



Accounting for food availability reveals contaminant-induced breeding impairment, food-modulated contaminant effects, and endpoint-specificity of exposure indicators in free ranging avian populations



Jabi Zabala ^{a,b,*}, Ignacio Rodríguez-Jorquera ^{a,c}, Joel C. Trexler ^{d,e}, Sophie Orzechowski ^a, Lindsey Garner ^a, Peter Frederick ^a

^a Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL, USA

^b Department of Zoology and Animal Cell Biology, University of the Basque Country, UPV/EHU, C/Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Araba, Basque Country, Spain

^c Centro de Humedales Río Cruces (CEHUM), Universidad Austral de Chile, Valdivia, Chile

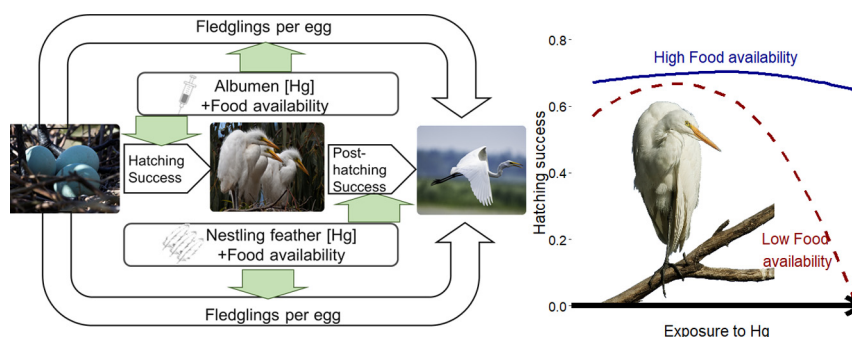
^d Department of Biological Sciences, Florida International University, Miami, FL, USA

^e Coastal & Marine laboratory, Florida State University, St. Teresa, FL, USA

HIGHLIGHTS

- Including food availability revealed widespread Hg-breeding endpoint associations.
- 75% of associations (9/12) were only detected when accounting for food availability.
- The influence of food was interactive with, rather than additive to, Hg.
- Albumen [Hg] explained more variation in hatching success, feather [Hg] in later endpoints.
- High food availability appeared to reduce deleterious effects of Hg.

GRAPHICAL ABSTRACT



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ABSTRACT

It remains unclear how sub-lethal effects of contaminants play out in relation to other stressors encountered by free-ranging populations. Effects may be masked or influenced by interactions with field stressors such as food availability. We predicted that (1) including food availability, and particularly its interaction with Hg, would reveal or enhance associations between Hg and breeding endpoints. We further predicted that (2) breeding impairment associated with Hg would be higher under food stress conditions. We monitored Hg and nest success of great egrets (*Ardea alba*) in eight breeding colonies in the Florida Everglades over 11 years. We characterized variation in local food availability among colonies and years using fish biomass and recession range—a proxy to fish vulnerability. We used two Hg exposure indicators (egg albumen Hg and nestling feather Hg) and six breeding endpoints (clutch-size, brood-size, fledged-size, hatching success, post-hatching success and fledglings per egg) to assess whether variation in food availability influenced associations between Hg and these endpoints. Accounting for interactions between Hg and food availability, we identified statistically significant associations in all 12 indicator-endpoint combinations, while only three were detectable without food. Further, 10 combinations showed interactions between Hg and components of food availability. Our results also indicated an endpoint-specific affinity, with albumen [Hg] explaining more variation in hatching success while nestling feather [Hg] explained more variation in post-hatching survival. Both Hg indicators accounted for relevant

* Corresponding author.

E-mail address: j.zabala@ehu.eus (J. Zabala).

(6–10%) amounts of variation in fledglings produced per egg laid, an integrative endpoint. Increased Hg exposure resulted in overall reduced reproductive success when food availability was low, but our models predicted low or no effects of increasing Hg exposure when food availability was high. Our results indicate that Hg induced impairment is strongly driven by food availability, providing a framework that accommodates previously contradictory results in the literature.

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1. Introduction

Pollution of natural systems by chemical contaminants as a consequence of human activities has reached all areas of the globe, and field concentrations of many pollutants and heavy metals are known to reach levels that could cause sub-lethal effects (Loganathan, 2011). Dose-dependent sub-lethal effects for some pollutants have been well delineated in laboratory conditions, and these highly controlled studies have also been invaluable in demonstrating the physiological mechanisms involved in animals. However, effects in natural environmental conditions, where other natural stressors occur, are not fully understood. Impairment due to exposure might make animals more sensitive to environmental stressors, and this relationship could result in strong net effects. Similarly, individuals experiencing harsher environmental conditions or under environmental stress could be more sensitive to the effects of pollutants (Bustnes et al., 2006; Holmstrup et al., 2010; Sih et al., 2004; Tartu et al., 2014). For instance, survival of *Hyla versicolor* tadpoles exposed to pesticides was severely reduced if they were also exposed to predator cues but with no real predation risk (Relyea and Mills, 2001). Interactions between exposure to pollutants and nutritional status have been proposed and reported in animal populations (Bustnes et al., 2015, 2008, 2005; Holmstrup et al., 2010). A review of interactions between toxic chemicals and natural stressors (temperature, moisture and dissolved oxygen) found significant interactions in 62.3% of the 61 experimental studies examined (Laskowski et al., 2010).

Variation in food availability is probably the most relevant stressor for animals in natural settings, and it may have direct or indirect influences on growth, breeding, immune response, health and survival (Demas and Nelson, 1998; Hoi-Leitner et al., 2001; Ostfeld and Keesing, 2000). The influence of food on breeding success and other traits is well known when there is marked inter-annual variation in the production or availability of food resources (Ostfeld and Keesing, 2000). Surprisingly, studies assessing interactions between pollutants and natural stressors have largely overlooked the possible influence of fluctuating food availability (Holmstrup et al., 2010). - To our best knowledge, a single experimental study assessed food and contaminant interactions in birds (Keith and Mitchell, 1993). That study found significant negative interactive effects of chemical contaminants and food stress in ovary weight and oviduct weight. They also reported evidence suggesting negative interactive effects of food restriction and contaminant exposure in breeding success, but they could not test these results for statistical significance (Keith and Mitchell, 1993). A possible reason for the lack of field studies assessing interactions between contaminants and variation in food availability is the difficulty of assessing food availability in the field. Most ecological studies use food abundance as a proxy for food availability, but prey availability may often be poorly related to abundance (Andruskiw et al., 2008; Gawlik, 2002; Quinn and Cresswell, 2004).

Mercury (Hg) is a global contaminant whose concentration in the environment increased markedly in the last century as a consequence of anthropogenic activities and whose organic form methylmercury (MeHg) is readily absorbed and retained by organisms and biomagnifies, especially in aquatic food webs (Scheuhammer et al., 2007; Slemr and Langer, 1992; Vo et al., 2011). Predatory species, particularly aquatic top predators such as piscivorous birds, are often exposed to high concentrations of Hg because of biomagnification. Effects of Hg

at sub-lethal concentrations include neurotoxic, immunotoxic, and genotoxic effects, endocrine disruption, reproductive impairment, and reduced growth and body condition, among others (Tan et al., 2009; Whitney and Cristol, 2017; Wolfe et al., 1998). Effects levels have been established for fish and birds in laboratory conditions where food availability and other environmental stressors are controlled (Frederick and Jayasena, 2011; Fuchsmann et al., 2017; Varian-Ramos et al., 2014). Effects of Hg in natural settings are less understood and may be more complex in natural systems. For instance, contrary to predictions, studies in two avian species reported better body or physiological condition in individuals or years with higher Hg concentration ([Hg] hereafter, Herring et al., 2009; Provencher et al., 2016). In tree swallows (*Tachycineta bicolor*) reproductive impairment associated with Hg was only observed during unusually high temperatures, with no detectable effect at cooler temperatures (Hallinger and Cristol, 2011). Another study with the same species only detected reduced reproductive success associated with Hg in young females breeding for the first time, which were arguably impaired due to inexperience (Brasso and Cristol, 2008). These results suggest the presence of interactions between uncontrolled stressors and Hg contamination.

We hypothesized that sub-lethal effects of field exposure to Hg in free ranging vertebrates are modulated by environmental stressors, particularly food availability. Thus, our null hypothesis is that Hg effects are independent of food availability. We predicted that accounting for variation in food availability among breeding areas and years would improve or reveal associations between reproductive endpoints and exposure to Hg in wild birds. Based on reported interactive effects of other contaminants and environmental stressors, and the apparent interaction between food stress and contaminants reducing bird success in experimental exposures (Keith and Mitchell, 1993), we further predicted that the influence of Hg would be interactive with food availability rather than additive, and that effects of the same exposure to Hg would be stronger when birds are under nutritional stress from low food availability conditions. Thus, we predicted that differences in food availability would modify the slope of the association between Hg exposure and breeding endpoints, and therefore modulate the intensity of Hg effects. Finally, we predicted the degree of breeding impairment associated with Hg would be higher under food stress.

To test these predictions, we analyzed reproductive responses to Hg exposure with and without accounting for variation in food availability. We were able to characterize both main components of food availability: food abundance (fish biomass, >130 sites sampled annually) and food vulnerability (surface water drying trends, >120 gaging station measurements) by breeding location. To test the prediction that food availability modulates sub-lethal Hg effects we employed a nest-approach using data from two years of individual great egret (*Ardea alba*) nest exposure and success to Hg assessed with two indicators (albumen and nestling feathers) and six different nest-level endpoints. To test the prediction that low food availability intensifies Hg impairment we used a colony-approach (individual nest success and average exposure in the colony), within 11 years of great egret nesting success during which food availability and Hg exposure varied temporally and geographically. We examined associations between three breeding endpoints while accounting for interactions with food availability. Finally, we used the best model of each endpoint to predict its values under contrasting food availability conditions.

2. Materials and methods

2.1. Study area and species

We monitored breeding success of great egrets in the Everglades of Dade, Broward and Monroe counties, Florida, USA. Great egrets are piscivorous and their breeding success is influenced by local food availability (Beerens et al., 2011; Powell, 1983). Great egrets establish breeding colonies on tree islands set widely apart (2–15 km) within the extensive graminoid Everglades wetland (9200 km²) where they are exposed to geographically and temporally variable Hg primarily through food (Frederick et al., 2002; Zabala et al., 2019b).

Food availability for piscivorous birds in the Everglades is influenced by fish abundance at the start of the breeding season and the rates of surface water recession, which concentrates fish in small depressions and make them vulnerable to capture (Beerens et al., 2015; Botson et al., 2016; Frederick et al., 2002). As water recession progresses during the November – May dry season, a continuous supply of shallow pools become exposed that contain concentrated fish. The recession is interrupted either by a seasonal rainfall pulses in the dry season, or by the onset of the rainy season, (June–July). In both cases, water levels rise and prey disperse, becoming much less easy for birds to capture (Botson et al., 2016; Gawlik, 2002; Lorenz, 2014).

2.2. Monitoring reproductive success

Beginning in 1998 we monitored great egret breeding success and Hg exposure in 5–10 colonies annually. We selected colonies based on location to ensure a geographically representative sample of breeding and Hg conditions within the study area (Frederick et al., 2002; Herring et al., 2013). We could not monitor the same colonies every year because some colonies were not used by birds every year or water levels did not allow access to some of them. In selected colonies (Fig. S1) we marked great egret nests within 4 m of a trail oriented from edge to center of the colony, with uniquely numbered flagging, and monitored nests during weekly visits recording number of eggs and nestlings at each nest. We visually detected nests by searching for nest platforms (0.5–1 m diameter) within the 4-m band around the trail. Lower detection of clutches that fail early can bias estimates of hatching success, particularly when presence of adults is used as the main cue to detect nests (Shaffer, 2004). In our case, nest detection was based on the weekly transect visits looking for nest platforms rather than adults, and we visited colonies as soon as pairs were settled and egg laying started. Great egret nesting is relatively synchronous and new nests were marked along transects as eggs were laid on existing nest platforms. To examine the possible influence of differential detectability of nests of different ages, we compared our raw estimates of hatching success with those that were generated using reproductive success pro-rated for exposure days (Mayfield estimates; Mayfield, 1961).

We considered nestlings to be successfully fledged if they survived to 21 days of age. Beyond that age, great egret young are highly mobile, and can no longer be reliably associated with particular nests. We considered six breeding endpoints defined as (1) clutch size: maximum number of eggs counted in a nest; (2) brood size: the maximum number of live young counted in the nest; (3) fledglings per nest: the number of nestlings surviving at least to the age of 21 days in the nest; (4) hatching success: proportion of eggs in a clutch that resulted in hatchlings; (5) post-hatching success: proportion of hatched nestlings that survive to fledgling stage (>21 days of age); and (6) fledglings per egg: proportion of eggs that resulted in successfully fledged offspring. We also analyzed correlations among endpoints to better understand our results.

2.3. Hg sample collection and determination

We measured Hg exposure in both nestling feathers and albumen. For nestling feathers, we chose growing or fully grown scapular nestling

feathers as a sampling medium because 1) they are among the first, and the largest body feathers that nestlings grow, 2) in this species, they strongly correlate with cumulative Hg intake during the nestling period (Spalding et al., 2000), 3) their [Hg] does not vary with age at sampling or among siblings (Zabala et al., 2019a), and 4) they are easy and quick to collect and less invasive than other methods. We randomly selected a minimum of 10 nests that successfully raised nestlings and when these were 20–28 days of age we collected 10 fully-grown feathers from the scapular area of the largest chick in the nest (Zabala et al., 2019a). Therefore, we assumed that nestling feathers approximate total Hg intake during the nestling phase. On the other hand, we assumed albumen [Hg] to come from maternal transfer (Ackerman et al., 2016) and represent parental Hg exposure and *in ovo* embryo exposure. In the colony-approach, we assumed that colony averaged albumen [Hg] and nestling feather [Hg] approximate overall Hg exposure in that colony in that breeding season. Before 2015, we sampled any nests with chicks of an appropriate age within the colony. From 2015 onwards we sampled nestling feathers mostly from nests monitored for reproductive success. In 2015, 2016 and 2017, we sampled thin albumen from eggs and nestling feathers in a randomly selected subset of the nests monitored for reproductive success (Zabala et al., 2019c, 2019b). We microsampled thin albumen (0.3–0.6 ml.) from the second laid egg in each nest during early incubation using a nondestructive technique (Stebbins et al., 2009). Thus, in many cases we had both feather and albumen information from the same nest.

In the field, we kept albumen samples refrigerated after extraction and froze them later the same day for storage until analysis. We stored dry feathers in sealed paper envelopes until analysis. Before analysis we washed feathers with deionized water to remove dirt and other non-feather materials from the surface and then oven dried them for 48 h at 65 °C. Prior to 2014, we sent nestling feather samples to the Florida Department of Environmental Protection Chemistry Section for [Hg] determination (see Sup. Mat. for further details on their methods). From 2014 onward, we measured mass of samples with a precision scale (Mettler Toledo AG204; 0–210 g ± 0.1 mg), and determined Total [Hg] using a Direct Mercury Analyzer (Milestone DMA 80; detection limit 0.001 ng Hg). We assumed the majority of Hg was in the form of MeHg (Ackerman et al., 2013; Spalding et al., 2000), but all samples are reported as total Hg [THg] on a wet weight (ww) basis for albumen ($N = 154$) and dry weight (dw) basis for nestling feathers ($N = 328$). Each run in the DMA consisted of 20–40 samples and included two blanks, 4–6 standard reference samples (DORM-2, DORM-4, DOLT-2, DOLT-5 and TORT-2) and 15–25% of samples had duplicates for quality control purposes. All duplicate concentrations were within 10% of the original sample. Averaged recoveries of the three certified reference materials measured were $97.0 \pm 6.6\%$ ($N = 34$) in albumen runs and $96.5 \pm 7.5\%$ ($N = 105$) in feather runs.

2.4. Food availability

We modeled food availability using two covariates, each approximating one component of food availability. For food abundance we used local fish biomass (fish g/m²) and for vulnerability we used local water recession range (Zabala et al., 2020), the variation in water depth during the breeding season.

To estimate fish biomass, we used annual systematic ecosystem-wide surveys of fish species typically consumed by wading birds. We identified Primary Sampling Units (PSUs) by a Generalized Recursive Tessellation Sampling (GRTS) draw with post-stratification by landscape subunits of the Greater Everglades following Stevens and Olsen (2004). We used a 1-m² throw trap and standardized methods to remove all animals from the trap to provide an estimate of biomass (g/m²). The method is efficient, and provides a relatively unbiased estimate of fish density and biomass over a range of prey sizes relevant for wading bird foraging (between 0.15 cm and 8 cm standard length) and in the diversity of habitats sampled (Gatto and Trexler, 2019; Jordan et al.,

1997). We collected three throw-trap samples at each site and sampled approximately 150 PSUs annually between mid-September and early December, the peak of annual system-wide inundation and fish recruitment (Gatto and Trexler, 2019), setting the stage for prey availability in the following dry-season nesting period. These wet-season fish density estimates have been shown to be a predictor of fish density in dry-season habitats where breeding wading birds forage (Botson et al., 2016).

We used averaged fish biomass in all PSUs within 20 km of any breeding colony as indicator of food abundance for each colony and year. We used the 20 km radius because the vast majority of foraging flights (>95%) of great egrets in the Everglades were within 20 km of the colony (Bancroft et al., 1994).

To calculate recession ranges we used data from the Everglades Depth Estimation Network (EDEN; <https://sofia.usgs.gov/eden/>). We downloaded daily average water-depth values from January 2006 to December 2018 from 128 gaging stations within the study area. For each station and year, we determined the highest water level during the first three months of the year and the lowest water level between February and June. The difference between these two measurements equaled the water level recession range during the time that egrets were likely to be nesting. To describe annual colony-specific conditions, we averaged recession ranges at the gaging stations within 20 km of each colony.

2.5. Analytical approach

In this study we used two different analytical approaches. First, we used a nest-level approach (nest-approach hereafter, Table 1) to test the prediction that accounting for variation in food availability would enhance or reveal associations between reproductive endpoints and

Table 1
Conceptual description of analyses, showing characteristics and key differences among the Nest- and Colony-approaches after endpoints, exposure indicators, and covariates. We indicate in parenthesis whether its value reflects individual nest values (Nest) or values estimated for the whole colony (Col).

Analysis	Nest-approach	Colony-approach
Key differences	Endpoints and exposure to Hg measured at each nest	Endpoints measured at each nest included in analyses but exposure to Hg averaged from a subset of randomly sampled nests in each colony.
Predictions tested	1- Accounting for variation in food availability will clarify associations between reproductive endpoints and exposure to Hg 2- Influence of Hg and food availability on endpoints will be interactive rather than additive	1- The degree of breeding impairment associated with Hg is higher under food stress conditions
Nb of nests in analyses	126-130 (albumen analyses) 67-70 (feather analyses)	782-1290 (feather only)
N of years	2	11
N of colonies	8	14
N col-years	11	41
Endpoints assessed	1- Clutch-size (Nest) 2- Brood-size (Nest) 3- Fledglings per nest (Nest) 4- Hatching success (Nest) 5- Post-hatching survival (Nest) 6- Fledglings per egg (Nest)	1- Hatching success (Nest) 2- Post-hatching survival (Nest) 3- Fledglings per egg (Nest)
Exposure indicator	1- Albumen [Hg] (Nest) 2- Nestling Feather [Hg] (Nest)	Averaged Nestling Feather [Hg] (Col)
Covariates	1- [Hg] (Nest) 2- Avg Fish biomass (Col) 2- Avg Rec range biomass (Col)	1- [Hg] (Col) 2- Avg Fish biomass (Col) 3- Avg Rec range biomass (Col) 4- Clutch-size (Nest)
Random factors	Colony Id	Colony Id Year

Hg in breeding birds. To do that, we re-analyzed nest success and Hg data used by Zabala et al. (2019c), but also accounted for food availability. We also included interaction terms between Hg and components of food availability to test our prediction that the influence of food availability would be interactive rather than additive. These data consisted of records of breeding success of great egret nests monitored in 2015, 2016 and 2017 in several colonies of the Everglades and exposure to Hg in nests estimated through albumen or nestling feathers [Hg] (Table 1). These years were the only ones in which we had reproductive success, food availability, and indicators of both feather and albumen [Hg]. Yet, 2016 was an atypical year, with high water throughout the breeding season and surface-water level reversals (increases >30 cm) in January and February resulting from unseasonably high rainfall events and early abandonments of major breeding colonies (Cook and Baranski, 2017). As these unusual conditions could hide or bias influence of Hg and food conditions in non-extreme environmental conditions, we discarded the few 2016 data from analyses. The remaining data set consisted of 130 nests sampled for albumen and 70 for feathers (Tables 1, S1, S2). These nests were monitored in nine colonies but as a consequence of variation in water levels and colony use between years, we only monitored three of these colonies in both years. (Table S2). Some of the predictor covariates (e.g. averaged fish biomass) had the same value for all nests in the same colony and the same year but varied among years. Therefore, we considered each unique combination of colony and year (colony-year hereafter) as independent samples for these covariates (but accounted for possible colony effects adding it as random factor, see below). In the nest-level approach, in addition to individual Hg exposure indicators for nests, we only had 11 unique combinations of fish biomass and recession-range, to model food availability we did not include interactions between its components to avoid over-parametrizing. Average albumen [Hg] in individual samples from 2015 and 2017 was 0.578 THg µg/g ww (range: 0.010–2.143 THg µg/g ww, N = 130). Average feather [Hg] in individual samples from 2015 and 2017 was 11.343 THg µg/g ww (range: 0.795–25.665 THg µg/g dw, N = 70; for further detail on [Hg] variation within colonies and among colonies and years see (Zabala et al., 2019b)).

Next, we used a colony-level approach (colony-approach hereafter, Table 1), which included many more years of information, but did not include egg albumen [Hg]. In this approach, exposure to Hg was estimated using nestling feathers for the breeding colony as a whole, not for individual nests. We averaged nestling-feather [Hg] from the same colony and year and assumed it to represent average exposure to Hg in nests in that colony-year. The colony-approach included 1300 nests monitored in several colonies over 11 years resulting in 41 colony-year combinations, and therefore 41 different food availability conditions (Tables 1, S1). In this analysis, we used colony-averaged feather [Hg] and food availability to examine the interactive effects of food and Hg exposure on nest success. We tested the prediction that the degree of breeding impairment associated with Hg would be higher under food stress. As the number of different food availability conditions observed was limited (N = 41), we only used two covariates and their interaction to describe it (fish biomass and recession range, see above). To assess the possible influence of the selection of covariates on our results, we ran parallel optimization of models with alternative parametrizations and covariates. In these model optimizations, we used hatching success as reference endpoint because it was the endpoint that accounted for a higher R² (see results). As with the 2015/2017 data set, we characterized food availability in each colony by using fish biomass from an average of >20 PSUs and water levels from >23 gages (Table S1). Averaged nestling feather [Hg] in colonies ranged between 3.54 and 19.335 THg µg/g dw and its average value was 9.246 THg µg/g dw (N = 41; for further detail in colony-averaged [Hg] values and their variation see (Zabala et al., 2019a, 2019b; Zabala et al., 2020)). Observed [Hg] values were within the range of values expected to cause a 10–20% reduction in breeding success (Fuchsman et al., 2017; Zabala et al., 2020).

In both cases, (nest- and colony-approach) the above referenced samples for each approach indicate maximum sample sizes, but lack of endpoint data for some samples resulted in varying sample sizes for specific analyses. In some nests we could not determine the value for one or more of the six endpoints, therefore sample sizes for specific analyses vary slightly. Collinearity among average fish biomass, recession range and nestling feather or albumen [Hg] in albumen and nestling feather datasets was ≤ 0.5 in all cases and lower (≤ 0.3) in the colony-approach dataset (Figs. S2, S3, and S4).

2.6. Statistical analyses

In the nest-approach, we modeled reproductive endpoints of individual nests as a function of their nestling feather or albumen [Hg] and colony-specific food availability indicators (Table 1). In the colony-approach, we modeled nest reproductive endpoints as a function of colony-averaged nestling feather [Hg] and colony-specific food availability indicators. To look for associations between breeding endpoints and explanatory covariates we used Generalized Linear Mixed Models (GLMMs). To account for possible colony-level effects we included colony as random factor in every analysis. In colony analyses, we added year as a crossed random factor. In individual nest analyses we did not include year because we had data from just two years, and we could not consider them as representative of inter-annual variation (Bolker, 2015). For analyses purposes we categorized breeding endpoints in two groups; 1) 'Count-based' endpoints, which are based in counts of discrete offspring-units produced such as clutch-size, brood size or number of offspring fledged or weaned; and 2) 'Probabilistic' endpoints that measure the probability of successfully completing one stage or process or the breeding cycle. These endpoints include hatching success or post-hatching survival of nestlings in birds, or probability of a neonate surviving to a given age. Count-based endpoints are known to be strongly influenced by food availability in birds (Powell, 1983; Ruffino et al., 2014) and they have some particular analytical problems (McDonald and White, 2010). In addition, probabilistic endpoints could better capture Hg effects on reproductive measures, with reduced confusing effects of food availability. For instance, number of eggs laid is likely to be strongly associated with food availability, potentially masking or confusing the influence of Hg, while survival of laid eggs to hatching seems more likely to be influenced by factors such as *in-ovo* embryonic exposure to Hg (Heinz and Hoffman, 2003) or aberrant incubating behavior associated with Hg (Evers et al., 2008). Similarly, using brood size to assess Hg effects may be influenced by food availability as number of nestlings is directly related to number of eggs. Survival of nestlings can be more directly affected by their exposure to Hg (Varian-Ramos et al., 2014; Zabala et al., 2019c). However, food availability could also strongly influence probabilistic endpoints through nest abandonment or starvation, particularly if food conditions change strongly during the breeding cycle.

A particular issue of count-based endpoints is that they often are problematic to analyze due to their discrete nature and constrained set of possible values (whole numbers < 5) (McDonald and White, 2010). Therefore, we assessed three possible error distributions: the Poisson, the Negative Binomial, both with a log link function, and a Gaussian error distribution with the identity link. For hatching success, post-hatching success and fledglings per egg, we used proportional models and a binomial distribution error with a logit link (Zuur et al., 2009). In proportional models, we used a combined response variable in which each nest was considered as a series of repeated binomial trials. For instance, in hatching success a nest with three eggs resulting in two nestlings was modeled as a set of three binomial trials (three eggs), two of them were successful (two nestlings) and the other failed (unhatched egg).

To test the prediction that accounting for variation in food availability would enhance or reveal associations between reproductive endpoints and Hg, in each of the 12 possible endpoint-indicator

combinations (6 endpoints and two indicators: albumen and nestling feather) in the nest-approach, we assessed 10 different models depicting different hypotheses. These models included: (1) a null model in which the output of the endpoint was constant assuming no influence of covariates; (2) a model using only a linear association between [Hg] in either nestling feather or albumen [Hg] and the endpoint of interest, representing a scenario in which Hg exposure is the main driver of breeding success regardless of food conditions; (3) a model assessing effects of components of food availability (fish biomass and recession range) in which endpoints are influenced by food availability but not by Hg; (4) a linear model that included a full interaction of Hg with fish biomass and recession range, in which the influence of Hg on breeding success is monotonic but influenced by food availability; (5) a log model that included a full interaction of log (Hg) with fish biomass and recession range, that assumed an influence of Hg on breeding success similar to the previous model but with a different shape. We also evaluated (6) a quadratic [Hg] model in which Hg is the main influence on breeding success, but its effect is not monotonic, and (7) a model with full interactions of Hg and its quadratic term with food availability covariates. Models 4, 5, and 7 test our prediction of interactive effects of Hg and food availability, but with different shapes for the association of Hg with the endpoint assessed. In some of the nest-level analyses models might be data limited, irrelevant parameters arising from interactions could increase the AICc hiding improvement caused by others. Thus, to assess for possible associations or partial interactions of Hg and food availability penalized by AICc in more general models by the large number of covariates included and to evaluate whether food availability and Hg effects are additive or interactive as we predicted, we performed (8) a stepwise backward optimization of the model with full interactions between food availability and linear Hg; (9) the same optimization of the model assessing interactions between food availability and log [Hg]; and (10) the optimization of the food interaction and quadratic Hg model. To optimize models, we inspected the output of each general model and sequentially removed the covariate with highest *p* value and compared the performance of that model with the previous one using AICc (Burnham and Anderson, 2002; Zuur et al., 2009). We repeated this step until no improvement, in terms of reduction in AICc, was achieved by removing covariates. We kept the number of models and covariates constant in the 12 endpoint-indicator combinations in the nest-approach to enhance comparability among results. We caution that all our models were nested: the model assessing interactions between food availability and linear and quadratic [Hg] was the most general one and all the others were models in which one or more of the terms have been set to zero. Therefore, the 2 AICc rule did not apply since such an increment (~ 2 AICc units) is to be expected from adding even a meaningless covariate (Arnold, 2010; Burnham and Anderson, 2002).

In colony-approach analyses, we only assessed the three probabilistic endpoints: hatching success; post-hatching survival; and fledglings per egg. We did not include count-based endpoints because of problems detected in the nest-approach analyses. In addition to recession range and fish biomass, we added clutch size as a predictor covariate to approximate maternal body condition and possibly model part of the intra-colony variation in food conditions. In the colony approach for each endpoint, we assessed a (1) null model; (2) a model with linear Hg as the only predictor; (3) a model with linear Hg and food availability in which food components and clutch size interacted among them but not with Hg; (4) a model with full interactions between food availability, clutch-size and linear [Hg]; (5) another model assessing log ([Hg]) as the only predictor; (6) a model with log ([Hg]) and food availability in which food components and clutch size interacted among them but not with Hg; (7) a model assessing full interactions of food availability, clutch-size and log ([Hg]); and (8) a model assessing quadratic Hg as the only predictor; (9) a model with quadratic Hg and food availability in which food components and clutch size interacted among them but not with Hg; (10) a model assessing full interactions

between food availability, clutch-size and [Hg] and its quadratic term. Finally, we performed stepwise backward optimization of models 4, 7 and 10 as described for individual nests. To approximate exposure at the colony level we used the [Hg] in nestling feathers from the same colony and year (average feather [Hg] hereafter). Some ecotoxicologists favor geometric means over the arithmetic mean to account for skewed data distributions, yet in our case arithmetic and geometric means were closely related (linear relationship of arithmetic average to geometric average $\beta = 1.015 \pm 0.007$, $P < 0.001$, $R^2 = 0.99$, $N = 130$). We have reported decreased accuracy in colony-averaged feather [Hg] when based on samples from less than six nests (Zabala et al., 2019a). Therefore, in colony-approach models, all of the colony-years whose average [Hg] was based on feather samples from 6 or more individuals ($N = 29$) had the same weight (weight = 1), while the others ($N = 12$) were weighted by the number of nests sampled divided by 6. Within the regression, weights influenced the fitting criterion and determined the contribution of their related observations to estimated parameters.

From the best model in each set we calculated the marginal (R_{marg}^2) and conditional (R_{con}^2) coefficient of determination accounted for by the fixed factors of the GLMM and by the fixed and random factors as well (Nakagawa and Schielzeth, 2013). We also calculated the semi-partial Coefficient of Determination (R_{part}^2), the proportion of observed variation explained in exclusivity by covariates and their interactions. We used commonality analysis (Nakagawa and Schielzeth, 2017; Nimon and Oswald, 2013; Ray-Mukherjee et al., 2014) to deconstruct the R^2 of a set of predictors into unique and common, or shared effects. To ease the interpretation and calculation of partial R^2 we grouped covariates in two blocks and estimated the value for the block. These blocks were Hg ([Hg] in albumen or nestling feathers, and its quadratic value when appropriate) and food availability (fish biomass and recession range). In colony-approach models, as they included more combinations of components of food availability, we also estimated the partial R^2 for fish biomass and recession range and the contribution of food and Hg interactions to the partial R^2 of Hg. We report all R^2 as adjusted R^2 values. To assess the possible influence of selected food availability covariates and specifications in the colony-approach models, we used the hatching success model as reference (because of its better performance, see results) and ran parallel model selections using recession rate instead of recession range; clutch size parametrized as a categorical covariate with two values (small vs large); reduced numbers of interactions between food availability components and Hg, and with simpler random structures (only year or only colony as random factor).

Finally, using the best colony-approach model of each set we predicted the expected value for the endpoint in the context of gradual increases ($[0.1] \mu\text{g/g}$ THg dw or ww) of feather [Hg] and contrasting food availability conditions. To determine contrasting food availability conditions, we used the 0.25 and 0.75 quantiles of values observed in fish biomass and recession range data sets. We plotted results and inspected change in the shape of associations between exposure to Hg and reproductive success to evaluate our prediction that breeding impairment associated with exposure to Hg would be higher under reduced food availability. We also produced similar plots for some of the endpoints using models of the nest-approach for comparison. We warn that shapes predicted from the nest-approach analyses should be cautiously assessed as they are based on only two years of data and the range of food availability conditions included is therefore reduced.

We performed all analyses using R 3.5.1 (Team, 2016). For GLMMs we used the package lme4 1.1–19 (Bates et al., 2015). We used PiecewiseSEM 1.2.1 (Lefcheck, 2016) to calculate R^2 of GLMMs, and AICcmodavg 2.1–4 (Mazerolle, 2017) for AICc values. We produced plots with corrplot 0.84 (Wei and Simko, 2017) and ggplot2 3.1.0 (Wickham, 2009) packages. Values reported in descriptive statistics are mean \pm 1 standard deviation with the range in parenthesis, unless stated otherwise.

3. Results

3.1. Nest-approach: influence of food availability in detecting associations between endpoints and Hg

As predicted, accounting for food availability revealed widespread associations between Hg and breeding endpoints. In models including only linear effects of Hg, significant associations between Hg and endpoints of breeding success were scarce, detected in only 3 of the 12 sets of models (Tables 2, 3). However, when we included interactions with food availability, we found associations with Hg in all the model sets and the best model of 11 of 12 model sets retained interactions among food conditions and Hg, particularly with its quadratic term. In the remaining model, the linear Hg model was also the best in the set (Table 2). For instance, the model for hatching success assessing interactions between quadratic albumen [Hg] outperformed models assessing linear Hg effects and food-only effects by, 13.66 and 9.01 AICc units respectively, and by 15.40 and 10.75 units respectively when compared to the optimized model of food and quadratic albumen [Hg] interactions (Table 2). In several endpoints, the model assessing quadratic Hg and food availability interactions seemed to perform poorly compared to the null model, but when we optimized it, it consistently outperformed the null (Table 2). This was because the full interaction model had many insignificant terms penalizing the AICc. Thus, the better performance of models with interactions supported the prediction that variation in food availability exerted its main influence in the association between Hg and endpoints in an interactive way.

The inclusion of interactions between food availability and exposure to Hg in the nest-approach also revealed endpoint-specific affinities of indicators of Hg exposure. Albumen [Hg] accounted exclusively for more variation in endpoints related to the egg phase (clutch size, hatching success, and fledglings per egg, partial R^2 's respectively: 0.057, 0.089 and 0.061), particularly in hatching success, while its relevance was comparatively low in post-hatching success indicators (brood-size and post-hatching survival, partial R^2 's respectively: 0.022 and 0.023; Tables 4, 5). Conversely, nestling feather [Hg] accounted for more variation in endpoints involving later stages of the breeding cycle (post-hatching survival, fledglings per egg, and fledglings per nest, partial $R^2 > 0.083$ in every case), while its relevance in earlier stages was smaller (partial $R^2 < 0.075$; Tables 5, 6). These indicator-endpoint affinities were not obvious until we accounted for food availability. While associations of nestling feather [Hg] with post-hatching endpoints were still detectable when food availability was not taken into account, all the other associations (9 of 12) and particularly that of albumen [Hg] with hatching success remained hidden.

Count-based endpoints (clutch-size, brood-size and fledglings per nest) were problematic as they did not clearly fit into any distribution (McDonald and White, 2010). They were right truncated (all values < 5), violating the assumptions of Poisson or negative binomial distributions, and their variance to mean ratio clearly departed from 1 in most cases (All < 0.8 and all feather values < 0.25 ; Tables 4, 6) further deviating from the assumptions of the Poisson distribution. Indeed, model selection and optimization for count-based endpoints using different error families and link functions yielded different results (Tables 2, S3). For instance, assuming a Gaussian distribution, the best model for clutch-size and albumen [Hg] retained [Hg] and two interactions with recession range and had a marginal R^2 of 0.068 (Table 4). Yet, when we used Poisson and negative binomial distributions the best model was the null (Table S3). Following McDonald and White (2010), we report results from Gaussian models but we caution that there is uncertainty associated with them and have included results for Poisson and Negative binomial models (Tables S3, S4). Further, count-based endpoint values indicated medium to high degrees of correlation (Fig. 1) and a strong association of Hg with just one endpoint could result in spurious associations with other endpoints.

Table 2

Relative performance of each model in the 12 sets of nest-approach analyses, expressed as increase in AICc with regards to the best model in the set. Hg indicates linear effect of Hg, while Food * Hg the model assessing a possible interaction between linear Hg and the two food availability components. Log (Hg) and (Hg + Hg²), indicate respectively models assessing log and quadratic shapes for the association between [Hg] and sub-lethal effects, while Food * log (Hg) and Food * (Hg + Hg²) indicate models assessing their interaction with food availability components. NA indicates that no covariate was retained in the optimization of that particular approach, resulting in a null model specification. Optimized models are models resulting from backwards stepwise optimization of models assuming different shapes for the influence of Hg in breeding response. For instance, Optimized Food * Hg is the model resulting from the optimization of the Food * Hg model. In the last line, we indicate the number of nests (N) included in each analysis. For clutch-, brood- and fledglings per nest, we report results assuming a Gaussian distribution of error and identity link. For results of models based on Poisson and negative binomial distributions, see Table S2. Food indicates both components, fish biomass and recession range, were included in the models.

Hg sample	Model	Clutch	Brood	Fledglings per nest	Hatching success	Post-hatching survival	Fledglings per egg
Albumen	Null	2.66	4.25	3.61	13.31	0.79	6.84
	Hg	4.79	6.38	5.56	15.40	2.53	8.68
	Food	5.00	3.04	3.28	10.75	1.69	4.41
	Food * Hg	9.48	7.44	7.67	12.84	8.01	8.09
	Food * log (Hg)	10.67	8.15	9.92	12.58	6.85	10.76
	Hg + Hg ²	2.22	7.11	5.39	15.99	4.51	9.03
	Food * (Hg + Hg ²)	10.21	5.37	5.52	1.74	13.87	3.75
	Optimized food * Hg	Na	1.36	3.28	10.35	0.40	4.41
	Optimized food * log (Hg)	Na	1.85	3.28	8.36	0	4.41
	Optimized food * (Hg + Hg ²)	0	0	0	0	0.40	0
	N	130	128	126	128	93	126
Feather	Null	1.05	4.40	2.75	1.28	7.64	7.74
	Hg	2.53	6.65	1.16	1.92	0.11	0
	Food	3.10	8.89	5.39	3.58	6.88	7.23
	Food * Hg	4.14	7.68	7.73	6.23	4.50	7.25
	Food * log (Hg)	5.34	9.24	9.47	5.93	8.66	9.96
	Hg + Hg ²	2.91	7.77	2.20	4.14	2.26	2.24
	Food * (Hg + Hg ²)	10.96	13.07	13.62	12.82	9.04	13.43
	Optimized Food * Hg	0.19	1.89	1.16	0.12	0.11	0
	Optimized Food * log (Hg)	1.50	3.16	2.61	0	4.10	3.31
	Optimized Food * (Hg + Hg ²)	0	0	0	0.12	0	0.96
	N	70	69	68	69	67	68

Among probabilistic endpoints, albumen [Hg] and its interactions with food availability were strongly associated with hatching success. This model accounted for a higher R² than any of the other 12 combinations assessed in the nest-approach, and the quadratic form of Hg and its interactions accounted for 8.9% of the observed variation (Tables 4, 5). However, albumen [Hg] and its interaction with food availability accounted for a comparatively small amount of the variation observed in post-hatching survival (R²_{partial} = 0.023). Indeed, the marginal R² of the best model for hatching success including food and albumen [Hg] was 7 times that of the best model for post-hatching survival based solely on albumen [Hg] (Table 5). Albumen [Hg] also accounted for 6.1% of the observed variation in fledglings per egg, an endpoint integrating pre- and post-hatching success, roughly in the mid-point of the previous two endpoints. Conversely, nestling feather [Hg], accounted exclusively for higher R² in endpoints associated with post-hatching

success than for hatching success (0.083 and 0.099 compared to 0.075; Tables 5, 6). Nestling feather [Hg] was linearly associated with post-hatching related endpoints (fledglings per nest, post-hatching survival and fledgling per egg, P > 0.021 in every case; Table 3). Association with other endpoints occurred only when accounting for interactions with food conditions. Nestling feather [Hg] and its interactions with food accounted in exclusivity for less of the observed variation in clutch size or hatching success than in post-hatching endpoints (Tables 5, 6). We plotted predicted change in hatching success and post-hatching success at contrasting environmental conditions and gradual increases of albumen [Hg] and nestling feather [Hg] respectively (Fig. S5). However, we caution that these plots are based on a small number (N = 11) of different food availability conditions and they necessarily show a partial picture that should not be extrapolated to other systems.

Table 3

Output of linear models of association between Hg measured in albumen and nestling feathers, and different breeding endpoints. We show the estimate of slope, its standard error, the value of z statistic and the P value associated. For relative performance in each case see Table 2 for nest-approach models and Table 7 for colony-approach analyses.

Hg sample	Endpoint	Est	SE	z Val.	P	N	ΔAICc
Albumen	Clutch size	-0.004	0.049	-0.084	0.933	130	2.13
	Brood size	0.006	0.116	0.048	0.962	128	2.13
	fledglings per nest	0.048	0.101	0.475	0.636	126	1.95
	Hatching success	0.013	0.149	0.087	0.931	128	2.09
	Post Hatching survival	0.151	0.241	0.626	0.532	93	1.74
	Fledglings per egg	0.077	0.153	0.503	0.615	126	1.84
	Feather	Clutch size	0.057	0.065	0.881	0.381	70
Brood size		0.009	0.083	0.103	0.918	69	2.25
fledglings per nest		-0.181	0.076	-2.370	0.021	68	-1.59
Hatching success		-0.299	0.236	-1.270	0.204	69	0.64
Post Hatching survival		-0.695	0.204	-3.402	0.001	67	-7.53
Fledglings per egg		-0.601	0.174	-3.450	0.001	68	-7.74
Hatching success		0.050	0.095	0.532	0.595	1290	1.73
Colony-approach	Post Hatching survival	0.171	0.107	1.601	0.109	782	-0.30
	Fledglings per egg	0.073	0.092	0.785	0.433	1101	1.39

Table 4

Output of the best model for associations between albumen [Hg] and breeding endpoints in the nest-approach. We indicate sample size for each analysis (N) and the R² accounted by the fixed factors (the R² of the entire model, R²_{marg}) and by fixed and random components of the model together (R²_{cond}). For endpoints based on counts we also indicate the variance to mean ratio (σ²/μ) of the response variable. For each covariate retained in the optimized model we show the estimate of slope, its standard error, the value of the z statistic and the P-value associated.

Endpoint	Variable		Var	Est	SE	z Val.	p
	Fixed	Random					
Clutch size							
N = 130	Colony (N = 8)		0		0		
R ² _{marg} = 0.068	Intercept			2.684	0.051	52.525	<0.001
R ² _{cond} = 0.068	Hg			0.098	0.061	1.618	0.061
σ ² /μ = 0.112	Rec. Range * Hg			0.407	0.157	2.594	0.011
	Rec. Range * Hg ²			-0.467	0.156	-2.988	0.003
Brood size							
N = 128	Colony (N = 8)		0		0		
R ² _{marg} = 0.010	Intercept			1.857	0.104	17.859	<0.001
R ² _{cond} = 0.010	Avg. Fish			-0.357	0.102	-3.502	0.001
σ ² /μ = 0.792	Rec. Range * Hg ²			-0.171	0.085	-2.020	0.045
Fledglings per nest							
N = 126	Colony (N = 8)		0.202		0.449		
R ² _{marg} = 0.068	Intercept			1.386	0.187	7.427	<0.001
R ² _{cond} = 0.254	Hg ²			0.374	0.154	2.418	0.017
σ ² /μ = 0.795	Rec. Range * Hg			0.593	0.296	2.001	0.048
	Rec. Range * Hg ²			-0.879	0.326	-2.698	0.008
Hatching success							
N = 128	Colony (N = 8)		0.047		0.216		
R ² _{marg} = 0.192	Intercept			0.906	0.188	4.821	<0.001
R ² _{cond} = 0.203	Rec. Range			-0.535	0.185	-2.889	0.004
	Hg ²			0.549	0.346	1.584	0.113
	Avg. Fish * Hg			-2.442	0.696	-3.507	<0.001
	Rec. Range * Hg			1.597	0.565	2.826	0.005
	Avg. Fish * Hg ²			3.424	1.079	3.173	0.002
	Rec. Range * Hg ²			-1.768	0.633	-2.794	0.005
Post-hatching survival							
N = 93	Colony (N = 8)		0.300		0.548		
R ² _{marg} = 0.026	Intercept			1.128	0.261	4.319	<0.001
R ² _{cond} = 0.108	Avg. Fish * log (Hg)			0.300	0.177	1.692	0.091
Fledglings per egg							
N = 126	Colony (N = 8)		0.197		0.444		
R ² _{marg} = 0.140	Intercept			0.230	0.222	1.037	0.300
R ² _{cond} = 0.189	Avg. Fish			-0.508	0.232	-2.190	0.029
	Rec. Range			-0.671	0.205	-3.266	0.001
	Hg ²			0.839	0.310	2.707	0.008
	Avg. Fish * Hg			-0.380	0.245	-1.548	0.122
	Rec. Range * Hg			1.413	0.573	2.467	0.014
	Rec. Range * Hg ²			-2.030	0.665	-3.052	0.002

Table 5

Marginal R² (R²_{marg}) of the best model in each set of nest-approach analyses. We also show the marginal R² of the same model considering only additive effects of Hg (Hg) covariates retained in the optimized model (Hg and/or Hg²) and the marginal R² of a model with only the additive effects of food availability (Food) covariates retained in the optimized model (fish biomass and/or recession range). The difference between the marginal R² of the optimized model and the model containing only the covariates of the other block (food for Hg and vice-versa) is shown in parentheses, which is an approximation of the amount of R² accounted exclusively by Hg or food availability.

Endpoint	Albumen			Feather		
	Hg	Food	R ² _{marg}	Hg	Food	R ² _{marg}
Clutch size	0.057	0.032	0.068	0.052	0.075	0.078
Brood size	0.022	0.099	0.100	0.125	0.124	0.125
Fledglings per nest	0.055	0.046	0.068	0.106	0.000	0.106
Hatching success	0.089	0.184	0.192	0.075	0.078	0.118
Post-hatching survival	0.023	0.024	0.026	0.083	0.032	0.141
Fledglings per egg	0.061	0.129	0.140	0.099	0.000	0.099

Table 6

Output of the best nest-approach model of associations between feather [Hg] and breeding endpoints in the same nest. We indicate sample size for each analysis (N) and the R² accounted by the fixed factors (R²_{marg}) and by fixed and random components of the model together (R²_{cond}). For endpoints based on counts we also indicate the variance to mean ratio (σ²/μ) of the response variable. We show the variance of each random effect (Var) and for each covariate retained in the optimized model we show the estimate of slope, its standard error, the value of z statistic and the P value associated.

Endpoint	Variable		Var	Est	SE	z Val.	p
	Fixed	Random					
Clutch size							
N = 70	Colony (N = 8)		0		0		
R ² _{marg} = 0.078	Intercept			2.857	0.072	39.895	<0.001
R ² _{cond} = 0.078	Hg ²			0.177	0.088	2.017	0.048
σ ² /μ = 0.106	Rec. Range * Hg ²			-0.177	0.075	-2.369	0.021
Brood size							
N = 69	Colony (N = 8)		0.032		0.179		
R ² _{marg} = 0.125	Intercept			2.645	0.107	24.788	<0.001
R ² _{cond} = 0.238	Hg ²			0.227	0.110	2.070	0.042
σ ² /μ = 0.172	Rec. Range * Hg ²			-0.278	0.090	-3.098	0.003
Fledglings per nest							
N = 68	Colony (N = 8)		0.052		0.228		
R ² _{marg} = 0.106	Intercept			1.934	0.107	18.131	<0.001
R ² _{cond} = 0.189	Hg ²			-0.203	0.075	-2.717	0.008
σ ² /μ = 0.220							
Hatching success							
N = 69	Colony (N = 8)		0		0		
R ² _{marg} = 0.118	Intercept			2.514	0.306	8.229	<0.001
R ² _{cond} = 0.118	Rec. Range * log (Hg)			0.662	0.432	1.531	0.126
	Avg. Fish * log (Hg)			-0.618	0.286	-2.161	0.031
Post-hatching survival							
N = 67	Colony (N = 8)		0		0		
R ² _{marg} = 0.141	Intercept			1.226	0.213	5.748	<0.001
R ² _{cond} = 0.141	Hg ²			-1.017	0.290	-3.503	<0.001
	Rec. Range * Hg ²			0.477	0.263	1.813	0.070
Fledglings per egg							
N = 68	Colony (N = 8)		0		0		
R ² _{marg} = 0.099	Intercept			0.957	0.170	5.633	<0.001
R ² _{cond} = 0.099	Hg			-0.601	0.174	-3.450	0.001

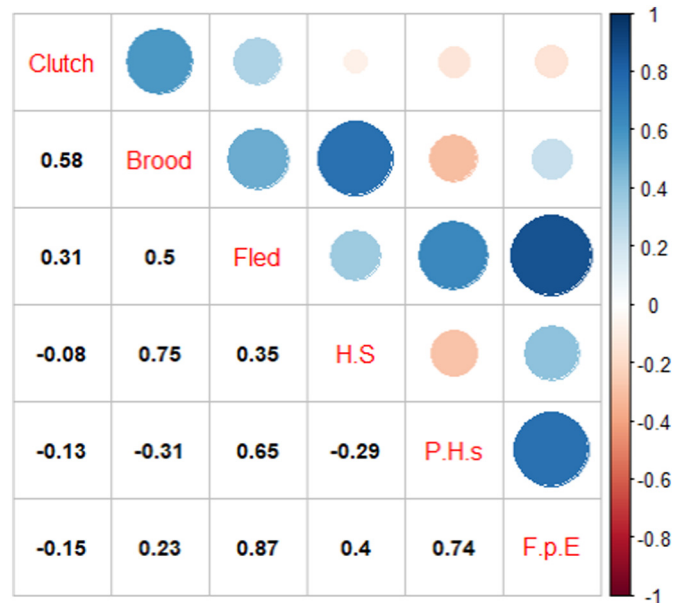


Fig. 1. Pearson correlation coefficient among endpoints assessed. We show the numeric value for each combination of endpoints (below the diagonal) and the circles indicating visually strength and trends of correlation are using circles above the diagonal: blue colors indicate positive correlation and orange negative correlations, while the size of the circle indicates the strength of the correlation. Clutch, Brood and Fled indicate respectively clutch, brood and fledglings per nest. H.S indicates hatching success, P.H.s for post-hatching success and F.p.E for fledglings per egg. The plot is based on nests included in the nest-approach and for which we had values of all endpoints assessed (N = 103).

3.2. Colony-approach: variation in Hg-associated breeding impairment at contrasting food availability conditions

Models with Hg and food interactions also performed best in colony-approach analyses (Table 7), supporting results of the nest-approach and our predictions. For hatching success and fledglings per egg, the optimized model based on a quadratic shape with interactions unequivocally performed better than optimized models based on log or linear associations ($\Delta AICc > 9.8$ in every case). For post-hatching success, the optimized log Hg with interactions model had the lowest AICc, but

Table 7

Relative performance of colony-approach models expressed as increase in AICc compared to the best model for each endpoint. Linear, Log-linear and Quadratic indicate respectively models assuming a linear, logarithmic or quadratic association between average nestling feather [Hg] in the colony and observed endpoint values in each monitored nest. Null is the model with no predictors- "Hg" a model including only Hg, as predictor. "Food + Hg" indicates models including food availability interacting among them and with clutch size but in which the effect of food is interactive. "Food * Hg" indicates models assessing full interactions between food availability, clutch size and Hg. Finally "Food * Hg optimized" is the result of stepwise backwards optimization of "Food * Hg" models. We show the conditional (R^2_{Cond}) and marginal (R^2_{Marg}) R^2 of each model and the number of parameters in it (k). The sample size (N) provided refers to the number of nests included- Detailed output of the best model of each set is provided in Table 8 and detailed information of parameters included/retained in other candidate models in Table S5.

Endpoint	Shape	Model	$\Delta AICc$	R^2_{Cond}	R^2_{Marg}	k
Hatching success N = 1290		Null	118.22	0	0.21	3
	Linear	Hg	119.95	0	0.21	4
	Linear	Food + Hg	83.67	0.08	0.23	11
	Linear	Food * Hg	25.63	0.14	0.28	18
	Linear	Food * Hg optimized	15.49	0.13	0.28	10
	Log-linear	Hg	120.13	0	0.20	4
	Log-linear	Food + Hg	83.62	0.08	0.24	11
	Log-linear	Food * Hg	23.69	0.15	0.3	18
	Log-linear	Food * Hg optimized	15.48	0.15	0.3	13
	Quadratic	Hg	121.83	0	0.21	5
	Quadratic	Food + Hg	84.78	0.08	0.24	12
	Quadratic	Food * Hg	10.50	0.18	0.3	26
Post-hatching survival N = 782	Quadratic	Food * Hg optimized	0	0.17	0.28	19
		Null	33.94	0	0.13	3
	Linear	Hg	33.64	0.01	0.16	4
	Linear	Food + Hg	23.05	0.06	0.24	11
	Linear	Food * Hg	22.31	0.09	0.19	18
	Linear	Food * Hg optimized	1.57	0.08	0.19	9
	Log-linear	Hg	34.39	0.01	0.16	4
	Log-linear	Food + Hg	24.03	0.05	0.23	11
	Log-linear	Food * Hg	11.35	0.10	0.18	18
	Log-linear	Food * Hg optimized	0	0.10	0.16	10
	Quadratic	Hg	35.49	0.01	0.16	5
	Quadratic	Food + Hg	24.79	0.06	0.24	12
Quadratic	Food * Hg	22.31	0.13	0.20	26	
Quadratic	Food * Hg optimized	0.32	0.09	0.18	12	
Fledglings per egg N = 1101		Null	50.28	0	0.14	3
	Linear	Hg	51.68	0	0.15	4
	Linear	Food + Hg	55.54	0.02	0.15	11
	Linear	Food * Hg	23.28	0.09	0.21	18
	Linear	Food * Hg optimized	8.02	0.09	0.21	10
	Log-linear	Hg	51.70	0	0.15	4
	Log-linear	Food + Hg	55.30	0.02	0.15	11
	Log-linear	Food * Hg	23.57	0.09	0.21	18
	Log-linear	Food * Hg optimized	8.71	0.09	0.21	10
	Quadratic	Hg	53.37	0	0.15	5
	Quadratic	Food + Hg	55.80	0.03	0.16	12
	Quadratic	Food * Hg	21.55	0.11	0.22	26
Quadratic	Food * Hg optimized	0	0.11	0.23	12	

Table 8

Output of the best model for endpoints assessed in the colony-approach. We indicate the R^2 accounted for by the fixed factors (R^2_{Marg}) and by fixed and random components of the model together (R^2_{Cond}). We show the variance of each random effect (Var) and for each covariate retained in the optimized model we show the estimate of slope, its standard error, the value of z statistic and the P value associated. We also report the number of groups for each of the crossed random factors and the variance (Var) and standard error of random effects.

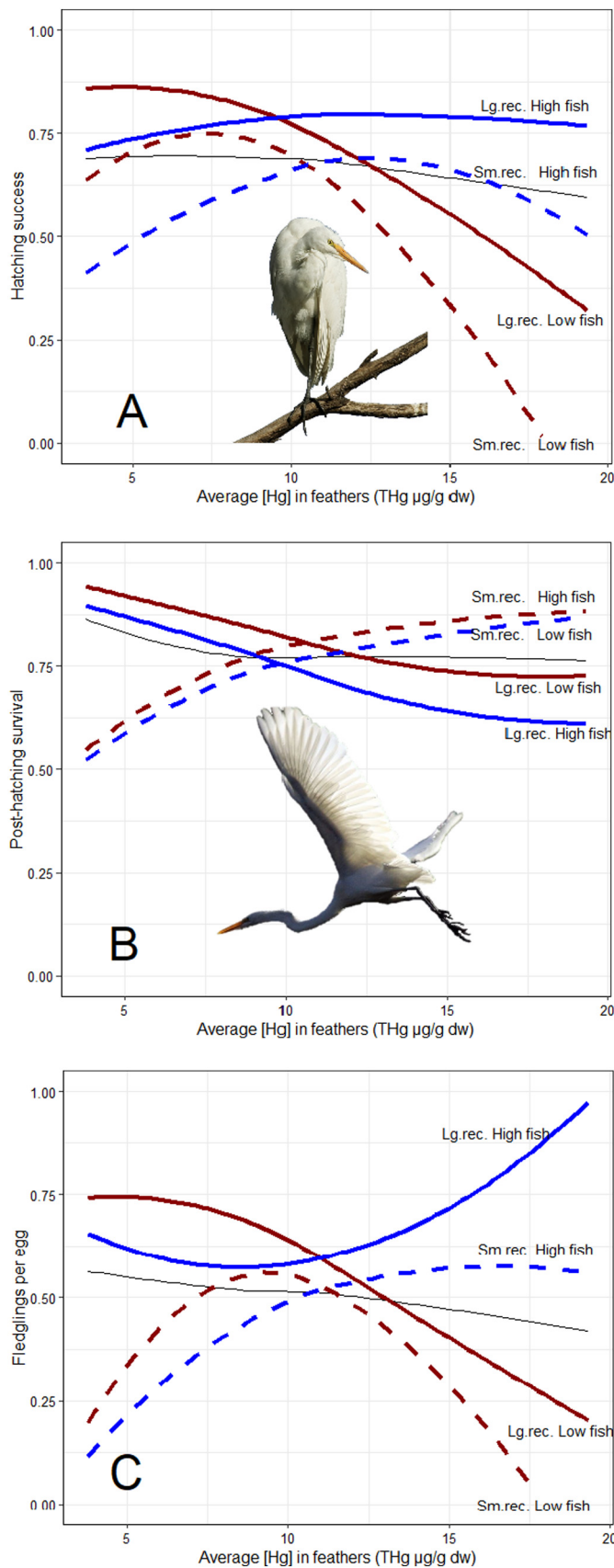
Endpoint	Variable		Est	SE	z Val.	p		
	Fixed	Random						
Hatching success $R^2_{Marg} = 0.169$ $R^2_{Cond} = 0.280$	Colony (N = 14)		0.179	0.423				
	Year (N = 11)		0.330	0.574				
	Intercept			0.849	0.239	3.56	<0.001	
	Rec. Range			0.569	0.134	4.24	<0.001	
	Clutch			0.202	0.058	3.49	<0.001	
	Hg			1.542	0.507	3.04	0.002	
	Hg ²			-1.649	0.521	-3.16	0.002	
	Rec. Range * Clutch			-0.220	0.075	-2.92	0.003	
	Avg. Fish * Hg ²			1.171	0.141	8.28	<0.001	
	Rec. Range * Hg			-1.370	0.507	-2.70	0.007	
	Rec. Range * Hg ²			1.454	0.514	2.83	0.005	
	Clutch * Hg			-0.725	0.395	-1.83	0.067	
	Clutch * Hg ²			0.746	0.411	1.82	0.069	
	Rec. Range * Avg. Fish * Hg			1.059	0.531	2.00	0.046	
	Rec. Range * Avg. Fish * Hg ²			-1.453	0.603	-2.41	0.016	
Clutch * Avg. Fish * Hg			-1.605	0.449	-3.57	<0.001		
Clutch * Avg. Fish * Hg ²			1.452	0.495	2.93	0.003		
Rec. Range * Clutch * Hg			0.972	0.378	2.57	0.010		
Clutch * Rec. Range * Hg ²			-0.812	0.398	-2.04	0.042		
Post-hatching survival $R^2_{Marg} = 0.099$ $R^2_{Cond} = 0.165$	Colony (N = 12)		0.260	0.510				
	Year (N = 11)		0	0				
	Intercept			1.318	0.173	7.61	<0.001	
	Avg. Fish			-0.376	0.129	-2.93	0.003	
	Rec. Range			0.264	0.104	2.53	0.011	
	Clutch			-0.379	0.083	-4.58	<0.001	
	Rec. Range * Avg. Fish			-0.170	0.092	-1.85	0.064	
	Rec. Range * log (Hg)			-0.526	0.095	-5.55	<0.001	
	Clutch * Rec. Range * Avg. Fish			0.132	0.046	2.84	0.005	
	Clutch * Rec. Range * Avg. Fish * log(Hg)			0.182	0.102	1.78	0.075	
	Fledglings per egg $R^2_{Marg} = 0.114$ $R^2_{Cond} = 0.228$	Colony (N = 12)		0.353	0.594			
		Year (N = 11)		0.131	0.363			
		Intercept			0.022	0.223	0.10	0.920
		Rec. Range			0.606	0.141	4.29	<0.001
		Hg			1.098	0.453	2.43	0.015
Hg ²				-0.903	0.455	-1.98	0.047	
Avg. Fish * Hg				-1.352	0.763	-1.77	0.076	
Avg. Fish * Hg ²				2.485	0.888	2.80	0.005	
Rec. Range * Hg				-2.093	0.720	-2.91	0.004	
Rec. Range * Hg ²				2.069	0.752	2.75	0.006	
Clutch * Avg. Fish * Hg				-1.191	0.360	-3.31	0.001	
Clutch * Avg. Fish * Hg ²				1.024	0.373	2.74	0.006	

the optimized quadratic model was within 2 AICc units of it. Models assessing interactions between food and Hg always outperformed models that did not include interactions between the two but with interactions among its components. The food-Hg interaction model

Table 9

Approximation of the amount of R^2 attributable exclusively to each group of covariates (Partial R^2) in the colony-approach. We indicate the R^2 accounted by the fixed factors (R^2_{Marg}) in the best model for each endpoint and R^2 attributable to Hg (Hg) covariates (additive and interactive effects), food availability (Food = fish biomass + Recession Range), to Hg through interactions with other covariates (i.e excluding possible contribution of Hg as additive covariate; Hg (int.)), and that of other covariates individually.

Partial R^2	Hatching success	Post-hatching survival	Fledglings per egg
R^2_{Marg}	0.169	0.099	0.114
Hg	0.088	0.057	0.096
Hg (int.)	0.085	0.057	0.094
Food	0.160	0.055	0.112
Fish biomass	0.058	0.049	0.083
Recession range	0.079	0.064	0.030
Clutch size	0.048	0.037	0.014



(linear, log-linear or quadratic) that performed best in each endpoint clearly outperformed the model based on the same shape that accounted for food and Hg effects but not for their interaction, and there was virtually no support from the data for models with no Hg-food interactions ($\Delta AICc > 10$ in every case). The evidence in our data supported the Hg-food interaction models even before optimization, as in every case they performed better than models with no interactions even before optimization. Simple linear models showed no association between any endpoint and Hg (Table 3, 8), further supporting our hypothesis that main effects of Hg are through interactions with food. The best model for each of the three endpoints included several interactions between components of food availability and the linear and quadratic terms of Hg (Table 8). The best colony-approach model for hatching success accounted for 28% of the variation observed. The fixed components of the model (the marginal R^2) accounted for 16.9%, while the partial R^2 attributable to Hg was 0.088 (Table 9). The marginal R^2 and the partial R^2 of Hg from the best colony-approach model for post-hatching survival were lower than those for hatching success (0.099 and 0.057 respectively; Table 9), while partial R^2 of Hg values of the best colony-approach model for fledglings per egg were intermediate (0.114 and 0.096 respectively; Table 9). Further, the partial R^2 of Hg was mostly (>95% in every case) attributable to interactions with food availability rather than to additive effects (Table 9). For instance, the partial R^2 of Hg in hatching success was 0.088 but 97% of that effect (0.085) was attributable to interactions. Thus, had we not considered interactions between Hg and food, Hg would only account for a partial R^2 of 0.003. Partial R^2 values of Hg itself were generally within the range of partial R^2 of food availability and its components (Partial R^2 ranges: 0.057–0.096 and 0.055–0.160 respectively; Table 9). In the case of hatching success, differences in detection of clutches of different ages did not seem to bias our results as raw estimates of hatching success and Mayfield estimates were strongly correlated ($\beta = 1.338 \pm 0.110, P < 0.001, N = 19, R^2 = 0.897$).

Using the best colony-approach model for each endpoint (Table 8), we modeled breeding success in association with Hg under contrasting food availability conditions (high fish and large recession vs. low fish biomass and small recession,). Predicted hatching success was always higher when food availability was high, with little variation as exposure to Hg increased. In contrast, increases in Hg exposure were associated with reduced hatching success when food availability was low (Fig. 2A). The best colony-approach model for post-hatching survival under high food availability predicted high success at low Hg values, with a gentle monotonic decrease as exposure increased. Post-hatching success under low food availability conditions was low at low Hg values and increased as exposure to Hg increased, converging with or exceeding the predicted response under high food availability (Fig. 2B). The latter part of this response did not support our predictions. Finally, our best colony-approach model for fledglings per egg predicted increased reproductive success with increasing Hg under high food availability conditions. At low food availability, our model predicted decreases in fledgling success as Hg increased. We also saw contrasting trends at low Hg values, depending on fish availability (Fig. 2C). The apparent increase in fledglings per egg associated with initial increases of Hg at low exposures was somewhat unexpected.

For colony-approach models, we assessed the validity of covariates selected, alternative parametrization of clutch-size and alternative

Fig. 2. Predicted hatching success (A), Post-hatching survival (B) and Fledglings per egg (C) in the colony-approach under contrasting scenarios of food availability. The thin line smooths observed endpoint values in relation to feather [Hg] without other covariates. The thick blue line shows predicted values under most favorable food conditions (large recession, high fish biomass) while the thick red dashed line shows predicted values under the most adverse conditions. Lines are smoothers fitted over model-predicted average hatching success for specified fish biomass and recession values while values of other covariates (clutch size, colony and year) were those observed in the field. For definitions of large and small recession and low and high fish biomass see text.

random structures by running parallel model optimization using hatching success as a case study. In every case, models produced similar results in terms of predicted shapes of hatching success under variation in Hg exposure and food availability, but in every case the alternative models performed worse in terms of AICc (Sup. Mat. Results and Tables SX1 – SX5, Figures SX1 – SX5).

4. Discussion

Our results strongly supported our predictions that, in field conditions, sub-lethal effects of exposure to Hg are modulated by food availability. Without accounting for variation in food availability, we would not have discovered associations between reproductive endpoints and Hg in 9 of 12 model sets of the nest-approach, and 3 of 3 of the colony-approach analyses. Generally, the influence of food availability and exposure to Hg on breeding impairment was interactive, as most of the best models (10 of 12 nest-approach and 3 of 3 colony-approach analysis-sets) retained interactions between Hg and components of food availability. Further, in colony-approach analyses most of the partial R^2 attributable to Hg was accounted for by interactive terms of Hg and food availability rather than by its additive terms. Our results also supported the prediction that impairment associated with Hg would be higher under conditions of food stress. The evidence was slightly contradictory in this last prediction. Results of two of the three endpoints assessed in the colony-approach supported the prediction, but the post-hatching success model predicted impairment associated to Hg was slightly higher under high food availability conditions, putatively because Hg had already exerted its effect on previous phases of the breeding cycle.

Our analyses also demonstrated strong evidence of endpoint-specific affinity of indicators of exposure. Generally, in the nest-approach analyses albumen [Hg] accounted for more variation of reproductive measures taken early in the breeding cycle (clutch size and particularly hatching success) and comparatively less of endpoints related to later stages (brood-size or post-hatching survival). Conversely, fledgling feather [Hg] explained proportionately more variation in later breeding stages (brood size, fledglings/nest, post-hatching survival) than in early stages of the breeding cycle. A plausible explanation for the lower association between nestling feather [Hg] and hatching success in individual nest analyses could be that we could not sample feathers from nestlings that had died earlier in the cycle, particularly from nests that failed to hatch any nestlings (Zabala et al., 2019c).. There is no general agreement on preferred standards for either indicator tissues to assess exposure to Hg or endpoints to assess sub-lethal effects of that exposure. Controlled dosage experiments and some field studies have reported good correlations among [Hg] in different tissues and between parents/offspring (Ackerman et al., 2019; Spalding et al., 2000). However, several field studies reported poor or absent correlations between [Hg] in samples from the same individual or breeding units (e.g., nests or territories; Akearok et al., 2010; Hartman et al., 2013; Zabala et al., 2019c). Our results suggest that, in field studies at least, there might not be an exposure indicator that is always best but that the indicator should be selected depending on the endpoint to assess.

Endpoint specificity may also stem from temporal variation in exposure to Hg within the breeding cycle. Elsewhere, we reported that while albumen and feather [Hg] were not correlated in samples from the same nest, there was high correlation between the two at the colony level (Zabala et al., 2019b). In this study, colony-approach models using nestling feather [Hg] explained more variation in hatching success than in post-hatching survival and had a marginal R^2 similar to that of the best albumen model for individual nest hatching-success (marginal R^2 of 0.169 and 0.192 for hatching success using feathers and albumen respectively, Tables 4, 8). It is also true that previously reported inter-colony variability was higher than intra-colony variability in this system (Zabala et al., 2019b), suggesting a mechanism by which colony-level

data represented a reliable geographic signal. Together, this information suggests that there may be within-season variation in Hg exposure, but that nestling feather [Hg] averaged at the colony level captures general Hg exposure trends at the temporal scale of the entire breeding cycle. Although the reported variation explained by Hg in our models might seem low (0.022–0.125, or 2.2–12.5%), it is important to remember that this study was carried out in a natural setting, with many other sources of variance that we could not account for. A review on the amount of variance explained in ecological and evolutionary studies concluded that reported results overall explained on average 2.5–5.2% of the observed variance (Møller and Jennions, 2002). Our ecological modelling also has some limitations that could influence the results. Although estimates of fish biomass are thorough and based on quality regular standardized sampling, we cannot know for sure where birds have been feeding. If, for instance, birds select patches with highest fish biomass, averaged fish biomass would at least in some cases underestimate fish biomass in areas used by birds. In the same line, fish biomass, which is a measure of density, might not fully capture total fish abundance as this varies with flooded surface too. For instance, the same biomass (g m^{-2}) in two different years could represent a twofold difference in total fish mass if the flooded area was halved in any of the years. Our modelling of water dynamics in the area is also partial. The variable we used captured the difference between maximum and minimum water depth in the season but was insensitive to relevant variation within those parameters. The same recession range can represent a slow and steady recession reliably providing favorable conditions for birds over a long breeding season, or a shorter and faster recession ended abruptly by a strong reversal forcing birds to abandon. In the same line, we could not account for the effects of small reversals within the breeding season that can cause partial or total abandonment (Frederick and Collopy, 1989). Considered together, all those limitations probably resulted in uncertainty around our estimates. Finally, lack of references for food availability in other Hg effects studies prevents us from translating our results to other systems or species. Although we measured changes in variables relevant for food availability for great egrets, we don't know how they translate into actual biomass availability and under what thresholds of fish biomass and recession range egrets become food deprived. In a related study, we reported higher numbers of breeding pairs in colonies with higher recession ranges (Zabala et al., 2020) but some birds nested, and in some cases successfully bred, even in the smallest recessions observed. Therefore, thresholds for environmental conditions causing nutritional stress probably vary among breeding pairs.

A distinct advantage of using colony-averaged nestling feather [Hg] in the colony-approach was the ability to include all monitored clutches including those that hatched no eggs at all. This was not possible in the nest-approach analyses when we used feather [Hg] as indicator of exposure. Inclusion of all nests in colony-approach analyses may have allowed representation of a relevant part of the variation in hatching success. Hatching failure might reflect not only *in-ovo* mortality (Heinz et al., 2009; Herring et al., 2010), but also effects of Hg on poor parental incubation behavior and early abandonment (Evers et al., 2008; Frederick and Jayasena, 2011). For these reasons, we believe that predictions from colony-approach models are closer to the true shapes of relationships between reproductive endpoints and exposure to Hg in natural systems. Colony-approach datasets included more variation in food availability conditions (11 vs 2 years) and may have encompassed more extreme values of other background variation than nest-approach models (14 vs 8 colonies included in the colony-approach and nest-approach respectively). A potential issue of the colony-approach is that is based on feathers, and thus Hg exposure is derived only from a subset of successful nests. If nests exposed to higher Hg values fail in early breeding stages, nestling feathers could underestimate exposure to Hg (Zabala et al., 2019c), and thus overestimate the effect. Such a bias could result in the overestimation of effects in our models but the different trends we report associated with variation in

food availability should remain unaffected. Thus, we emphasize the relevance of the reported patterns of variation in effects associated with different food availability conditions rather than the apparent absolute values of dose-response relationships in our results. The latter should be regarded as indicative, at best. In addition, within a range of 5.26 to 19.34 mg/g dw THg in nestling feathers, colony-averaged egg albumen and nestling feather [Hg] in Great Egrets in the Everglades showed a good correlation ($r = 0.703$; (Zabala et al., 2019c). The THg range assessed in that paper is very similar to the one in this study (3.54 to 19.34 mg/g dw THg) and it encompasses its high-end, which presumably could be more sensitive to possible bias. Therefore, it seems that possible effects of underestimation are minor in this study. The research unit of the study, individual vs population, is a relevant feature of ecotoxicological field studies. Some studies have used individuals or breeding units with their respective individual exposure values as the level of analysis, (Goutte et al., 2015; Tartu et al., 2013; Zabala et al., 2019c), while others have compared breeding in one or more contaminated areas with values from a reference area, or along a gradient of contamination, assuming similar exposure to all individuals within each of the areas defined (Brasso and Cristol, 2008; Hallinger and Cristol, 2011; Meyer et al., 1998). While the individual approach is often deemed, a priori, as more accurate, the population approach usually encompasses more individuals over larger areas. However, the population approach depends on clear differences in exposure to pollutants among areas and may result in reduced power resulting from variation in individual exposure within areas and inherent differences in breeding potential among areas (Akearok et al., 2010; Zabala et al., 2019a). Our results seem to support the use of population level analyses, such as our colony-approach work, in which exposure to Hg for the whole area or population is estimated from a subset of individuals. This seemed to lift, at least partially, possible limitations of indicator-specific affinity of Hg indicators found in nest-approach analyses. There may have been a cost to the colony-approach though, since it depends on a higher variance in Hg exposure between rather than within populations, as demonstrated for this study area (Zabala et al., 2019a, 2019b), but may not be the case in all studies. Therefore, we caution about applying this result without careful assessment of the study population or area and, when possible, assessment of the inter- and intra-population variability in exposure among areas or populations.

Count-based endpoints were analytically problematic in our study and we caution that results of count-based endpoints may be inherently unreliable. The counts did not meet the assumptions of any of distributions and results were not coherent across the three distributions assessed. In addition, count-based endpoints were highly correlated (Fig. 1). This is not surprising as brood size is dependent on clutch size, and number of fledglings per nest is dependent on brood size. In contrast, the probabilistic measures (hatching success and post-hatching survival), showed a low and negative correlation, suggesting that success in these two endpoints was independent of each other. These characteristics of count-based and probabilistic endpoints appear to be inherent, suggesting that our findings may have broad applicability.

Most of the statistical effect of Hg appeared to be expressed through an interaction with food availability. Our predicted effects were not monotonic, suggesting that there is a wide variety of outcomes that could occur at any single Hg exposure value, depending on food availability (Figs. 2, S5). In general, our results demonstrate that when food is highly available, the effects of Hg were muted within the observed Hg exposures, probably due to better nutritional status. While this was true for hatching success and fledglings per egg, the response of post-hatching survival (Fig. 2B) seemed to contradict the trend. However, post-hatching survival does not include eggs of clutches that failed to hatch. In this winnowing process, most of the deleterious effect attributable to Hg already took place before hatching, and nestlings exposed to Hg are therefore a small and pre-selected group that could represent particularly Hg resistant eggs or parents (Buck et al., 2016;

Varian-Ramos et al., 2013). We lack information on Hg dynamics on fish and on fish fat content in different years and locations, which affect the Hg content and food quality ratio and probably is adding uncertainty in our results. Hydrological conditions could affect Hg dilution in fish during favorable growing seasons with deep inundation and result in a correlation between fish biomass and Hg content, and affect the relationship between food availability and exposure to Hg. Hydrological conditions can also influence Hg methylation and therefore MeHg concentrations. However, our results (Figs. S2, S3 and S4) suggest that was not the case, as the correlation between fish biomass and Hg in birds was low in every case and the correlation between Hg in birds and recession range was mid-low and particularly low in the largest data set. Analyses of a longer data set in the same system showed that while water depth and fish biomass were highly correlated, Hg in birds did not strongly correlate with either of them or with recession range (Zabala et al., 2020). This does not mean that such patterns do not exist but, at minimum there was no clear trend suggesting relationships that could bias our results or cause spurious correlations. These variables more likely show random interannual variation that simply adds uncertainty to our estimates.

In the case of low Hg exposures and low food availability, we saw an increase in predicted hatching success and fledglings per egg with increasing exposure to Hg (Fig. 2). In our study population, Hg exposure is probably influenced directly by food intake because food is the primary source of Hg for great egret nestlings. Therefore, within a certain range of food availability, [Hg] can reflect both nutritional condition and Hg exposure simultaneously. We believe that positive associations of nesting success with Hg in the low range of Hg values is therefore a marker for higher food intake and better nutritional status, rather than a hormetic effect (Heinz et al., 2012).

Generally, we caution that that the predicted variation in endpoints (Fig. 2) is relative rather than absolute, becoming less reliable at the extreme values of Hg of each scenario. We emphasize that our overall interpretation of these results is that interactions between food availability and Hg appear to be critical to understanding sub-lethal effects of Hg in field situations, and that the shapes and general trends we describe of the predicted relationships are more important than the absolute estimated values we provide for any given scenario. We also caution that our results show interactions between Hg exposure and food availability and variability in sub-lethal effects attributable to Hg at different food conditions, not net effects of Hg on reproduction.

Our results show that the associations of Hg exposure and reproduction can vary from positive to negative, depending on food availability. This provides a framework that potentially reconciles apparently contradictory results from natural settings. These contradictions have included better physiological status or body condition, or better performance in some breeding success metrics of individuals with higher pollutant concentrations (Bustnes et al., 2008; Herring et al., 2009; Provencher et al., 2016; Tartu et al., 2014); lack of detectable associations, at least in some breeding endpoints, in some individuals or populations exposed to relevant toxic concentrations (Pollet et al., 2017; Tartu et al., 2014; Taylor and Cristol, 2015; Zabala et al., 2019c); variability in effects of similar pollutant concentrations among years or categories of individuals (Brasso and Cristol, 2008); and historic records of successful breeding under higher concentrations of Hg (Frederick et al., 2002; Zabala et al., 2020).

Our results have several applied and relevant consequences for the design and interpretation of studies aiming to understand breeding impairment associated with exposure to contaminants in field conditions. **First**, we caution that studies that do not account for food availability (including all its components and not just abundance) or other relevant stressors are likely to fail to detect contaminant effects or to underestimate their role. Other stressors (thermal, behavioral, predator presence, disease, human disturbance) are also candidates that may strongly modulate the effects of contamination in the field (Brasso and Cristol, 2008; Bustnes et al., 2006; Holmstrup et al., 2010; Sih et al., 2004).

However, food stress is likely to be ubiquitous and paramount for understanding and predicting contaminant effects in natural settings. **Second**, there may often be no single best indicator of exposure to contaminants. Indicators of exposure to contaminant should be matched as closely in time as possible with endpoints, since exposure may change markedly with time or stage of reproduction. This assumption might be partially relaxed if there is a clear geographic structure in exposure and/or concentration of the relevant contaminant(s) in the environmental is stable in the short- or mid-term. **Third**, when there is no information on indicator-endpoint affinity, or the best indicator cannot be sampled, researchers should favor integrative, probabilistic endpoints that include elements of multiple parts of the reproductive cycle, over those that are indicative of success in only one stage of reproduction. **Lastly**, we advise against the use of count-based endpoints such as clutch-size, brood-size or fledglings per nest because of their inherent intractability for analysis.

CRediT authorship contribution statement

JZ and PF conceived and designed the study; IRJ designed study and field procedures of the nest-approach; JCT designed and performed fish monitoring; IRJ, SO, LG, PF and JZ performed field work, nest monitoring and Hg determination and processed data; JZ and PF analyzed data; and JZ wrote a first draft that was extensively revised, rewritten and amended by PF, JCR, IRJ, SO and LG.

Declaration of competing interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.148322>.

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