organic pollutants (compare Galbán-Malagón et al. 2013).

For DDX, we did not find significantly different concentrations between time periods among all age groups. A study comparing several penguin species showed that DDX concentrations in adult fat tissue were lower around 2010 than they were in the 1980s and 1990s (Ellis et al. 2018). Lower 4,4'-DDT/4,4'-DDE ratios in 2000 to 2009 than in 1960 to 1969 suggest that more recently found DDX concentrations are from DDX that reached the Antarctic through long-distance transportation and is persistent to the environment now (Ellis et al. 2018). In this study, we neither detected 2,4'- or 4,4'-DDT in 2001 and 2003, nor in 2014 to 2016. This is in accordance with the suggestion that detected concentrations do not originate from recent input, and reflect persistent concentrations in the Antarctic environment. Similar concentrations between the two time intervals could hint to an equilibrium between transfer to Antarctic waters and sedimentation of DDX, but additional studies are needed to delineate whether this could be a time trend.

In the present study, egg membrane Hg concentrations were highest in 1998, and were higher in 2003 than in 2014 to 2016. In Adélie penguins, feather Hg concentrations were significantly lower in 2007 than in museum specimen from the 1950s (Carravieri et al. 2016), and prey animals like Southern Ocean squids from subantarctic waters showed decreasing Hg concentrations over a decade of monitoring (Seco et al. 2020). Other species feeding in Antarctic waters like emperor penguins (*Aptenodytes forsteri*) showed no change of Hg feather concentrations compared to museum samples (Carravieri et al. 2016). However, emissions in the southern hemisphere were at best stable or even increased within the past years (Soerensen et al. 2012; Streets et al. 2017). High emissions originate from artisanal small-scale gold mining that is increasing with rising gold prices. Gold mining is for example found in Ghana or Peru, and hence, no decrease of emissions in the southern hemisphere is expected in the near future (Eagles-Smith et al. 2018). In sub Antarctic regions, increasing atmospheric Hg concentrations are also reflected in Hg concentrations in feathers of several seabird species compared to years or decades earlier (Carravieri et al. 2016; Mills et al. 2020). Interestingly, even with decreasing atmospheric Hg concentrations, animals may not reflect these trends, because several environmental factors, e.g., warming climate, and biogeochemical processes, may promote Hg methylation and its subsequent incorporation in food webs (Wang et al. 2019). Hence, even though our results showed higher Hg concentrations in the early 2000s than in 2014 and 2016, caution is necessary, as due to atmospheric Hg deposition in oceanic waters (Krabbenhoft and Sunderland 2013; Cossa et al. 2011) an increase of Hg concentrations in seabirds like the Wilson's storm-petrel should not be excluded (Carravieri et al. 2016).

Foraging behavior of wildlife can often explain diverging pollutant concentrations, because of prey from different trophic positions or geographic areas (Braune et al. 2005; Carravieri et al. 2014, 2016; Corsolini and Sará 2017; Cherel et al. 2018; Mills et al. 2020). We used stable isotope $\delta^{13}C$ and $\delta^{15}N$ values to analyze if differences of pollutant concentrations among time periods could be caused by foraging behavior. Stable isotope values of the years 2014 to 2016 did not differ from 1998 or 2003, while 1998 and 2003 were significantly different from each other. Specifically, there is a high divergence of $\delta^{15}N$ values, but a high overlap of $\delta^{13}C$ values that can be used as a proxy for latitude (Fig. 5; Cherel and Hobson 2007; Quillfeldt et al. 2010). However, trophic positions calculated from $\delta^{15}N$ values differed in all years more within than among years. Additionally, Hg concentrations did not correlate to $\delta^{13}C$ or $\delta^{15}N$ values. Hence, we think that observed concentration differences in this study of Hg in 1998, 2003 and 2014 to 2016, and of PCBs in 2001 and 2003 compared to 2014 to 2016, are unlikely to be caused by diverging pre-laying exodus latitudes or prey trophic positions, and rather reflect a decrease of the pollutants in the environment over time. However, our available data covered only a small number of breeding seasons, and more data are needed to delineate a significant decreasing trend of POPs and Hg concentrations in Wilson's storm-petrel eggs (Rigét et al. 2011; Bignert et al. 2004).

Conclusions and outlook

Overall, the pollutants in embryos and egg membranes of Wilson's storm-petrels were similar to those often reported for biota in Antarctic waters. Concentrations of Hg in the Wilson's storm-petrel egg membranes were in similar ranges than those of seabirds with similar diets like Adélie penguins, but POP concentrations measured in Wilson's storm-petrel embryo samples were higher than those of the larger seabird. In general, concentrations of POPs and Hg decreased over the observed decade, and POP concentrations increased with advancing embryonic development. Our study established a first basis of pollutant concentrations found in embryos and egg membranes of Wilson's storm-petrels breeding in the Antarctic. Also, our results show the importance of considering the developmental stage of the embryo when analyzing pollutants. Further studies are needed to investigate potential impacts of these pollutants, e.g., on reproductive success and toxicity effects on nestlings. It is important to examine whether the low concentrations during the first weeks of embryonic development are negligible in terms of toxic impact on embryo development compared to higher pollutant loads transferred in later stages of development, or whether even low concentrations could be detrimental in sensitive phases of development. With melting ice masses due to climate change, trapped pollutants may be released into coastal Antarctic waters and incorporated in the food chain, likely increasing the toxicity risks for seabirds.

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Author contributions NK, PQ, LB, and RD conceived and designed the research. NK, LB, and PB conducted or were involved in pollutant analyses. ML facilitated permits and access to the field station in the framework of our collaboration between Argentina and Germany. CB, ML, NK, and PQ collected samples. NK and LB analyzed data. NK wrote the manuscript. All authors reviewed and approved the manuscript.

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Data availability We declare that data archiving is not mandated but data will be made available upon reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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Appendix

Electronic Supplementary Material for Chapters

In the following the electronic supplementary material for Chapter 2 and Chapter 3 is described. Links and QR-Codes to access the material are provided below the description. Raw data for chick measurements (body mass, wing and tarsus length, age, body temperature) and physiological parameters measured and analyzed for Chapter 1 & 2 is published at the PANGAEA Database. Links and QR-Codes for published data are provided below the description of the supplementary material.

Chapter 2 *Electronic Supplementary Material*

Figures F1 & F2 All individual graphs for logistic tarsus (F1) and wing (F2) growth that were considered for statistical analyses. Single measurements, phase of linear growth, at maximal growth rate, age at maximal growth rate, 90 % of maximal tarsus or wing length and age at 90 % of maximal tarsus or wing length as well as age and wing length at onset of steep wing growth.

Table T1 Estimates and test statistic for general linear model of maximal tarsus growth rate using mean body temperature until inflection point, age at inflection point and sex as independent variables.

Table T2 Estimates and test statistic for general linear model of 90 % maximal tarsus length using mean body temperature until asymptote, age at asymptote and sex as independent variables.

Table T3 Estimates and test statistic for general linear model of maximal wing growth rate using mean body temperature until inflection point, age at inflection point and sex as independent variables.

Table T4 - T15 Non significant or similar results of analyses mentioned in the main paper using a different temperature variable (short-, medium-, or long-term mean body temperature).

Table T4 & T5 Analogue results for analyses of haematocrit values using mean body temperature of past five days (T4) and mean body temperature of past ten days

(T5).

Table T6 & T7 Analogue results for analyses of triglyceride concentrations using mean body temperature (T6) and mean body temperature of past ten days (T7).

Table T8 & T9 Analogue results for analyses of bacterial killing activity using mean body temperature (T8) and mean body temperature of past five days (T9).

Table T10 & T11 Analogue results for analyses of high IgY values using mean body temperature of past five days (T10) and mean body temperature of past ten days (T11).

Table T12 & T13 Analogue results for analyses of low IgY values using mean body temperature of past five days (T12) and mean body temperature of past ten days (T13).

Table T14 & T15 Analogue results for analyses of PHA swellings using mean body temperature (T14) and mean body temperature of past ten days (T15).

Table T16 - T18 Results for analyses of lymphocyte count using mean body temperature (T16) and mean body temperature of past five days (T17) and mean body temperature of past ten days (T18).

Table T19 - T21 Results for analyses of heterophile count using mean body temperature (T19) and mean body temperature of past five days (T20) and mean body temperature of past ten days (T21).

Table T22 - T24 Results for analyses of eosinophile count using mean body temperature (T22) and mean body temperature of past five days (T23) and mean body temperature of past ten days (T24).

Table T25 - T27 Results for analyses of basophile count using mean body temperature (T25) and mean body temperature of past five days (T26) and mean body temperature of past ten days (T27).

Chapter 3 *Electronic Supplementary Material*

Online resource 1 Parameters used for age estimation of embryos by wet weight based on embryonic growth curves of the Leach's Storm-petrel (*Oceanodroma leucorhoa*) by Ricklefs, 2010.

Online resource 2 Figure of approximate mass growth of embryos and embryo age groups presented in this study. The curve was fitted analogue to Ricklefs, 2010, using the parameters presented in Online Resource 1.

Online resource 3 Table of age groups by wet weight of embryos used in this study.

Online resource 4 Table of temperature program of gas chromatography oven used for pollutant measurement.

Online resource 5 Table of parameters of test substances used in mass spectrometry detection, including substance, function, structure, chemical abstracts service registry number, molar mass, retention time and target ions.

Online resource 6 Table of calculated limits of detection and quantification for all test substances.

Online resource 7 Table with results from Dunn' post-hoc test for pairwise comparisons between single PCB pollutants.

Online resource 8 Table with an overview of PCB and DDX concentrations in eggs of other bird species breeding across the Antarctic Peninsula from other studies.

Figure 4.1: Electronic supplementary material for Chapter 2
https://ars.els-cdn.com/content/image/1-s2.0-S1095643326000188-mmc1.pdf

Figure 4.2: Electronic supplementary material for Chapter 3
https://static-content.springer.com/esm/art%3A10.1007%2Fs00300-022-03065-w/MediaObjects/300_2022_3065_MOESM1_ESM.pdf

Figure 4.3: Data at Pangaea for Chapters 1 & 2
https://doi.pangaea.de/10.1594/PANGAEA.987818

Peer Reviewed Publications

Peer Reviewed Publications

Kuepper ND, Bauch C, Libertelli MM, & Quillfeldt P. Cold blood lives longer? Telomere dynamics during heterothermy in storm-petrel chicks. [in preparation]

Kuepper ND, Czirják GÁ, Libertelli MM, & Quillfeldt P. Consequences of heterothermy during development on physiology and growth for Antarctic storm-petrel nestlings. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 314, 111983; https://doi.org/10.1016/j.cbpa.2026.111983

Ausems ANMA, **Kuepper ND**, Braun C, Gębczyński A, Hahn S, P. Jadwiszczak P, Jakubas D, Kraemer P, Lorenz S, Richter B, Ruß A, Schmoll T, Thébault J, Thorn S, Turner J, Wojczulanis-Jakubas K & Quillfeldt P (2023). Population dynamics of Antarctica's smallest endotherm, the Wilson's storm-petrel, over the last four decades. *Polar Biology*, 46(7), 655-672. https://doi.org/10.1007/s00300-023-03154-4

Kuepper ND, Böhm L, Braun C, Bustamante P, Düring RA, Libertelli MM, & Quillfeldt P (2022). Persistent organic pollutants and mercury in a colony of Antarctic seabirds: higher concentrations in 1998, 2001, and 2003 compared to 2014 to 2016. *Polar Biology*, 45(7), 1229-1245. https://doi.org/10.1007/s00300-022-03065-w

Kuepper ND, Marek C, Coria N, Libertelli MM, & Quillfeldt P (2018). Facultative hypothermia as a survival strategy during snowstorm induced food shortages in Antarctic storm-petrel chicks. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 224, 76-83. https://doi.org/10.1016/j.cbpa.2018.06.018

Kuepper ND, Melber M & Kerth G (2016). Nightly clustering in communal roosts and the regular presence of adult females at night provide thermal benefits for juvenile Bechstein's bats. *Mammalian Biology*, 81, 201–204. https://doi.org/10.1016/j.mambio.2015.11.003