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Contemporary premature feather loss (PFL) among common tern chicks in Lake Ontario: the return of an enigmatic developmental anomaly

JENNIFER M. ARNOLD[1*], DONALD J. TYERMAN[2], DOUG CRUMP[3], KIM WILLIAMS[3] & STEPHEN A. OSWALD[1]

[1]Division of Science, The Pennsylvania State University, Berks Campus, 2080 Tulpehocken Road, Reading, PA 19610, USA.

[2] Presqu'ile Provincial Park, Ontario Parks, 328 Presqu'ile Parkway, R.R. #4 Brighton, Ontario, K0K 1H0, Canada.

[3] National Wildlife Research Centre, Environment Canada, 1125 Colonel By Drive, Ottawa, Ontario, K1A 0H3, Canada.

Short Title: Premature feather loss revisited

KEYWORDS: avian virus, algal toxins, contaminants, corticosterone, feather loss, Great Lakes, induced moult, nutritional stress, pathogens, premature moult

[*]Corresponding author:
Jennifer M. Arnold
Division of Science,
The Pennsylvania State University, Berks Campus,
2080 Tulpehocken Road,
Reading, PA 19610, USA
Tel: +1-610-396-6002
E-mail: jma25@psu.edu

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Abstract

In July 2014, we observed premature feather loss (PFL) among non-sibling, common tern *Sterna hirundo* chicks between 2 and 4 weeks of age at Gull Island in northern Lake Ontario, Canada. Rarely observed in wild birds, to our knowledge PFL has not been recorded in terns since 1974, despite the banding of tens of thousands of tern chicks across North America since then. The prevalence (5% of chicks) and extent of feather loss was more extreme than in previous reports but was not accompanied by other aberrant developmental or physical deformities. Complete feather loss from all body areas (wing, tail, head and body) occurred over a period of a few days but all affected chicks appeared vigorous and quickly began to grow replacement feathers. All but one (recovered dead and submitted for post-mortem) most likely fledged 10-20 days after normal fledging age. Secondary covert feather samples were collected from PFL chicks (n=6; including shed feathers and re-growing live feathers) and normal individuals (n=8; plucked live feathers) and were analyzed for corticosterone concentrations.

There was striking temporal association between the onset of PFL and persistent strong southwesterly winds that caused extensive mixing of near-shore surface water with cool, deep lake waters. We found no evidence of feather dystrophy, concurrent developmental abnormalities or nutritional shortfall among affected chicks. Thus, the PFL we observed among common terns in 2014 was largely of unknown origin but may have been caused by unidentified pathogens or toxins welling up from these deep waters along the shoreline.

PFL was not observed among common terns at Gull Island in 2015, although we did observe similar feather loss in a herring gull *Larus argentatus* chick in that year. Comparison with sporadic records of PFL in other seabirds suggests that PFL may be a rare, but non-specific response to a range of potential stressors. Its reemergence in penguins, and now gulls and terns, may indicate widespread environmental changes that could lead to health risks for birds and other wildlife.

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Introduction

In July 2014 we observed premature feather loss (PFL) among common terns *Sterna hirundo* at Gull Island in northern Lake Ontario, Canada. This condition, whereby developing chicks lose their wing, tail, head and body feathers has rarely been documented in wild birds (although feather loss has been well studied in domestic poultry; Hughes 1985, Leeson and Walsh 2004). To our knowledge, PFL has only been reported for terns in coastal, eastern North America and at Indian Ocean breeding islands between 1970 and 1974 (Gochfeld 1971, 1975, Hays and Risebrough 1972, Feare 1974). At that time, it was cautiously associated with contaminant burdens, chiefly mercury and polychlorinated biphenyls (PCBs) (Hays and Risebrough 1972, Gochfeld 1980), and pathogenic organisms and their toxins (Bourne et al. 1977). However, researchers were unable to rule out the possibility of other stressors (such as trauma, cancers, allergens, infections, and genetic factors) (Gochfeld 1971).

Colonial seabirds and waterbirds are recognized as indicators of aquatic environments (Kushlan 1993, Piatt et al. 2007, Durant et al. 2009) in two main capacities: population-level/demographic effects and physiological/biochemical effects (Kushlan 1993). In most cases, physiological and biochemical endpoints are quicker to quantify and more widely used (Kushlan 1993, Furness and Camphuysen 1997, Burger and Gochfeld 2004). However, gross physiological endpoints, such as cases of aberrant or retarded development (including feather loss, e.g. Hays and Risebrough 1972) or reduced growth rates (e.g. Lyons and Roby 2011) may be more easily quantified, especially when birds are easy to catch (such as waterbird chicks prior to fledging age). While these do not necessarily permit absolute diagnosis, they allow immediate detection (although not clear causality) of rare events and can help to narrow down potential causal factors (e.g. Bourne et al. 1977).

Substantial feather loss can be induced in poultry by periods of starvation, nutritional restrictions, as well as a consequence of feather pecking, shock molt or Psittacine Beak and Feather Disease (Spearman 1980, Hughes 1985, Leeson and Walsh 2004, Møller et al. 2006). However, it is rarely reported in wild birds except for some pathogenic infections (e.g. in psittacines; Ha et al. 2007; but see Table 1). In North America, PFL in common terns was reported locally in the vicinity of Long Island, New York, as well as a few locations in coastal Connecticut and Massachusetts between 1970 and 1974 (Hays and Risebrough 1972, Nisbet 1972, Gochfeld 1980), but to our best knowledge, and despite tens of thousands of common terns being banded in the interim, no further cases have been reported anywhere subsequently (M. Gochfeld and I.C.T. Nisbet pers. comm.).

The re-emergence of PFL in common terns may be of conservation concern and, given their role as indicators of the aquatic environment, may indicate acute health risks for birds and other wildlife in the Lake Ontario region. Here, we describe the chronology and progression of PFL at Gull Island, Ontario, and review associated evidence to narrow down potential causes of this aberrant development. We aim to increase awareness of this phenomenon among ornithologists

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and the wider scientific community to ensure timely reporting of future occurrences that may lead to a better understanding of its underlying causes.

Materials & Methods

Since 2008, we have studied the reproductive biology of common terns annually at Gull Island, Presqu'ile Provincial Park, ON, Canada (43°59′N, 77°45′W) under appropriate authorizations (see Acknowledgements). Following standard protocols (Arnold et al. 2015), each year nearly all chicks were banded and chicks from all study nests (~80 – 130) were recaptured and weighed regularly (every ~1-4 d) from hatching until fledging. In 2014, chicks were recaptured near-daily until 24 July and then weekly until 20 August.

On discovery of premature feather loss (PFL) in multiple individual chicks at Gull Island, we took a range of photographs (head, tail, wing, body) and also measured wing length (maximum wing chord) and tail length (maximum length of longest outer tail feather) at each subsequent recapture using a 300 mm wing rule. The same was done for a sample of normally developing tern chicks during this time period. For corticosterone analysis, secondary coverts (5-6/bird on average) were obtained (by plucking) from most chicks exhibiting PFL (n=6 chicks; 5 with regrowing feathers and 1 with original feathers) and from normal (control) chicks (n=8; original feathers) during this period. Mainly regrowing feathers were available from PFL chicks as protocols were developed following initial identification of this condition. The carcass of one subsequently dead PFL chick was sent to D. Campbell, of the Canadian Wildlife Health Cooperative, Ontario Veterinary College for post-mortem.

Secondary covert feathers were analyzed for corticosterone as per the method of Bortolotti et al. (2008). Briefly, the calamus was removed and the remaining feather was cut into small pieces and extracted with methanol overnight at 50°C. After centrifugation at 13,000 rpm for 20 minutes, the supernatant was transferred to a fresh tube and re-extracted with 1 ml methanol. The methanol fraction was evaporated to dryness overnight before final reconstitution in 200 µl steroid diluent. The sample was analyzed using a corticosterone EIA kit (Assay Designs product no. 900-097) as per the manufacturer's protocol. Data are presented as pg corticosterone/mm feather.

Repeated mass measurements from eight chicks that exhibited PFL were used to construct a composite growth curve for comparison to larger samples of normally developing chicks. Median wing and tail growth rates (slopes of measurements within individuals) for chicks exhibiting PFL and normal chicks were compared using Mann Whitney U-tests. For these analyses, only chicks with multiple measurements and of similar age (24 d – 33 d) were included in analyses (6 PFL chicks and 3 control chicks).

Local, hourly weather data from the Trenton, ON, weather station (44°7′N, 77°32′W; 21 km to the northwest) were downloaded from Environment Canada (http://climate.weather.gc.ca/). From these we calculated daily (24 h) means from 1 May to 31 July for air temperature, relative

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humidity, wind speed, wind direction, visibility and standard atmospheric pressure, and also minimum nighttime temperatures and maximum daytime air temperatures. Lake surface temperature was retrieved from Environment Canada (http://weather.gc.ca) for the nearest near-shore, weather buoy (Ajax, ON, Station 45159, 43°46' N 78°59' W, 105 km E of Gull Island).

Analyses were conducted in R (R Core team 2015). All means are reported with ±1 SD, all medians with quartiles [upper, lower].

Results

Premature feather loss (PFL) was first discovered in two non-sibling, common tern chicks at Gull Island on 5 July, 2014. Initial symptoms noted at this time were missing feathers on the head and body, similar in extent to that sometimes resulting from territorial aggression by neighboring adults or chicks (but without the associated laceration, bruising or hemorrhaging). However, by 8 July these two chicks had lost down, primaries and most feathers from all areas of the head, body, tail and wings, and in one chick, pin feathers were already growing back in places (Fig 1). On this same day, two other chicks exhibited PFL symptoms for the first time. Between 9 July and 11 July a further 3 chicks were found with PFL and we noted a final case in this month on 24 July. However, we also discovered an unbanded, medium-sized chick (estimated as 13-15 d of age) exhibiting PFL on 15 Aug (which is excluded from the analyses that follow). Although many affected chicks were from 2- or 3-chick broods, none had siblings that showed signs of PFL.

No evidence of feather dystrophy was found in the dead PFL chick sent for post-mortem analysis, reducing the likelihood that this PFL resulted from a viral infection (such as that from Circovirus and Papovavirus) (D. Campbell, pers. comm.). It also showed no obvious signs of infection or abnormality in heart, lung, liver or kidney tissues (D. Campbell, pers. comm.). There was a high level of cellularity in the pulp of the feathers, but the significance of this is unclear (D. Campbell, pers. comm.).

Corticosterone concentrations were above the limit of detection (0.5 pg/mm) for all feather samples analyzed. There were no significant differences between the levels of corticosterone measured in the feathers of the control birds and the PFL birds (Figure 2).

The mean age of chicks when first exhibiting PFL was 18 (± 3.7) d (range: 15 – 26 d). Comparison between normal plumage development and that of chicks exhibiting PFL is shown in Fig 1. Although growth in mass for chicks exhibiting PFL appeared normal initially, from about 10 d of age until normal fledging age (~25 d) masses were generally lower than the colony average (Fig 3). Although median rates of both wing and tail growth were slightly higher for PFL chicks than normal chicks at similar ages, these differences were not statistically significant (wing: PFL = 5.5 [4.3, 6.2] mm/d (n = 6), Normal = 4.0 [2.8, 5.3] mm/d (n = 3), W = 5, P = 0.38; tail: PFL = 3.8 [2.9, 4.9] mm/d (n = 6), Normal = 3.3 [2.2, 3.5] mm/d (n = 3), W = 3, P = 0.17).

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The seven surviving chicks that exhibited PFL in July were last seen between 21 and 42 d of age (mean: 29.1 ± 6.5 d). Mean body mass at their last recapture date was 119.3 (± 9.2) g, effectively identical to the colony average for birds of fledging age (118.3 ± 8.1, n = 29). Mean wing length of the PFL chicks was 125 (± 23) mm when last seen (well before actual fledging). Using the observed rates of daily wing growth (slope of maximum chord) we projected fledging dates for each of these chicks as the date at which wing lengths would have equaled 180 mm (the smallest wing length for a normal, fledged common tern chick in 2014). The earliest and latest projected fledging dates for the PFL chicks were 26 July and 20 Aug (mean: 2 Aug ± 9 d), respectively. During a visit to the colony on 11 Aug, we estimated there to be between 15 and 25 active common tern broods in the colony at this time.

The period in which PFL was first detected in common tern chicks (and three days immediately prior to it; 2 – 10 July) was characterized by plummeting near-shore, lake surface temperatures (Fig 4a) and falling air temperatures (Fig 4b) as well as stronger southwesterly winds (Fig 4c & d) and rising atmospheric pressure (not shown). Minimum nighttime temperatures and maximum daytime temperatures were highly correlated with mean air temperature (r_{89} = 0.82 and r_{90} = 0.99, respectively) and showed the same response (not shown). However, there were no obvious changes in relative humidity or visibility at this time (also not shown).

Discussion

The premature feather loss (PFL) that we observed in common tern chicks at Gull Island in 2014 is similar in two ways to that described by researchers working on the Atlantic coast of North America in the early 1970s. Firstly, we only observed it in chicks when they were between 2 and 4 weeks of age, the same age as noted by Hays & Risebrough (1972) and similar to Gochfeld (1971) [3-5 weeks], although in this latter case PFL was restricted to chicks closer to normal fledging age (Gochfeld 1971, Gochfeld pers. comm.). Secondly, in all our cases, shed feathers were replaced in all areas of the body but chicks appeared otherwise healthy and vigorous (Gochfeld 1971, Hays and Risebrough 1972). In fact, our PFL chicks, although they consequently had shorter wings and tails than normal chicks (Fig 1), showed no reduction in feather growth rates following feather loss. This is unsurprising, since feather growth appears highly conserved (e.g. under conditions of nutritional stress; Bize et al. 2006, Lyons and Roby 2011).

There are a few interesting differences between the PFL we observed and that previously documented both in terns and other birds (Table 1). Complete feather loss (Fig 1) is the extreme among terns in previous reports, as some birds only lost primaries and/or tail feathers (Gochfeld 1971, 1975, Hays and Risebrough 1972, Feare 1974, Bourne et al. 1977). Similar complete feather loss and regrowth has been observed in other species, e.g. greater black-backed gulls (*Larus marinus*), African (*Spheniscus demersus*) and Magellanic (*S. magellanicus*) penguin chicks (Kane et al. 2010). The incidence of PFL at our colony in 2014 was 5% (9/167) of all chicks, higher than that reported for common terns (0.5 – 1.1%; Hays and Risebrough 1972,

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Gochfeld 1975), although similar rates have been noted for other terns and gulls (Feare 1974, Roy et al. 1986) and even higher incidences in rehabilitated penguins (Kane et al. 2010). Unlike all other reports for terns, we did not observe concurrent developmental abnormalities (e.g. crossed-bills, absence of down, aberrant limb development; Feare 1974, Gochfeld 1975, Bourne et al. 1977) in PFL chicks or any other common tern chicks at our site. Lack of concurrent abnormalities is, however, consistent with contemporary reports among penguins (Kane et al. 2010, Barbosa et al. 2015, Grimaldi et al. 2015). Colony-wide hatching success also did not appear any different from in previous years (Arnold & Oswald unpublished data), suggesting an absence of gross embryonic deformity (such situations were previously linked with PFL; Hays and Risebrough 1972). Interestingly, previous studies in the lower Great Lakes between 1971 and 1974 detected a high prevalence of deformity among common tern chicks (including one chick at Presqu'ile Provincial Park) but no cases of PFL (Gilbertson et al. 1976).

Although high mortality among chicks developing PFL has been previously reported (Hays and Risebrough 1972) or assumed (Gochfeld 1980, Kane et al. 2010), we only recovered one dead chick with this condition despite intensive searching. Dead chicks can desiccate and decompose quickly (Gochfeld 1971); however, we visit each nest on a near-daily basis at this site and thus, recover nearly all chicks that die at this late stage of development. Given this, and the seemly vigorous condition of PFL chicks when last seen (our intensive searches finished on 24 July for logistic reasons), we expect that many of these chicks eventually fledged. This would not be completely unprecedented since successful fledging of tern chicks that exhibit PFL during development has been previously reported (Hays and Risebrough 1972).

While growth rates of wings and tails of common tern chicks recovering from PFL in 2014 appeared normal, PFL chicks had lower masses than normal chicks between 10 d of age and normal fledging age (~25 d). Although some of this may be directly due to the absence of plumage, the maximum differences we observed (~30 g on average at 18 d of age; Fig 3) likely exceeded the weight of lost plumage. As chicks recovering from PFL appeared vigorous and highly aggressive in some cases, this is unlikely to result from a competitive disadvantage during provisioning events (e.g. Oswald et al. 2012) but instead a preferential channeling of energy to feather regrowth. Such a relationship between PFL and growth has been previously documented for penguins (Kane et al. 2010) but not terns (Gochfeld 1971). PFL did not appear to affect the known tendency for tern chicks to overshoot adult mass and subsequently lose mass at this site, as the proportion of PFL chicks exhibiting this growth trajectory (25% [2/8 chicks]) was similar to that previously reported (34-38%, Arnold et al. 2015).

There was no difference between the normal and PFL chicks in terms of corticosterone levels in secondary covert feathers, although values were slightly higher for feathers from PFL chicks (Fig 2). It is important to note that feather corticosterone represents a measure of chronic stress (accumulation of corticosterone in the feather over time) (Bortolotti et al. 2008) and therefore an acute stress event, such as that potentially behind the observed feather loss, may not be detected, especially in regrowing feathers.

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Other reports of PFL have been for chicks late in the breeding season (Gochfeld 1971) but at Gull Island in 2014, PFL was first observed in early July (mean hatching date across all chicks in 2014 was 30 June) and an active colony persisted well into mid-August. There was a striking association between the timing of occurrence of onset of PFL at Gull Island in 2014 and plummeting (up to 13 C˚) near-shore, lake surface temperatures, falling air temperatures, and strong southwesterly winds (Fig 4). Sustained strong winds may cause substantial mixing of cold, deep lake waters with warmer surface waters which, after a period of warm lake surface temperatures, can bring anoxic bacteria (such as Type E *Clostridium botulinum*) to the surface, causing botulism outbreaks (Perez-Fuentetaja et al. 2004, Chun et al. 2013). However, we did not see much evidence of botulism among ring-billed gulls *L. delawarensis* or Caspian terns *Hydroprogne caspia* at this time (we have never encountered botulism in common terns at Gull Island), and systematic beach surveys at the Park did not report any unusual mortality events. It is possible, however, that the cooler, deep waters that mixed with near-shore waters contained, or vectored, sediment contaminated with pathogenic organisms or historical pollutants. In the early 1970s, there was evidence that PCBs might be responsible for gross deformities in chicks in Lake Ontario (although not PFL) (Gilbertson et al. 1976) and elsewhere (Ludwig et al. 1996), but research has largely debunked this idea (Kuiken et al. 1999). Contaminant levels in common tern eggs have declined following legislative action (Weseloh et al. 1989) and recent monitoring (via long-term monitoring of contaminants in herring gull eggs) has not indicated a return to problematic contaminant levels of these legacy organohalogen contaminants (Hebert 2000, Jermyn-Gee et al. 2005). Since PFL in 2014 was observed within a matter of days after falling near-shore lake temperatures (Fig 4), this largely precludes bioaccumulation of contaminants (PCBs [Hays and Risebrough 1972] or mercury [Gochfeld 1980]) as causal agents (C. Custer, pers. comm.).

Exposure to as yet unknown toxins or pathogens between 2 and 10 July 2014 seems the most likely current explanation of PFL in common tern chicks at Gull Island. Most affected chicks were between 1-2 weeks of age during this time period, although one chick was hatched at this time and the egg of another chick that developed PFL in August was laid at this time. Thus, it is possible that the causal agent persisted in the environment or was communicated to the developing embryo from the parent. As in most other cases of PFL, chicks showing this condition had normal siblings, suggesting a low rate of transmission and an absence of a strong genetic link (Roy et al. 1986, Kane et al. 2010, Barbosa et al. 2015). Pathogens such as algal toxins and bacteria (Bourne et al. 1977) or tick-borne (Feare 1974) or other viruses (Leeson and Walsh 2004, Ha et al. 2007) have been suggested as causes of PFL but we found no evidence of known viral (e.g. Psittacine Beak and Feather Disease [Circovirus]; Katoh et al. 2010, Varsani et al. 2014) or bacterial agents or tick infection. Feather loss, deformity and onset of molt, although not necessarily complete molt, are well studied in poultry and have also been associated with periods of starvation and nutritional deficiency (e.g. low protein diets, zinc, Vitamin E, and selenium; Leeson and Walsh 2004), but there was no strong evidence of acute nutritional

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deficiency in the PFL chicks in our study. Thus, we currently favor exposure to unknown toxins or pathogenic organisms as the causal mechanism.

Comparison with sporadic records of PFL in other birds (Table 1) suggests that PFL may be a rare, but non-specific response to a range of potential stressors, including environmental contaminants, viral and bacterial infections, tick-borne disease or nutritional deficiency. Given that PFL is very obvious in affected chicks (Fig 1) but is seldom reported despite the great number of studies that should detect it, suggests that the low incidence of reporting represents a rare condition rather than simply underreporting (Gochfeld 1971, Roy et al. 1986, Barbosa et al. 2015, Grimaldi et al. 2015). In 2015, we found no evidence of PFL in common terns despite similar nesting numbers and research protocols, but we did observe a single herring gull (*L. argentatus*) chick exhibiting premature feather loss (Arnold & Oswald unpubl. obs.).Thus, the reemergence of PFL in penguins (including adults; Grimaldi et al. 2015), and now gull and tern chicks, may perhaps indicate widespread environmental changes that could lead to health risks for birds and other wildlife, especially if pathogenic organisms are responsible (Bourne et al. 1977).

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Table 1. Reports of premature feather loss (PFL) in waterbirds. In all reports, feather-loss in some individuals studied was extensive.

Species	Location	Years	Explanation	Source
Common tern (*Sterna hirundo*)	Long Island, NY	1970-1974	Proposed link to contaminants (e.g. PCBs, mercury)	Gochfeld 1971, 1975; Hays & Risebrough 1972
Sooty tern (*Sterna fuscata*)	Seychelles	1973	Tick-borne soldado-virus	Feare 1974
Greater Black-backed gull (*Larus marinus*)	Witless Bay, Newfoundland	1984	Unknown cause	Roy et al. 1986
Herring gull (*Larus argentatus*)	Witless Bay, Newfoundland	1984	Unknown cause	Roy et al. 1986
African penguin (*Spheniscus demersus*)	South Africa	1989	Malnutrition	van Heezik and Seddon 1992
Emperor penguin (*Aptenodytes forsteri*)	Cape Washington, Antarctica	mid-1990s	Unknown cause	reported in Varsani et al. 2014
Magellanic penguin (*Spheniscus magellanicus*)	Argentina	2007	Unknown cause	Kane *et al.* 2010
African penguin (*Spheniscus demersus*)	South Africa	2009	Unknown cause	Kane *et al.* 2010
Adelie penguin (*Pygoscelis adeliae*)	Cape Crozier, Antarctica	2011	Unknown cause	reported in Varsani et al. 2014
Common tern (*Sterna hirundo*)	Gull Island, Lake Ontario	2014	Unknown cause, possible link to environmental conditions	This study
Adelie penguin (*Pygoscelis adeliae*)	Ross Is. & Antarctic Peninsula	2011-2015	Proposed avian virus (polyomavirus, novel astrovirus)	Barbosa *et al.* 2015; Grimaldi *et al.* 2015
Herring gull (*Larus marinus*)	Gull Island, Lake Ontario	2015	Unknown cause	Arnold & Oswald unpubl. obs.

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Figure 1. Plumage characteristics resulting from premature feather loss (PFL) in common tern chicks at Gull Island in 2014 (left hand panel) versus normal development (right hand panel; photo with white background taken from Common Tern Aging Guide: Wails et al. 2014). In each case, whole body (a,b), wing (c,d) and tail (e,f) are shown (pictures taken between 9 and 18 July). Chicks shown are between 21 and 27 d of age (fledging usually occurs between 21-29 days; Nisbet 2002).

Figure 2. Mean (±SE) corticosterone concentrations (pc/mm) in secondary covert feathers collected from common tern chicks exhibiting normal feather growth (control; n=8) and premature feather loss (PFL; n=7) at Gull Island in 2014. Differences among groups are not statistically-significant (t_{13} = 0.96, P = 0.36). The method limit of detection was 0.5 pg/mm.

Figure 3. Changes in mass throughout development of the eight chicks exhibiting premature feather loss [PFL] (black lines = 95% confidence intervals) superimposed over the range of weights for normal chicks (n = 159 chicks, grey shading = area between 95% confidence intervals). For PFL chicks measured later in development (> 30 d of age), when fewer measurements were available, individual data points are plotted.

Figure 4. Changes in mean daily weather conditions, (a) near-shore lake surface temperature, (b) air temperature, (c) wind speed, and (d) wind direction (maximum gust), and correspondence with distribution of hatching dates (blue boxplots and outlier) and dates of first exhibiting premature feather loss [PFL] (green boxplots and outlier). Trend lines are 7-day running average of the weather variable. Grey shading highlights the period of plummeting near-shore surface water temperatures (2 -10 July).

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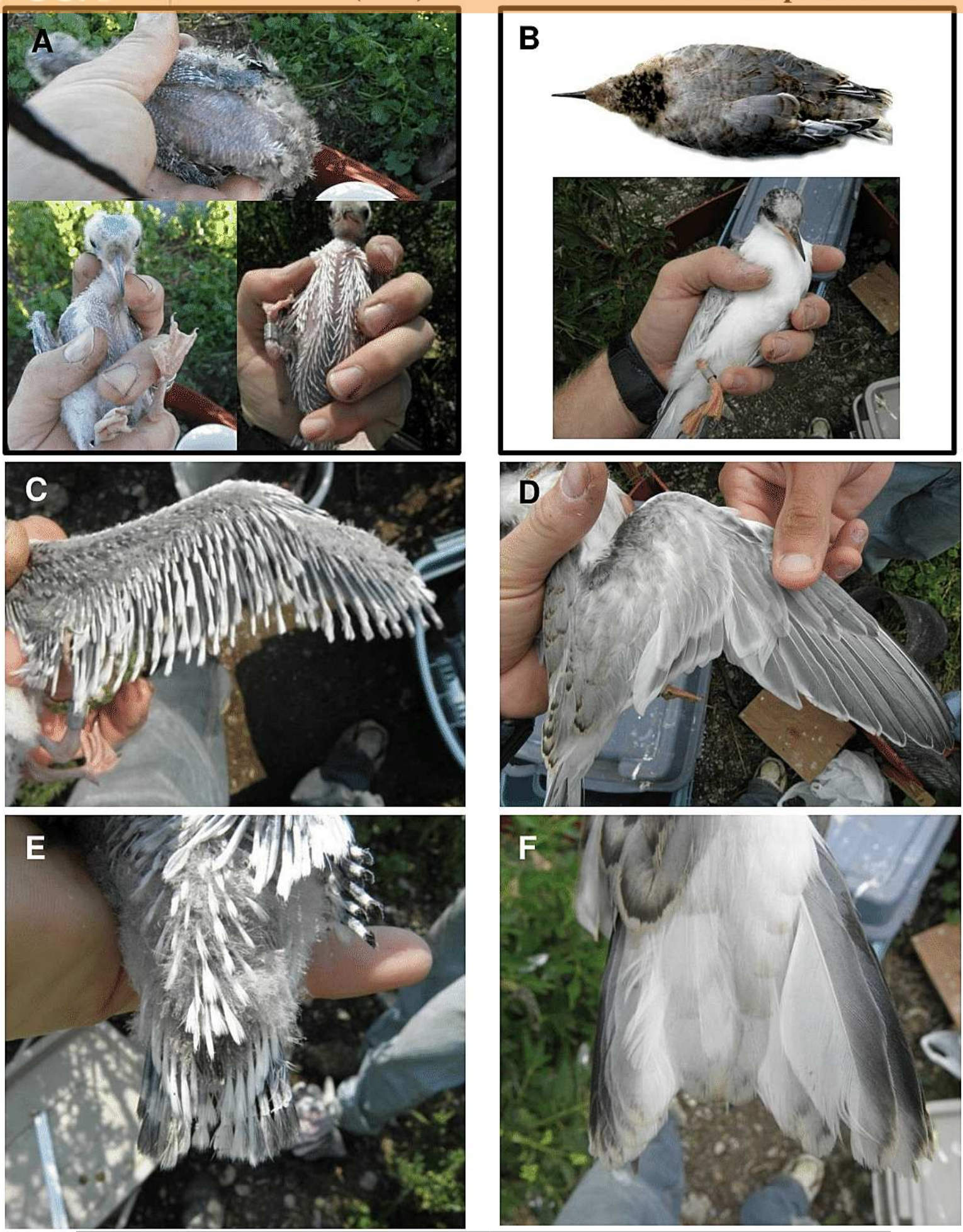
Premature feather loss (PFL) **Normal development**



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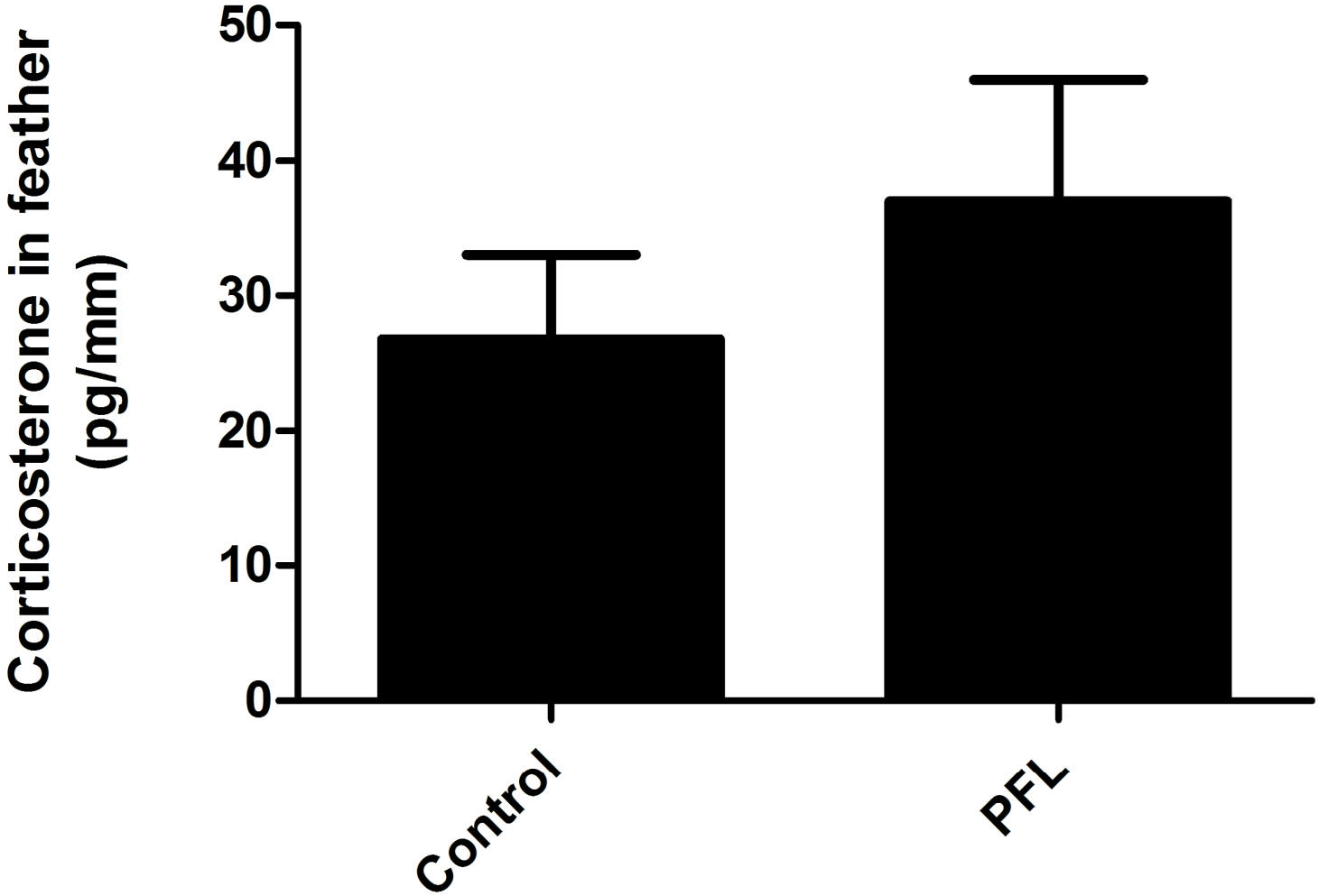

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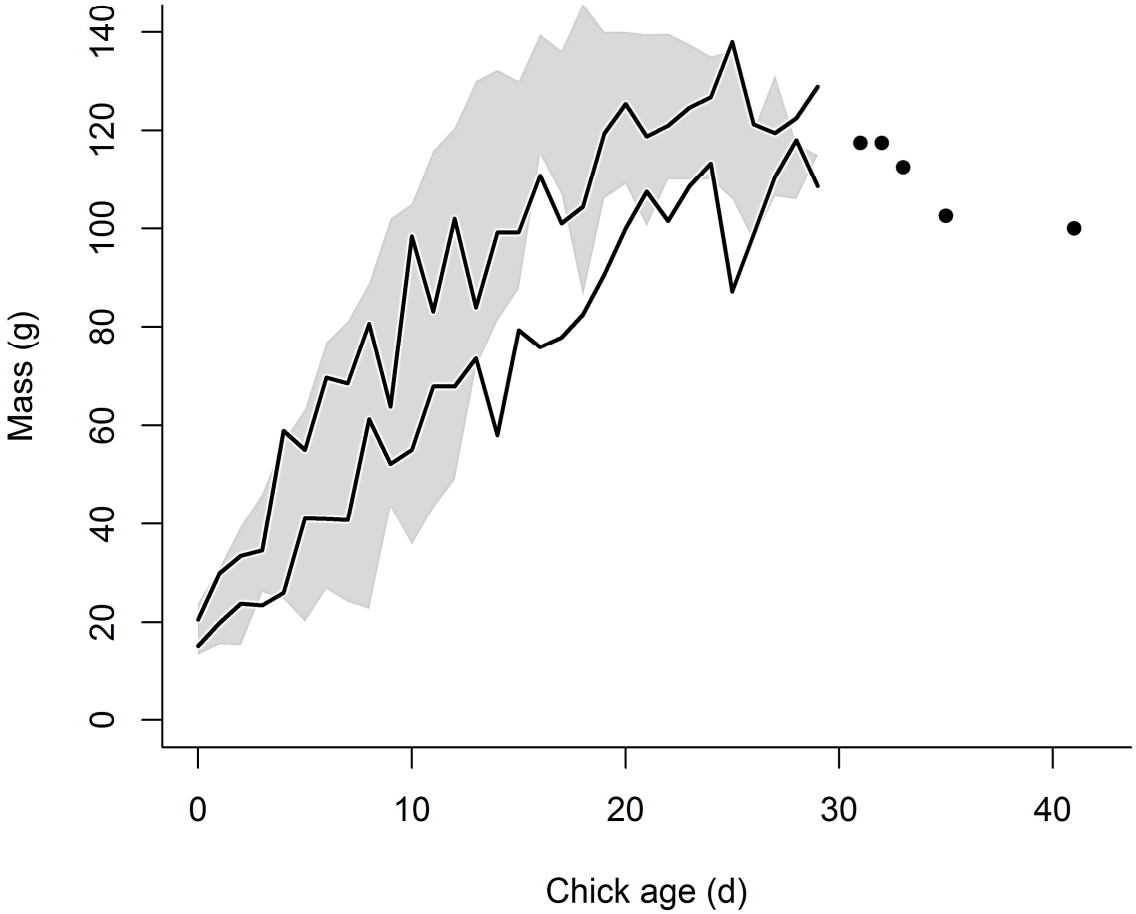

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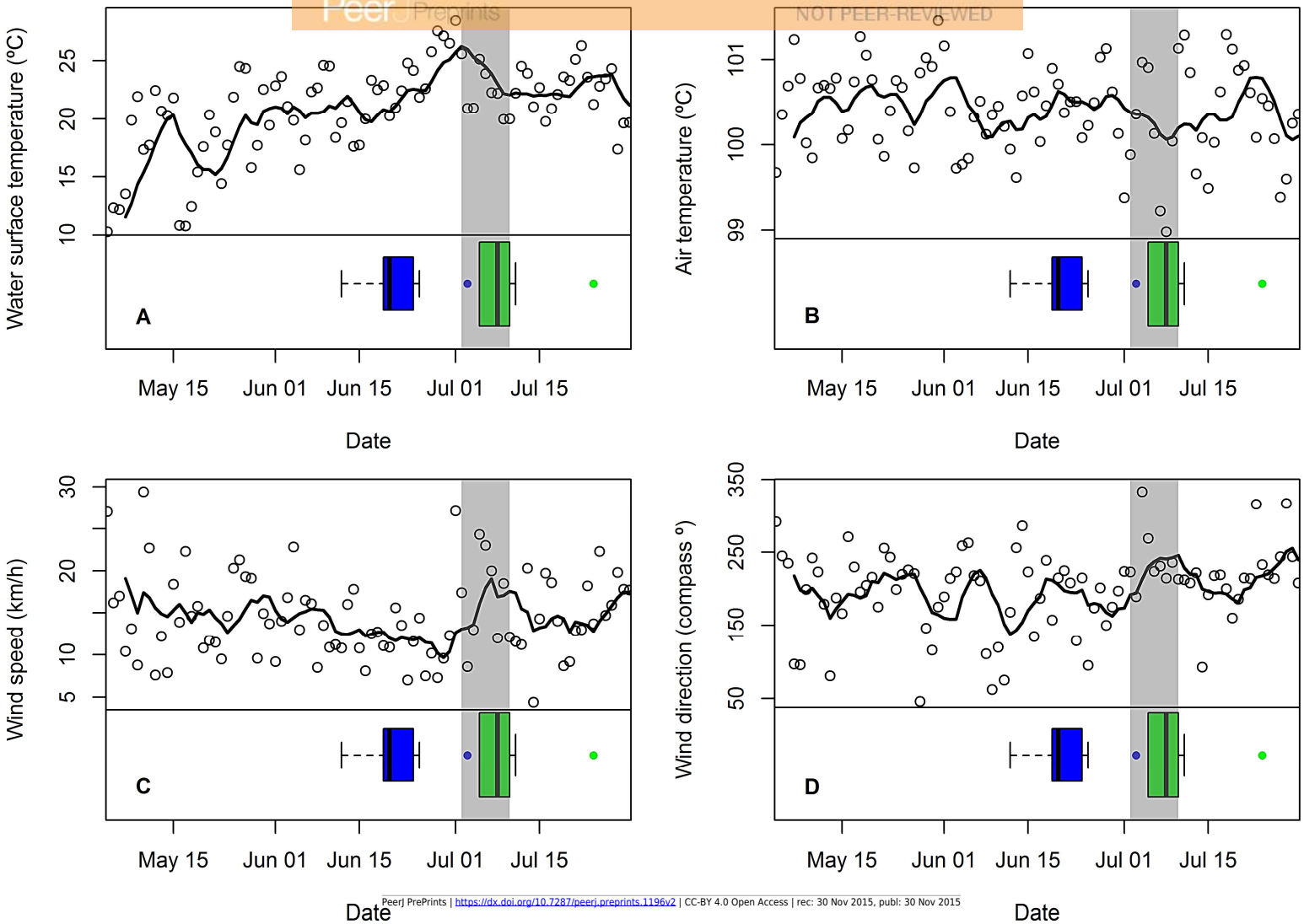

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