Plasma Biochemistries and Morphometric Indices of Body Condition in Imperial Cormorant (Phalacrocorax atriceps) Chicks

Authors: Gallo, Luciana, Quintana, Flavio, Svagelj, Walter S., and Uhart, Marcela

Source: Waterbirds, 40(2) : 118-128

Published By: The Waterbird Society

URL: https://doi.org/10.1675/063.040.0204

Plasma Biochemistries and Morphometric Indices of Body Condition in Imperial Cormorant (*Phalacrocorax atriceps*) Chicks

Luciana Gallo[1,*], Flavio Quintana[1], Walter S. Svagelj[2] and Marcela Uhart[3]

[1]Instituto de Biología de Organismos Marinos (IBIOMAR)-Concejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Boulevard Brown 2915, Puerto Madryn (U9120ACD), Chubut, Argentina

[2]Instituto de Investigaciones Marinas y Costeras (IIMyC)-Concejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Mar del Plata (UNMdP), Dean Funes 3250, Mar del Plata (B7602AYJ), Buenos Aires, Argentina

[3]One Health Institute, School of Veterinary Medicine, University of California, 1089 Veterinary Medicine Drive, VM3B, Davis, California, 95616, USA

[*]Corresponding author; E-mail: gallo@cenpat-conicet.gob.ar

Abstract.—Plasma biochemistries provide a complementary method for assessing physiological and nutritional status of free-ranging wild birds. Triglycerides, total protein and alkaline phosphatase were determined in 110 free-living Imperial Cormorant (*Phalacrocorax atriceps*) chicks aged 16-35 days, at Punta León (Argentina) during 2010 and 2011. Body mass at 30 days of age ("pre-fledging body condition", 2010 only) and body mass corrected by tarsus length at the time of blood sampling ("current body condition", 2011 only) were also determined. Variability of parameters by sex, hatching order, survival, age and breeding season was assessed, and the relationship between biochemical and morphometric indices was also explored. Morphometric indices were higher in A-chicks (pre-fledging body condition also varied with sex), and explained 35-55% of B-chick survival. Biochemistries differed significantly between breeding seasons, being higher in 2011. Alkaline phosphatase increased with age, and total protein was higher in A-chicks. Triglycerides and total protein accounted for 26% and 30%, respectively, of variation in current body condition; however, they did not forecast pre-fledging body condition. Lastly, total protein levels predicted B-chick survival (higher levels in surviving B-chicks), but their prognostic value was relatively low. The results suggest that unlike morphometric indices, the biochemistries chosen are valuable to assess individual body condition at the time of sampling, yet their applicability for predicting chick survival requires further evaluation. *Received 22 July 2016, accepted 29 December 2016.*

Key words.—body condition, Imperial Cormorant, morphometric indices, Patagonia Argentina, *Phalacrocorax atriceps*, plasma biochemistries.

Waterbirds 40(2): 118-128, 2017

Morphometric indices of body condition (e.g., body mass or size-adjusted body mass) are traditionally used as indicators of nutrient or energy reserves (mainly fat mass) in birds (Labocha and Hayes 2012), and numerous studies have shown their influence on fitness components of life-histories, such as survival (Bowers *et al.* 2014) and reproductive success (O'Dwyer *et al.* 2006). Plasma biochemistries provide a complementary method for assessing the physiological and nutritional status of free-ranging wild birds (Dawson and Bortolotti 1997; Jenni-Eiermann and Jenni 1998; Alonso-Alvarez *et al.* 2002). While plasma biochemistries are regulated within a relatively narrow homeostatic range (Kaneko *et al.* 2008), they may also respond to the animal's immediate physiological state in minutes (i.e., nutritional or anthropogenic stressors) (Milner *et al.* 2003). Significant adjustments in blood parameters including plasma biochemistries related to nutritional state accurately reflect demands from stages in the annual cycle such as migration and incubation-phase fasting (Navarro *et al.* 2007).

Triglycerides and total proteins are frequently used to indirectly assess the body condition of individuals, as they may reflect changes in body mass and food quality (Jenni-Eiermann and Jenni 1996; Dawson and Bortolotti 1997; Alonso-Alvarez *et al.* 2002). In particular, triglyceride levels are affected by the qualitative composition of the diet (Ferrer and Dobado-Berrios 1998) and have been used as indicators of energy reserves (Merilä and Svensson 1995). Total plasma protein reveals nutritional and health status since plasmatic proteins exert various critical transport, hormonal, enzymatic and im-

mune functions (Jenni-Eiermann and Jenni 1996; Dawson and Bortolotti 1997). Fasting-adapted species rely on structural proteins as energy sources only when fat reserves are depleted after an acute fasting period (Alonso-Alvarez *et al.* 2002). The enzyme alkaline phosphatase (ALP) is commonly used as a suitable marker of development in many altricial bird species because it varies with age and is related to bone growth (Viñuela *et al.* 1991; Tilgar *et al.* 2008). However, ALP can also vary rapidly in response to food availability (Viñuela and Ferrer 1997; Villegas *et al.* 2002; Amat *et al.* 2007). Previous studies found a relationship between ALP blood levels and body condition in birds of prey, as indicated by mass growth rate and body mass corrected by body size (Viñuela and Ferrer 1997; Villegas *et al.* 2002).

The applicability of blood parameters for assessing condition of free-living birds requires understanding of their natural drivers of variation (Fair *et al.* 2007). In chicks, triglycerides, total protein and ALP activity have been influenced by individual characteristics such as age, sex and hatching order (Viñuela *et al.* 1991; Masello and Quillfeldt 2004; Muriel *et al.* 2013; Minias and Kaczmarek 2013). Birds of different sexes are known to differ not only in their morphology, but also in their physiology, mainly due to differences in sexual hormones (Norte *et al.* 2009a; Jerzak *et al.* 2010). The asymmetry in size due to hatching asynchrony or sexual dimorphism may generate differences in competitive abilities between siblings (Oddie 2000). This might result in resource allocation favoring larger and more dominant nestlings (Mock and Parker 1997), which could lead to differences in the condition of chicks according to hatching order. This may be reflected in blood parameters associated with nutritional state, growth rates and fledgling mass (Platteeuw *et al.* 1995; Masello and Quillfeldt 2004; Minias and Kaczmarek 2013). Extrinsic factors like environmental conditions and anthropogenic disturbances that may vary between years and sites have also affected plasma biochemistries in birds (Seiser *et al.* 2000; Amat *et al.* 2007; Acevedo-Whitehouse and Duffus 2009; Norte *et al.* 2009b). Therefore, biochemical parameters could be useful bioindicators of individual condition, parental investment, and the quality of the environment in which chicks are reared (Horak *et al.* 2002; Norte *et al.* 2008, 2009b).

The Imperial Cormorant (*Phalacrocorax atriceps*) is a colonial seabird widely distributed along the Patagonian coast in Argentina (Frere *et al.* 2005). This species shows sexual dimorphism in size, with males larger than females (Svagelj and Quintana 2007). Even though modal clutch size is three eggs, less than 1% of the breeding pairs generate broods of three fledglings (Svagelj and Quintana 2011a, 2011b). Such strong brood reduction is a direct consequence of hatching asynchrony (Svagelj 2009). Both parents play an active role in chick rearing, feeding them twice daily (one time each) throughout the breeding cycle (Svagelj and Quintana 2011a, 2011b). These life-history features are suitable for exploring the variability of biochemical and morphometric parameters according to extrinsic and intrinsic factors in Imperial Cormorant chicks.

Our objectives were to: 1) determine biochemical parameters related to nutritional status and growth (triglycerides, total protein and alkaline phosphatase), coupled with two temporally distinct morphometric indices of body condition in free-living Imperial Cormorant chicks; 2) analyze the variability of these parameters according to sex, hatching order, survival, age and breeding season; and 3) evaluate the utility of select biochemical parameters in assessing the immediate body condition of Imperial Cormorant chicks, as well as predict their body condition at another stage of their development (e.g., prior to fledging).

METHODS

Study Area

The study was conducted at a colony of Imperial Cormorants located in Punta León (43° 03′ 51.3″ S, 64° 27′ 32.4″ W), Chubut, Argentina. This colony comprises ~3,400 breeding pairs homogeneously distributed in a flat and elliptical area (~130 m long, 15 m wide) (Svagelj and Quintana 2011a).

Data Collection

Fieldwork was conducted from September to December during two breeding seasons (2010-2011). We monitored a total of 85 nests (39 and 46 nests for the 2010 and 2011 breeding seasons, respectively). During the hatching period, all study nests were checked every 1-3 days to mark the tarsus of hatchlings with fiber-tape bands labeled with their nest number and associated hatching order. At the beginning of our study, 42% of monitored nests (n = 85) had three chicks, 41% had two chicks (first and second hatched chicks) and the remaining 17% had only one chick (first hatched chicks). During chick rearing, we visited nests every 3-5 days to determine the fate of chicks until it became impossible to capture them, at an age of ~35 days. Chicks were considered to have fledged if they reached 30 days of age, due to the high probability of chick survival to independence at that age (Svagelj and Quintana 2011a).

A total of 110 blood samples (< 1 ml; 2010: n = 50, 2011: n = 60) were taken from the jugular vein of Imperial Cormorant chicks (first (A-chicks): n = 84 (2010: n = 38, 2011: n = 46) and second hatched (B-chicks): n = 26 (2010: n = 12, 2011: n = 14)) at 16-35 (mean 25 ± 3) days of age using 1 to 3 cc heparinized syringes and 25 G x 1-inch needles. Blood sampling was performed in the morning, prior to the return of females from their first foraging excursion (see Harris *et al.* 2013), which implied a minimum of about 4-6 hr of fasting. At the time of blood collection, 31% (n = 85) of monitored nests had two chicks (A- and B-chicks), and the remaining nests had only one chick (A-chicks). The mortality of B- and C-chicks soon after birth was 41% (n = 77), mainly due to brood reduction (Svagelj and Quintana 2011a, 2011b). Additionally, 10 B-chicks (2010: n = 5, 2011: n = 5) that were sampled died at ~35 days of age (mean time interval between sampling and death: n = 5 days). A-chicks from these broods were sampled prior to the death of their siblings, and A- and B-chicks from the same nest were sampled at the same age to assess variation with hatching order and avoid age-related differences. During 2010, we also recorded chick body mass each time the nest was inspected (3-5 days, prior to food intake) until it became impossible to capture them (~35 days of age). This was performed using spring scales (Pesola) according to chick weight to estimate a morphometric index of "pre-fledging body condition". In 2011, a single measure of body mass and tarsus length was recorded at the time of blood sampling, coinciding with the linear growth phase (Svagelj 2009). This was to determine a morphometric index of "current body condition", with the aim of minimizing disturbance in the colony.

Laboratory Analysis

Blood samples were stored in plain vacuum tubes (Benton-Dickinson) and kept cool on ice until processing, within 4-6 hr post-collection. In addition, three or four drops of blood were placed on a small (50 × 20 mm) piece of filter paper (Whatman, GE Healthcare Argentina S.A.), air-dried and then stored separately for molecular sexing. Blood samples were centrifuged in a portable 12-volt centrifuge at 1,000 XG for 20 min (Mobilespin, Vulcan Technologies). Plasma was removed and stored at -80 °C until blood chemistry analysis for triglycerides, total protein and ALP were performed on a wet automated analyzer (Hitachi Model 902 Automatic Analyzer, Hitachi Science Systems) at a commercial veterinary laboratory. Chicks (female: n = 56, male: n = 53) were sexed by the DNA-based technique described by Quintana *et al.* (2008). Only one chick was not sexed due to poor sample quality.

Statistical Analysis

All body-mass measurements (n = 4-12 measurements per individual; mean = 9) were fitted to the Richards growth function (Tjørve and Tjørve 2010) using non-linear mixed models (Pinheiro and Bates 2000), considering the lack of independence between repeated measures. In this way, we estimated the body mass at 30 days of age for all chicks, and we considered it as a morphometric index of "pre-fledging body condition". For chicks sampled in 2011, we calculated the residuals of the regression between body mass and tarsus length (O'Dwyer *et al.* 2006; Minias *et al.* 2013) at the time of blood sampling (corrected by the age of chicks), and considered it as a morphometric index of "current body condition".

To examine possible sources of variation in plasma biochemistries and morphometric body condition indices, we employed linear mixed models, considering the non-independence between siblings (Pinheiro and Bates 2000). Models included biochemical parameters and morphometric indices as response variables (five models), sex and hatching order as fixed factors, and brood identity ("nest") as the random effect. Also, for models with biochemical parameters as response variable, we included the breeding season (2010-2011) and age of chicks at sampling (mean = 25 ± 3 days) as covariates.

In this study, most B- and all C-chicks died soon after birth due to brood reduction and were not sampled. Therefore, to explore the effect of selected parameters on chick survival, we considered the subset of sampled B-chicks and tested for the differences between B-chicks that did and did not survive. We employed logistic regression (Crawley 2007) with "survival probability" (0/1) as a response variable, and biochemical parameters and morphometric body condition indices as explanatory variables (one model for each to avoid multicollinearity). For plasma biochemistries, we fitted models considering data from the two breeding seasons because survival of B-chicks did not differ significantly between years ($\chi^2_1 = 0.55$, $P = 0.46$).

To investigate the relationship between selected plasma biochemistries and morphometric body condition indices in Imperial Cormorant chicks, we applied linear mixed models, considering the non-independence of chicks from the same brood. The models included morphometric indices as response variables (one model for each), plasma biochemistries as fixed effects, and sex and hatching order as covariates as

appropriate. Due to correlations between plasma biochemistries (triglycerides-total protein: r = 0.44, $P < 0.001$; total protein-ALP: r = 0.43, $P < 0.001$; ALP-triglycerides: r = 0.10, $P = 0.51$), separate models were run to avoid multicollinearity (six models in total).

We evaluated the significance of random effects ("brood") in all models with a likelihood-ratio test (LRT; Pinheiro and Bates 2000). Because only 31% (n = 85) of nests had two chicks, in those models where the term was not significant we analyzed significance of fixed effects using linear models (LM; Crawley 2007). We employed a backward selection procedure, removing non-significant terms from the model, one by one, in decreasing order of P-value (Crawley 2007). For all statistical analyses, we used the NLME package from the statistical software R (R Development Core Team 2013). Statistical significance was established at $P < 0.05$.

RESULTS

Biochemical Parameters and Morphometric Indices

Descriptive statistics for biochemical parameters are presented in Table 1. The breeding season was the factor that best explained the variability of plasma biochemistries, with particularly higher levels in 2011 (Table 2). None of the biochemistries varied according to sex (Table 2). Total protein levels were significantly higher in A-chicks (hatching order explained 3.4% of the variation in total protein), while none of the other biochemical parameters differed with hatching order (Table 2). ALP activity increased somewhat with age ($r^2 = 0.068$; Fig. 1), yet the other two biochemical parameters did not vary with the age of chicks (Table 2).

Estimated body mass at 30 days old (pre-fledging body condition) ranged from 1,052 to 1,996 g (mean = 1,565, SD = 241; n = 47), and was significantly higher in male and A-chicks (Table 3). Sex and hatching order explained 40.3% of variation in the pre-fledging body condition. Body mass adjusted for tarsus length at the time of blood sampling (current body condition) ranged from -575.7 to 310.3 g (mean = -2.4, SD = 154; n = 59), with negative values reflecting lower body mass than expected for tarsus length. This parameter also varied with hatching order, with A-chicks significantly higher than B-chicks, but did not differ between sexes (Table 3).

Considering the subset of sampled B-chicks, there were no significant differences in ALP activity and triglyceride levels between surviving and non-surviving chicks (Table 4). However, total protein and both morphometric body condition indices were significantly higher in surviving B-chicks (Table 4). Models including total protein levels, current body condition, and pre-fledging body condition explained 14.7%, 34.8% and 54.9% of the variation, respectively, of B-chick survival.

Relationship Between Biochemistries and Morphometric Indices

Biochemical parameters showed no significant relationship with the estimated pre-fledging body condition (Table 5). However, triglycerides and total protein levels explained 26.1% and 29.7% of the variance, respectively, of the current body condition (Table 5; Fig. 2). ALP activity was not a good indicator of current body condition, resulting in no significant relationship between the two parameters (Table 5).

DISCUSSION

This study provides information about sources of variation of selected plasma bio-

Table 1. Plasma biochemistries of free-living Imperial Cormorant chicks from Punta León, Argentina, over two breeding seasons (2010-2011). Assumption of normality was not met for biochemical parameters (Shapiro-Wilks test $P < 0.05$).

Parameter	Mean	Median	SD	2.5 Percentile	97.5 Percentile	Range	n
Total protein (mg/dl)	2.8	2.9	0.4	1.9	3.4	1.6-3.6	110
Triglycerides (mg/dl)	138.0	129.0	68.9	36.0	329.0	31-377	109
Alkaline phosphatase (IU/l)	1,846.8	1,753.5	817.5	650.0	3,757.0	560-4,235	110

Table 2. Variability of plasma biochemistries of Imperial Cormorant chicks according to sex, hatching order, age and breeding season. Nest identity (random factor) explained between <1% and 32% of the total variation of plasma biochemistries, and in all cases was not significant ($P > 0.05$). F-tests were used to assess the significance of the fixed factors included in the models.

	Triglycerides (mg/dl)			Total Protein (mg/dl)			Alkaline Phosphatase (IU/l)		
Fixed Factors	Mean ± SD	F	P-value	Mean ± SD	F	P-value	Mean ± SD	F	P-value
Hatching order									
A-chicks	140.6 ± 61.7	$F_{1,101} = 0.05$	0.83	2.9 ± 0.4	$F_{1,103} = 4.11$	0.045	1,802.8 ± 792.0	$F_{1,102} = 0.99$	0.32
B-chicks	36.3 ± 89.1			2.7 ± 0.3			1,934.5 ± 889.4		
Sex									
Female	145.1 ± 78.7	$F_{1,102} = 0.59$	0.44	2.8 ± 0.4	$F_{1,101} = 1.01$	0.32	1,810.9 ± 794.4	$F_{1,101} = 0.07$	0.79
Male	133.7 ± 55.4			2.8 ± 0.4			1,856.0 ± 838.9		
Breeding season									
2010	113.3 ± 56.7	$F_{1,104} = 11.70$	< 0.001	2.8 ± 0.4	$F_{1,104} = 24.7$	< 0.001	1,342.4 ± 581.5	$F_{1,104} = 53.30$	< 0.001
2011	158.9 ± 71.8			2.6 ± 0.4			2,267.2 ± 747.3		
Age		$F_{1,103} = 0.90$	0.34		$F_{1,102} = 2.30$	0.13		$F_{1,103} = 3.97$	0.049

Figure 1. Age-related variation in plasma levels of alkaline phosphatase activity (ALP) in Imperial Cormorant chicks. The regression lines (solid) and 95% confidence intervals (dotted line) are shown.

chemistries in Imperial Cormorant chicks from one of the largest colonies in Patagonia, Argentina. It also offers insights on the utility of physiological parameters as supplementary indicators and predictors of body condition and survival in this species. As expected, morphometric body condition indices varied according to hatching order (pre-fledging body condition also with sex), and were significantly lower in B-chicks (second hatched) that did not survive to fledging (compared with surviving B-chicks), confirming their applicability as indicators of individual body condition and predictors of chick survival. Likewise, biochemical parameters also varied with individual traits, yet much more subtly. Significant differences found between breeding seasons, age and hatching order explained a significant, but low proportion (< 10%) of the variation in ALP and total protein, respectively (total protein higher in A-chicks). All biochemical parameters were lower in non-surviving B-chicks (compared with surviving B-chicks), although these differences were significant only for total protein. Notwithstanding, the biochemical parameters assessed showed inconsistent applicability as indicators of body condition in Imperial Cormorant chicks. While triglycerides and total protein explained as much as 26% and 30%, respectively, of variation in current body condition (at the time of blood sampling), they did not

predict body condition at another moment (i.e., prior to fledging).

We found marked inter-annual variation in all biochemical parameters analyzed in Imperial Cormorant chicks. Although previous studies in the same colony showed that foraging behavior and feeding locations of breeding Imperial Cormorants are highly consistent within and between breeding seasons (Quintana *et al.* 2011; Harris *et al.* 2014), part of the inter-annual variability in chick chemistries may be explained by differential nutrient profiles of prey consumed by parents (see also González Miri and Malacalza 1999). Also contrary to our expectations, the breeding season that showed the highest mean values for plasma biochemistries (2011) coincided with that of lower productivity of the colony in terms of number of chicks fledged (based on average estimates over a period of 9 years; W. S. Svagelj and F. Quintana, unpubl. data). It is possible that repeated handling of chicks for morphometric measurements in 2010 may have affected their overall condition yet not to the extent of impacting survival or fledging. In 2011, handling of chicks was reduced to a single event, translating into a more robust individual physical condition, but insufficient for compensating for other unknown factors leading to colony-level lower fledging success. In absence of evidence for physiological causes of lower survivability in 2011, the use of an estimated morphometric index of body condition built from a single handling event seems to result in improved biochemical parameters of individuals. Nevertheless, because the survival of chicks in poor physical condition is generally low in bad breeding seasons with low food availability (Williams and Croxall 1990; Kato *et al.* 2001), parental favoritism toward a single chick in good condition could represent a strategy to ensure offspring in bad years (Kato *et al.* 2001). Thus, it seems possible that during the 2011 breeding season Imperial Cormorants would have made adjustments in parental investment to guarantee the independence of at least one chick in as good physiological condition as possible. Our data indicate that in 2011 body condition at the time of sampling in single A-chicks (mean = 44.8, SD = 129.6) tended to be higher than body condition of A-chicks from two-chick broods (mean = 10.7, SD = 84.8), although the difference was not significant ($F = 0.89$, $P = 0.35$). To further ascertain this hypothesis, future studies should include a larger number of two-chick nests and consider the "time with sibling" as a covariate in the analysis.

Regarding individual characteristics of chicks, age and hatching order showed a weak relationship with ALP and total protein levels, respectively (explained < 10% of the variation), whereas triglycerides did not differ with any of the intrinsic factors evaluated. ALP activity is considered a suitable marker for skeletal development in many altricial bird species as it increases during the growth stage (Viñuela *et al.* 1991; Tilgar *et al.* 2008). The small proportion of ALP variability explained by chick age in our study may be due to the narrow range in chick ages (16 to 35 days) in our sample, which also coincided with their highest growth period (Svagelj 2009). It may also be the result of opposite direction ALP variations caused by nutritional stress in deteriorating B-chicks as described below (Viñuela *et al.* 1991; Viñuela and Ferrer 1997).

The weak or nonexistent association of hatching order with total protein and triglycerides, respectively, is harder to understand unless fasting-related compensation mechanisms are considered (Alonso-Alvarez and Ferrer 2001). These biochemistries are affected by the qualitative and quantitative composition of the diet (Boismenu *et al.* 1992; Jenni-Eiermann and Jenni 1996; Alonso-Alvarez and Ferrer 2001). Therefore, differences according to hatching order and survival are anticipated, particularly in species like the Imperial Cormorant with brood reduction. However, in our study, hatching order explained a significant, but low percentage (3.4%) of the variation in total protein of chicks. This is likely due to underfed B-chicks using food protein as an energy source to avoid rapid depletion of limited fat reserves (Alonso-Alvarez and Ferrer 2001). In fact, sampled chicks that did not survive to independence were all B-chicks (most B- and all C-chicks died soon

Table 3. Morphometric body condition indices of Imperial Cormorant chicks according to sex and hatching order. Nest identity (random factor) explained 40% and 17% of the total variation of pre-fledging body condition and current body condition, respectively, and in all cases was not significant ($P > 0.05$). F-tests were used to assess the significance of the fixed factors included in the models.

	Pre-fledging Body Condition (g)			Current Body Condition (g)		
Fixed Factors	Mean ± SD	*F*	*P*-value	Mean ± SD	*F*	*P*-value
Hatching order						
A-chicks	1,640.0 ± 187.6	$F_{1,44} = 27.77$	< 0.001	32.9 ± 116.2	$F_{1,58} = 13.33$	< 0.001
B-chicks	1,317.4 ± 236.9			-127.4 ± 205.7		
Sex						
Female	1,528.4 ± 200.7	$F_{1,44} = 5.49$	0.02	-3.6 ± 155.5	$F_{1,57} = 0.08$	0.78
Male	1,605.5 ± 278.9			-1.1 ± 155.2		

after birth and were not sampled), and had significantly lower total protein yet only somewhat inferior triglyceride levels than survivor B-chicks. This matches what was seen by Alonso-Alvarez and Ferrer (2001) in Yellow-legged Gulls (*Larus cachinnans*) subject to experimental food restriction. Nonetheless, in our study the predictive power of total protein was relatively poor (i.e., it explained < 15% of B-chick survival). This most likely reflects that we sampled B-chicks before they had reached sufficiently advanced physical deterioration (i.e., death by starvation) (Jenni Eiermann and Jenni 1997; Alonso-Alvarez *et al.* 2002, 2003; Milner *et al.* 2003). Notwithstanding, we also found that ALP activity tended to be lower in non-surviving vs. survivor B-chicks, something that was likewise seen in fasting Yellow-legged Gulls in the studies described above (Alonso-Alvarez and Ferrer 2001).

While chosen biochemical parameters reflected the state of chicks at the time of sampling relatively well, they largely failed to suggest the most possible fate of individuals. Our results imply that either the time of sampling of B-chicks (not near enough terminal life-stages) or our small sample size impaired our capacity to identify differences in biochemical parameters of sufficient magnitude to ascertain their potential as predictors of survival to fledging (Nadolski *et al.* 2006). Assessing other parameters more closely linked to muscle catabolism and starvation (i.e., ketones, uric acid) would have had a higher prognostic value than the ones chosen in our study (Alonso-Alvarez and Ferrer 2001). While body mass steadily declines with food restriction and fasting, changes in biochemical parameters are masked by compensatory mechanisms until they reach a critical state (Alonso-Alvarez and Ferrer 2001). Further investigation, including a larger number of chicks in various stages of deteriorating condition and a broader set of

Table 4. Morphometric body condition indices and plasma biochemistries of Imperial Cormorant B-chicks (second hatched) according to survival. Pre-fledging body condition = estimated body mass at 30 days old. Current body condition = body mass corrected by tarsus length at the time of blood sampling.

	Surviving	Non-surviving	
Parameter (units)	Mean ± SD	Mean ± SD	Significance
Pre-fledging body condition (g)	1,467.2 ± 212.0	1,137.6 ± 100.5	$\chi^2_1 = 5.58$ $P = 0.02$
Current body condition (g)	-49.5 ± 156.0	-302.8 ± 212.5	$\chi^2_1 = 8.33$ $P = 0.004$
Triglycerides (mg/dl)	148.3 ± 97.1	116.3 ± 74.7	$\chi^2_1 = 0.80$ $P = 0.37$
Total protein (mg/dl)	2.8 ± 0.3	2.5 ± 0.3	$\chi^2_1 = 4.66$ $P = 0.03$
Alkaline phosphatase (IU/l)	2,021.1 ± 895.3	1,790.2 ± 913.3	$\chi^2_1 = 0.41$ $P = 0.52$

Table 5. Relationship between biochemical parameters and morphometric body condition indices of Imperial Cormorant chicks. Hatching order was included as covariate in all models, and sex only in those with pre-fledging body condition as response variable. Pre-fledging body condition = estimated body mass at 30 days old. Current body condition = body mass corrected by tarsus length at the time of blood sampling. Nest identity (random factor) explained 15% and 54% of the total variation of pre-fledging body condition and current body condition, respectively, and in all cases was not significant ($P > 0.05$). F-tests were used to assess the significance of the fixed factors included in the models.

	Pre-fledging Body Condition			Current Body Condition		
	Estimate ± SE	*F*	*P*	Estimate ± SE	*F*	*P*
Triglycerides	0.34 ± 0.50	$F_{1,43} = 0.44$	0.51	0.65 ± 0.24	$F_{1,56} = 6.89$	0.011
Total protein	4.15 ± 6.67	$F_{1,43} = 0.39$	0.54	235.54 ± 74.22	$F_{1,56} = 10.07$	0.002
Alkaline phosphatase	-0.01 ± 0.05	$F_{1,43} = 0.04$	0.83	$-6.59^{-03} \pm 2.53^{-0.2}$	$F_{1,56} = 0.07$	0.80

plasma biochemistries, is required to confirm the reliability of the latter to forecast Imperial Cormorant chick survival.

On the other hand, body condition indices performed extremely well, both as indicators of current status as well as in predicting survival to fledging. A high and significant percentage (19-33%) of the variation in morphometric indices of body condition was explained by hatching order. Moreover, body condition was significantly lower in B-chicks that did not survive to independence compared to surviving B-chicks (models including morphometric indices explained 35-55% of chick survival outcome). Morphometric parameters have been shown to be correlated with post-fledging survival of chicks, as well as recruitment probability, in a wide variety of species (Bowers *et al.* 2014). Our results confirm their usefulness in assessing individual body condition and predicting chick survival in Imperial Cormorants. Furthermore, our results are consistent with Svagelj (2009), who indicated that hatching asynchrony generates asymmetry in body size and motor skills between siblings, favoring first hatched chicks (A-chicks), and thus determining Imperial Cormorant chick survival. Similarly, only pre-fledging body condition (estimated by body mass at 30 days old) varied according to sex (males heavier). This result coincides with previous findings concerning sexual dimorphism in body mass of Imperial Cormorant chicks that occurs after 15 days of age and peaks at 20 days (Svagelj 2009).

Overall, the plasma biochemistries assessed in our study showed inconsistent applicability as indicators of body condition in Imperial Cormorant chicks. Triglycerides and total protein accurately reflected chick body condition at the time of sampling, explaining

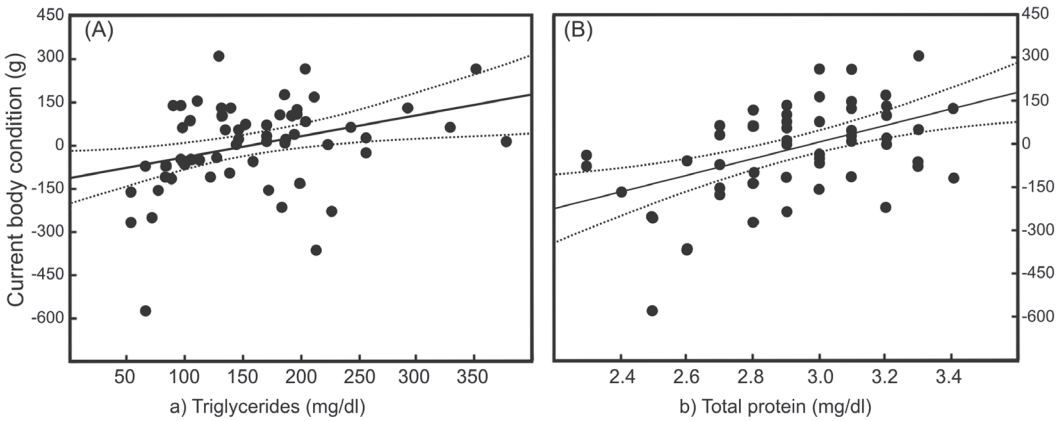

Figure 2. Relationship between biochemical parameters and current body condition of Imperial Cormorant chicks. This was measured by body mass adjusted for the tarsus length at the time of blood sampling. Triglycerides (A) and total protein (B) reflected 26% and 30% of variation in current body condition, respectively.

26% and 30% of variation, respectively, but did not foretell the condition of individuals at another moment (i.e., prior to fledging). Only total protein signaled B-chicks that would not survive. This is most likely linked to the capacity of food-deprived chicks to compensate their failing health until near death as detailed above, and our lack of terminal stage B-chick samples to assess this more thoroughly. On the other hand, it is also likely that the parameters chosen were incapable of detecting changes associated with catabolism and emaciation. The insufficient clarity on which biochemical parameters are the best indicators (higher predictive value) of body condition in chicks suggests that not all parameters are relevant to all species or contexts (Villegas *et al.* 2002; Minias and Kaczmarek 2013). Thus, appropriate validation in the study species is a necessary step before they may be applied in ecological studies. Further studies would benefit from selecting a broader scope of parameters as well as considering a multivariate approach to condense the information of biochemical parameters into a scalar variable.

ACKNOWLEDGMENTS

The authors gratefully acknowledge M. Di Martino, P. Giudici, A. Gómez Laich, S. Harris, V. Rago, R. Saenz Samaniego and G. Somoza for their contributions in the field and laboratory analysis. Permits were granted by Subsecretaría de Turismo y Áreas Protegidas (Nº 140–SSTyAP/10, N° 247-SSTyAP/11) and Dirección de Fauna y Flora Silvestre of Chubut Province (Nº 24/2010, Nº 98/2011 DFyFS-SSRN). This study was funded by the Wildlife Conservation Society, Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET), and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT). L. Gallo was supported by a doctoral fellowship from CONICET. Our methods meet all ethical guidelines for the use of wild birds in research, as stipulated by the standards and policies of the Government of Argentina.

LITERATURE CITED

Acevedo-Whitehouse, K. and A. L. J. Duffus. 2009. Effects of environmental change on wildlife health. Philosophical Transactions of the Royal Society B: Biological Sciences 364: 3429-3438.

Alonso-Alvarez, C. and M. Ferrer. 2001. A biochemical study of fasting, subfeeding, and recovery processes in yellow-legged gulls. Physiological and Biochemical Zoology 74: 703-713.

Alonso-Alvarez, C., M. Ferrer and A. Velando. 2002. The plasmatic index of body condition in the Yellow-legged Gulls *Larus cachinnans*: a food controlled experiment. Ibis 144: 147-149.

Alonso-Alvarez, C., M. Ferrer, J. Viñuela and J. A. Amat. 2003. Plasma chemistry of the chinstrap penguin *Pygoscelis antarctica* during fasting periods: a case of poor adaptation to food deprivation? Polar Biology 26: 14-19.

Amat, J. A., F. Hortas, G. M. Arroyo, M. A. Rendón, J. M. Ramírez, M. Rendón-Marto, A. Pérez-Hurtado and A. Garrido. 2007. Interannual variations in feeding frequencies and food quality of greater flamingo chicks (*Phoenicopterus roseus*): evidence from plasma chemistry and effects on body condition. Comparative Biochemistry and Physiology Part A 147: 569-576.

Boismenu, C., G. Gauthier and J. Larochelle. 1992. Physiology of prolonged fasting in greater snow geese (*Chen caerulescens atlantica*). Auk 109: 511-521.

Bowers, E. K., C. J. Hodges, A. M. Forsman, L. A. Vogel, B. S. Masters, B. G. Johnson, L. S. Johnson, C. F. Thompson and S. K. Sakaluk. 2014. Neonatal body condition, immune responsiveness, and hematocrit predict longevity in a wild bird population. Ecology 95: 3027-3034.

Crawley, M. J. 2007. The R book, 2nd ed. John Wiley and Sons Ltd, Chichester, U.K.

Dawson, R. D. and G. R. Bortolotti. 1997. Total plasma protein level as an indicator of condition in wild American kestrels (*Falco sparverius*). Canadian Journal of Zoology 75: 680-686.

Fair, J., S. Whitaker and B. Pearson. 2007. Sources of variation in haematocrit in birds. Ibis 149: 535-552.

Ferrer, M. and P. Dobado-Berrios. 1998. Factors affecting plasma chemistry values of the Spanish Imperial Eagle, *Aquila adalberti*. Comparative Biochemistry and Physiology Part A 120: 209-217.

Frere, E., F. Quintana and P. Gandini. 2005. Cormoranes de la costa patagónica: estado poblacional, ecología y conservación. Hornero 20: 35-52. (In Spanish).

González Miri, L. and V. Malacalza. 1999. Perfil nutricional de las principales especies en la dieta del Cormorán Real (*Phalacrocorax albiventer*) en Punta León (Chubut, Argentina). Ornitología Neotropical 10: 55-59. (In Spanish).

Harris, S., A. Raya Rey, R. A. Phillips and F. Quintana. 2013. Sexual segregation in timing of foraging by Imperial Shags (*Phalacrocorax atriceps*): is it always ladies first? Marine Biology 160: 1249-1258.

Harris, S., A. Raya Rey, C. Zavalaga and F. Quintana. 2014. Strong temporal consistency in the individual foraging behaviour of Imperial Shags *Phalacrocorax atriceps*. Ibis 156: 523-533.

Horak, P., L. Saks, I. Ots and H. Kollist. 2002. Repeatability of condition indices in captive greenfinches (*Carduelis chloris*). Canadian Journal of Zoology 80: 636-643.

Jenni-Eiermann, S. and L. Jenni. 1996. Metabolic differences between the post breeding, moulting and migratory periods in feeding and fasting passerine birds. Functional Ecology 10: 62-72.

Jenni-Eiermann, S. and L. Jenni. 1997. Diurnal variation of metabolic responses to short term fasting in passerine birds during the postbreeding, molting and migratory period. Condor 99: 113-122.

Jenni-Eiermann, S. and L. Jenni. 1998. What can plasma metabolites tell us about metabolism, physiological state and condition of individual birds? An overview. Biological Conservation Fauna 102: 312-319.

Jerzak, L., T. H. Sparks, M. Kasprzak, M. Bochenski, P. Kaminski, E. Wiśniewska, S. Mroczkowski and P. Tryjanowski. 2010. Blood chemistry in white stork *Ciconia ciconia* chicks varies by sex and age. Comparative Biochemistry and Physiology Part B 156: 144-147.

Kaneko, J. J, J. W. Harvey and M. L. Bruss (Eds.). 2008. Clinical biochemistry of domestic animals, 6th ed. Academic Press, San Diego, California.

Kato, A., Y. Watanuki and Y. Naito. 2001. Foraging and breeding performance of Japanese cormorants in relation to prey type. Ecological Research 16: 745-758.

Labocha, M. K. and J. P. Hayes. 2012. Morphometric indices of body condition in birds: a review. Journal of Ornithology 153: 1-22.

Masello, J. F. and P. Quillfeldt. 2004. Are haematological parameters related to body condition, ornamentation and breeding success in wild burrowing parrots *Cyanoliseus patagonus*? Journal of Avian Biology 35: 445-454.

Merilä, J. and E. Svensson. 1995. Fat reserves and health state in migrant goldcrest *Regulus regulus*. Functional Ecology 9: 842-848.

Milner, J. M., A. Stien, R. J. Irvine, S. D. Albon, E. Langvatn and E. Ropstad. 2003. Body condition in Svalbard reindeer and the use of blood parameters as indicators of condition and fitness. Canadian Journal of Zoology 81: 1566-1578.

Minias, P. and K. Kaczmarek. 2013. Concentrations of plasma metabolites as predictors of nestling condition in the Great Cormorant (*Phalacrocorax carbo sinensis*). Ornis Fennica 90: 142-150.

Minias, P., K. Kaczmarek, T. Janiszewski and J. Markowski. 2013. Hematology and plasma biochemistry values of great cormorant (*Phalacrocorax carbo sinensis*) nestlings. Journal of Wildlife Diseases 49: 194-196.

Mock, D. and G. Parker. 1997. The evolution of sibling rivalry. Oxford University Press, Oxford, U.K.

Muriel, M., D. Schmidt, C. P. Calabuig, J. Patino-Martinez and M. Ferrer. 2013. Factors affecting plasma biochemistry parameters and physical condition of Osprey (*Pandion haliaetus*) nestlings. Journal of Ornithology 154: 619-632.

Nadolski, J., J. Skwarska, A. Kaliński, M. Bańbura, R. Śniegula and J. Bańbura. 2006. Blood parameters as consistent predictors of nestling performance in great tits (*Parus major*) in the wild. Comparative Biochemistry and Physiology Part A 143: 50-54.

Navarro, J., J. González-Solís and G. Viscor. 2007. Nutritional and feeding ecology in Cory's shearwater *Calonectris diomedea* during breeding. Marine Ecology Progress Series 351: 261-271.

Norte, C. A., B. Sheldon, J. Paulo Sousa and J. Albino Ramos. 2008. Repeatability and method-dependent variation of blood parameters in wild-caught Great Tits *Parus major*. Acta Ornitholica 43: 65-75.

Norte, C. A., J. A. Ramos, J. P. Sousa and B. C. Sheldon. 2009a. Variation of adult Great Tit *Parus major* body condition and blood parameters in relation to sex, age, year and season. Journal of Ornithology 150: 651-660.

Norte, C. A., B. C. Sheldon, J. P. Sousa and J. A. Ramos. 2009b. Environmental and genetic variation in body condition and blood profile of great tit *Parus major* nestlings. Journal of Avian Biology 40: 157-165.

Oddie, K. R. 2000. Size matters: competition between male and female great tit offspring. Journal of Animal Ecology 69: 903-912.

O'Dwyer, T. W., W. A. Buttemer, D. M. Priddel and J. A. Downing. 2006. Prolactin, body condition and the cost of good parenting: an interyear study in a long-lived seabird, Gould's Petrel (*Pterodroma leucoptera*). Functional Ecology 20: 806-811.

Pinheiro, J. C. and D. M. Bates. 2000. Mixed-effects models in S and S-Plus. Springer, Berlin, Germany.

Platteeuw, M., K. Koffijberg and W. Dubbeldam. 1995. Growth of cormorant *Phalacrocorax carbo sinensis* chicks in relation to brood size, age ranking and parental fishing effort. Ardea 83: 235-245.

Quintana, F., G. López and G. Somoza. 2008. A cheap and quick method for DNA-based sexing of birds. Waterbirds 31: 485-488.

Quintana, F., R. Wilson, P. Dell'Arciprete, E. Shepard and A. Gomez Laich. 2011. Women from Venus, men from Mars: inter-sex foraging differences in the imperial cormorant *Phalacrocorax atriceps* a colonial seabird. Oikos 120: 350-358.

R Development Core Team. 2013. R: a language and environment for statistical computing v. 2.14.3. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/, accessed 12 October 2013.

Seiser, P. E., L. K. Duffy, A. D. McGuire, D. D. Roby, G. H. Golet and M. A. Litzow. 2000. Comparison of pigeon guillemot, *Cepphus columba*, blood parameters from oiled and unoiled areas of Alaska eight years after the Exxon Valdez oil spill. Marine Pollution Bulletin 40: 152-164.

Svagelj, W. S. 2009. Ecología reproductiva de aves marinas dimórficas en relación a las teorías de inversión parental y proporción de sexos en las nidadas. Ph.D. Dissertation, Universidad de Buenos Aires, Buenos Aires, Argentina. (In Spanish).

Svagelj, W. S. and F. Quintana. 2007. Sexual size dimorphism and sex determination by morphometric measurements in breeding imperial shags (*Phalacrocorax atriceps*). Waterbirds 30: 97-102.

Svagelj, W. S. and F. Quintana. 2011a. Breeding performance of the Imperial Shag (*Phalacrocorax atriceps*) in relation to year, laying date and nest location. Emu 111: 162-165.

Svagelj, W. S. and F. Quintana. 2011b. Egg-size variation in the Imperial Cormorant: on the importance of individual effects. Condor 113: 528-537.

Tilgar, V., P. Kilgas, M. Mägi and R. Mänd. 2008. Age-related changes in the activity of bone alkaline phosphatase and its application as a marker of prefledgling maturity of nestlings in wild passerines. Auk 125: 456-460.

Tjørve, E. and K. M. C. Tjørve. 2010. A unified approach to the Richards-model family for use in growth analyses: why we need only two model forms. Journal of Theoretical Biology 267: 417-425.

Villegas, A., J. M. Sánchez, E. Costillo and C. Corbacho. 2002. Blood chemistry and haematocrit of the black vulture (*Aegypius monachus*). Comparative Biochemistry and Physiology Part A 132: 489-497.

Viñuela, J. and M. Ferrer. 1997. Regulation of growth in red kites and imperial eagles. Wilson Bulletin 109: 92-101.

Viñuela, J., M. Ferrer and F. Recio. 1991. Age-related variations in plasma levels of alkaline phosphatase, calcium and inorganic phosphorus in chicks of two species of raptors. Comparative Biochemistry and Physiology Part A 99: 49-54.

Williams, T. D. and J. P. Croxall. 1990. Is chick fledging weight a good index of food availability in seabird populations? Oikos 59: 414-416.