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

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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.

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

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-

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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 x 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 (χ² = 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 bio-
chemistries (triglycerides-total protein: \( r = 0.44, P < 0.001 \); total protein-ALP: \( r = 0.43, P < 0.001 \); ALP-triglyc-
erides: \( r = 0.10, P = 0.51 \)), 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, remov-
ing 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 pa-
rameters 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 or-
der 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 \(( r^2 = 0.068; \text{Fig. 1})\), 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 \(( \text{mean} = 1,565, \text{SD} = 241; n = 47)\), 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 sam-
ping (current body condition) ranged from 
-575.7 to 310.3 g \(( \text{mean} = -2.4, \text{SD} = 154; n = 59)\), with negative values reflecting lower 
body mass than expected for tarsus length. 
This parameter also varied with hatching or-
der, 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 differ-
ences 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 (Ta-
ble 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 sig-
nificant relationship with the estimated 
pre-fledging body condition (Table 5). How-
ever, triglycerides and total protein levels 
explained 26.1\% and 29.7\% of the variance, 
respectively, of the current body condition 
(Table 5; \text{Fig. 2}). ALP activity was not a good 
indicator of current body condition, result-
ing in no significant relationship between 
the two parameters (Table 5).

**Discussion**

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

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**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 \( P < 0.05 \)).**

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

<table>
<thead>
<tr>
<th>Fixed Factors</th>
<th>Triglycerides (mg/dl)</th>
<th>Mean ± SD</th>
<th>F</th>
<th>P-value</th>
<th>Total Protein (mg/dl)</th>
<th>Mean ± SD</th>
<th>F</th>
<th>P-value</th>
<th>Alkaline Phosphatase (IU/l)</th>
<th>Mean ± SD</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatch order</td>
<td>A-chicks</td>
<td>145.6 ± 61.7</td>
<td>F1,101 = 0.05</td>
<td>0.83</td>
<td>2.9 ± 0.4</td>
<td>F1,102 = 1.01</td>
<td>0.32</td>
<td>1,802.8 ± 792.0</td>
<td>F1,102 = 0.99</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B-chicks</td>
<td>36.5 ± 89.1</td>
<td>F1,102 = 2.30</td>
<td>0.13</td>
<td>2.8 ± 0.4</td>
<td>F1,102 = 2.47</td>
<td>0.049</td>
<td>1,934.5 ± 889.4</td>
<td>F1,102 = 0.97</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>145.1 ± 78.7</td>
<td>F1,101 = 1.01</td>
<td>0.32</td>
<td>2.8 ± 0.4</td>
<td>F1,102 = 2.47</td>
<td>0.93</td>
<td>1,856.0 ± 889.4</td>
<td>F1,102 = 0.97</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>183.2 ± 55.4</td>
<td>F1,101 = 0.59</td>
<td>0.44</td>
<td>2.8 ± 0.4</td>
<td>F1,102 = 2.47</td>
<td>0.93</td>
<td>1,856.0 ± 889.4</td>
<td>F1,102 = 0.97</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding season</td>
<td>2010</td>
<td>113.3 ± 56.7</td>
<td>F1,104 = 11.70</td>
<td>&lt; 0.001</td>
<td>2.8 ± 0.4</td>
<td>F1,104 = 24.7</td>
<td>&lt; 0.001</td>
<td>1,934.5 ± 889.4</td>
<td>F1,104 = 53.30</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>158.9 ± 71.8</td>
<td>F1,104 = 11.70</td>
<td>&lt; 0.001</td>
<td>2.8 ± 0.4</td>
<td>F1,104 = 24.7</td>
<td>&lt; 0.001</td>
<td>1,934.5 ± 889.4</td>
<td>F1,104 = 53.30</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>10</td>
<td>145.1 ± 78.7</td>
<td>F1,104 = 11.70</td>
<td>&lt; 0.001</td>
<td>2.8 ± 0.4</td>
<td>F1,104 = 24.7</td>
<td>&lt; 0.001</td>
<td>1,934.5 ± 889.4</td>
<td>F1,104 = 53.30</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>158.9 ± 71.8</td>
<td>F1,104 = 11.70</td>
<td>&lt; 0.001</td>
<td>2.8 ± 0.4</td>
<td>F1,104 = 24.7</td>
<td>&lt; 0.001</td>
<td>1,934.5 ± 889.4</td>
<td>F1,104 = 53.30</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
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 Malarcaza 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
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>
<thead>
<tr>
<th>Fixed Factors</th>
<th>Pre-fledging Body Condition (g)</th>
<th>Current Body Condition (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>F</td>
</tr>
<tr>
<td>Hatching order</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-chicks</td>
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<td>F = 27.77</td>
</tr>
<tr>
<td>B-chicks</td>
<td>1,317.4 ± 236.9</td>
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<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Female</td>
<td>1,528.4 ± 200.7</td>
<td>F = 5.49</td>
</tr>
<tr>
<td>Male</td>
<td>1,605.5 ± 278.9</td>
<td></td>
</tr>
</tbody>
</table>

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.

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.
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
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.

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Literature Cited


