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# Estimation of Crayfish Abundance and Size-structure in Diets of White Ibis Chicks

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**Abstract.**—We investigated the use of crayfish gastroliths and exoskeleton pieces for quantifying crayfish abundance and size-structure in diets of White Ibis (*Eudocimus albus*) chicks in the Everglades. We then quantified crayfish and fish abundance from various small hard parts and intact fish heads in 23 boluses, taken from two nesting colonies in Water Conservation Area 3 (WCA 3) and Arthur R. Marshall Loxahatchee National Wildlife Refuge (Lox) in the Florida Everglades during May 2006. We determined that using gastroliths to estimate crayfish numbers in boluses had significant drawbacks; only a small fraction of field-caught crayfish bear gastroliths and the two crayfish species in the Everglades differ in percentage bearing gastroliths. In contrast, counts of crayfish rostrums and chelae pairs gave simple and similar estimates of crayfish in the boluses. The two colonies had strikingly different diets in May 2006; New Colony 3 (Lox) boluses were dominated by crayfish while birds from the Alley North (WCA 3) boluses were fish-dominated and had few crayfish. Using measurements of the crayfish rostrums we determined the size-structure of crayfish found in the diets of the New Colony 3 birds, and determined that the crayfish in the diet were relatively large (mean = 19 mm carapace length) when compared to the available crayfish in the marsh. These crayfish were also large relative to previous reports of crayfish found in White Ibis diets in the Everglades. Received 12 October 2007, Accepted 5 February 2008.

**Key words.**—crayfish, diet, *Eudocimus albus*, Everglades, gastrolith, *Procambarus alleni*, *Procambarus fallax*, rostrum, White Ibis.

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Whole bodies or skeletal remains of animal prey in boluses, esophagus, proventriculus, gizzard, and feces are frequently used to determine the dietary habits of waterbirds (e.g., Findholt and Anderson 1995; Falk *et al.* 2006; Seefelt and Gillingham 2006). Comparisons of diet data based on the source of information such as gut contents, pellets, or boluses, are common (Derby and Lovvorn 1997; Figueroa and Stappung 2003; Seefelt and Gillingham 2006), but the justification for using different prey parts or wholes, and the value of the information gleaned from each, is a subject of lesser investigation.

Some studies of waterbirds report only intact/identifiable animals found in the boluses (e.g., Kushlan 1979; Findholt and Anderson 1995; Smith 1997). However, intact prey found in boluses only offer information about prey that were expelled without being digested by adult or nestling. Unless the intact prey items are a representative sample of everything ingested, using intact prey alone, may provide biased estimates of diet composition. Furthermore if intact animals are scarce, many boluses will have to be collected

to gain adequate information about diet composition of a population.

In contrast to the use of intact animals, the various skeletal fragments and exoskeletons, while initially difficult to identify, may offer more information and a more representative sample of the thoroughly digested prey. For example, fish otoliths have been used to assess fish species composition in pellets of Double-crested Cormorants (*Phalacrocorax auritus*; Seefelt and Gillingham 2006).

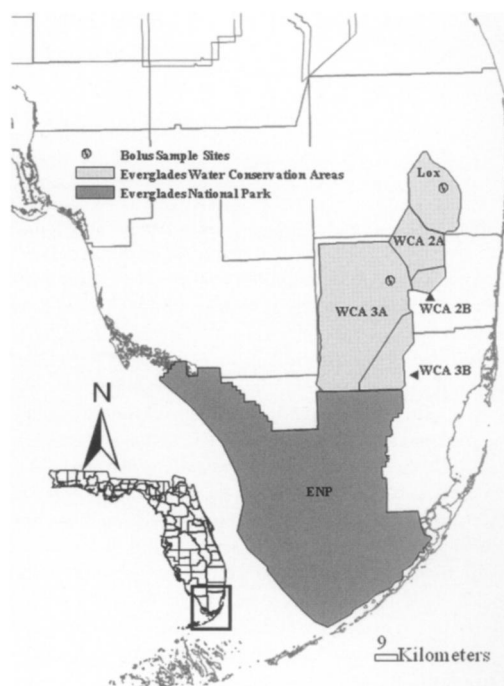
While crustaceans are consumed by a variety of waterbirds, their exoskeletons are shredded in boluses, making them difficult to count (Derby and Lovvorn 1997; Figueroa and Stappung 2003). Researchers quantifying crayfish in avian boluses have used different approaches; Derby and Lovvorn (1997) used exoskeleton incidence to determine the percent occurrence of crayfish in Double-crested Cormorant boluses, but they noted that they “could not be counted based on miscellaneous fragments.” Figueroa and Stappung (2003) used pairs of gastroliths (calcium pellets) to count crayfish in Great Egret (*Ardea alba*) boluses. Gastroliths are dy-

namic, paired calcified parts that serve a calcium storage function for molting crayfish (Pavey and Fielder 1989; Zanutto and Wheatley 2003). Because of their dynamic nature, not all crayfish are expected to have gastroliths, and their usefulness for quantifying crayfish in bird diets may be limited. To determine how many crayfish have been consumed the gastrolith counts need to be adjusted for the frequency of gastrolith-bearing crayfish. If different species of crayfish are present, as in the Everglades (Hendrix and Loftus 2000), the different species must also be examined to determine whether gastrolith incidence is equivalent.

Here we report our efforts to develop methods allowing quantification of crayfish (*Procambarus* spp.) from the remains found in White Ibis (*Eudocimus albus*) boluses collected from nestlings in the Florida Everglades. Crayfish are a key constituent of White Ibis diets in the Everglades (Kushlan 1979; Kushlan and Bildstein 1992) but ibis may switch to consuming fish when they are highly concentrated (Kushlan 1979). Using gastroliths, exoskeleton remains, and otoliths we contrasted the abundance of crayfish and fish in boluses taken from two White Ibis colonies in different regions of the Florida Everglades. By measuring exoskeleton parts, we also reconstructed the size-structure of consumed crayfish and compared it to the size-structure of crayfish available in the adjacent wetlands.

#### METHODS

Research was conducted during the 2006 dry season (Nov-May) in the Florida Everglades on wading birds nesting in the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Lox), Palm Beach County, and in Water Conservation Area 3 (WCA 3), Broward County, Florida (Fig. 1). The Alley North (26°11' N, 80°31' W) and New Colony 3 (26°31' N, 80°17' W) colonies in WCA 3 and Lox were visited routinely (Fig. 1). WCA 3 is dominated by ridge and slough topography, with shallower water on sawgrass ridges and deeper water in sloughs, which contain both submerged and emergent aquatic plants. Lox has relatively shallower sloughs dominated by emergent rushes and extensive tree islands. The colonies are 45 km apart and the water depths in the two regions are independently managed. Observations of White Ibis foraging flights from these two colonies indicate that their feeding grounds did not overlap; New Colony 3 birds flew  $6.3 \pm 0.7$  km (mean  $\pm$  SE) to forage while Alley North birds flew  $16.5 \pm 0.5$  km (J. Beerens, unpublished data). White Ibis were nesting in large numbers (>3,000 individuals) at each site.



**Figure 1.** Bolus sampling sites of White Ibis chicks during the 2006 breeding season in the Florida Everglades. Lox = Arthur M. Loxahatchee Wildlife Refuge. WCA 3A = Water Conservation Area 3A. ENP = Everglades National Park.

#### Gastroliths in Field-caught Crayfish

Six hundred and eighty-six crayfish were collected from the Everglades wetlands during the dry seasons of 2005 and 2006. All of them were dissected to determine the frequency with which naturally occurring crayfish bear gastroliths. The dry season is when most ibis occur in the Everglades and is thus when most crayfish are consumed by the birds (Kushlan 1979). Crayfish were captured using 1-m<sup>2</sup> throw traps (Kushlan 1981) during the dry season (ibis nesting season) as part of a prey-concentration study taking place in multiple water management units throughout the ecosystem. Throw trapping gives good estimates of crayfish density and size structure in these wetlands (Dorn *et al.* 2005). Each crayfish was preserved in 70% ethanol after being fixed in PREFER (Anatech, Battle Creek, Minnesota). All crayfish were identified as either slough crayfish (*Procambarus fallax*) or Everglades Crayfish (*P. alleni*), measured to the nearest 0.1 mm carapace length (CL) and dissected to look for gastroliths. Both sides of each crayfish were checked for gastroliths; gastroliths were found associated with the cardiac stomach just posterior to the eyes. Although all crayfish have small soft membranes near the place that gastroliths develop, the gastroliths were identified as hardened calcareous pellets by squeezing them with the forceps. Gastrolith diameters (largest dimension) were measured (rounded to the nearest 0.05 mm) using a dissecting microscope. Gastrolith frequency of occurrence for each species was determined, and the relationships between crayfish length

and gastrolith frequency, as well as crayfish length and gastrolith size were explored.

#### Crayfish and Fish Counts in White Ibis Boluses

Twenty-three boluses from two colonies of nesting White Ibis were collected between 2-17 May 2006 when the nestlings were between 10-20 d old (*sensu* Desanto *et al.* 1990). Boluses were collected from eleven and twelve chicks respectively from Alley North and New Colony 3, and the contents were preserved in 70% ethanol. Boluses were collected opportunistically while conducting concurrent physiological research in each of the two nesting colonies. White Ibis chicks that were sampled were captured after boluses were detected in their esophagi. In most cases chicks readily regurgitated the entire bolus upon capture as evidenced by the lack of bolus present when their esophagus was palpated. Bolus collection from several chicks required gentle massaging of the esophagus and touching of their bill to induce regurgitation. Occasional observations of boluses being regurgitated in other parts of the colony indicated that the boluses looked similar to the sampled boluses. The diets likely reflect diets of both chicks and adults as the condition of the prey remains (lots of bare otoliths, fish bones, and crayfish fragments) suggests partial digestion occurred in the adults.

In the lab the boluses were poured through a 0.5-mm mesh net, washed, and then sorted to find crayfish parts and fish otoliths. Each sample was sifted twice; each time crayfish chelae, rostrums, and gastroliths (Fig. 2) and fish otoliths and intact fish heads/bodies were removed. The rostrum of a crayfish is the portion of the carapace anterior to the eyes and terminating in the acumen.

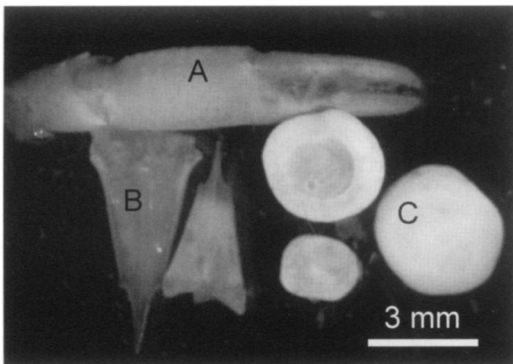
Four estimates of crayfish abundance were made. Chelae-based crayfish estimates were made by assuming that each crayfish had two chelae when captured and fed to the chick, so the total count was divided by 2 (rounding up to the nearest whole crayfish in the case of odd counts). Because each crayfish has only one rostrum, the count was used as an estimate of the crayfish in each bolus. Two gastrolith estimates of crayfish were made. The first estimate was a simple count of gastroliths divided by 2, given their paired nature. The first estimate only quantified gastrolith-bearing crayfish but not total crayfish in each bolus. Given the small percent-

ages of gastrolith-bearing crayfish, extrapolation on the basis of gastroliths, up to the total crayfish in each bolus was impossible (i.e., some diets had no gastroliths, but clearly contained crayfish). The extrapolation of total crayfish consumed based on gastroliths was scaled up to the entire group within a colony (i.e., could not estimate bolus-bolus variation) for purposes of comparison with other estimates. After estimating how many gastrolith-bearing crayfish were in each bolus, the total crayfish eaten by the group was determined. This extrapolation was made by assuming adult ibis do not selectively forage based on gastrolith presence. Because gastrolith frequency of occurrence in wild-caught crayfish differed between the two crayfish species, a determination of the species present in the boluses was necessary. Three pieces of evidence suggested that all of the crayfish in the boluses were *P. fallax*. First, five individual *P. fallax* were identifiable on the basis of crayfish reproductive organs found on partially digested animals. Second, during the time of the year when these birds were feeding (early May) most *P. alleni* habitats were dry and most *P. alleni* would have been well below ground. Third, the large slough habitats near these colonies are dominated by *P. fallax* (N. Dorn and J. Volin, unpublished data). For each colony the summed estimate from gastrolith counts was multiplied by an extrapolation factor taken from our empirical estimate of gastrolith incidence for *P. fallax*.

Fish estimates were made by summing counts of otoliths (counts / 2) and heads/bodies. Preliminary observations of the fish species composition were made by comparing otoliths to a reference collection of otoliths extracted from common species of freshwater fish in the Everglades. Fish and crayfish counts per bolus were compared for the two colonies treating each bolus as a unit of observation. Because the variances and residuals departed from linear model assumptions the data were analyzed with adjusted ANOVAs (Welch's weighted ANOVA—Sokal and Rohlf 1995) and rank-based non-parametric Wilcoxon tests. The tests gave equivalent results and only the Wilcoxon tests are mentioned in the Results section.

#### Crayfish Size-structure

Size-structure of the consumed crayfish from New Colony 3 was estimated and compared to available prey collected from nearby marshes within the range of normal foraging flights (i.e., crayfish population size-structure in Lox). Rostrum widths were measured to the nearest 0.05 mm using the dissecting scope. Rostrum widths were measured perpendicular to the long axis of the carapace at the base of the acumen. A regression that predicts crayfish CL from rostrum width ( $R^2 = 0.94$ ,  $P < 0.001$ ,  $N = 38$ ) was constructed using an independent sample of intact *P. fallax*. The regression was used to estimate sizes of crayfish in the boluses. Each crayfish was then placed into one of four five-mm size classes (10-15, 15-20, 20-25, and >25 mm). The first category was started at ten mm because the smallest crayfish estimated from the samples was 9.7 mm; indicating that ten mm crayfish are potential prey. The available crayfish sizes were estimated using crayfish size-structure data from throw trap samples taken from Lox during mid-April 2006 (T. Robertson and J. Trexler, unpublished data). Chesson's  $\alpha$  (Chesson 1983) for each size-class was calculated for New Colony 3 to look for evidence of size-selective foraging:  $\alpha = r_i/p_i/\sum r_i/p_i$ , where  $r_i$  is the proportion of con-



**Figure 2.** Crayfish chela (A), rostrums (B) and gastroliths (C) used to quantify crayfish abundance in boluses.

sumed crayfish in size-category  $i$ , and  $p_i$  is the proportion of available crayfish in the size-category  $i$ . A chi-square goodness-of-fit test was used to compare crayfish sizes to expected sizes under a null hypothesis of non-selective foraging (Sokal and Rohlf 1995).

RESULTS

Gastroliths in Field-caught Crayfish

Gastroliths were not common in field-caught crayfish of either species and differed markedly between *P. fallax* and *P. alleni*. Thirty-three percent of *P. fallax* (35 of 107) had gastroliths while only 7.5% (44 of 581) of *P. alleni* had gastroliths. Gastrolith frequency in field-caught crayfish also tended to be greater among smaller size-classes, especially for *P. alleni* (Table 1). Gastrolith frequency also differed somewhat by year of collection (2005 vs. 2006: *P. alleni* 4.7% vs. 11%, *P. fallax* 37% vs. 29.5%) and these differences could not be simply attributed to annual variation in crayfish size. For *P. fallax*, gastrolith size and crayfish size were positively related (crayfish CL predicted from gastrolith size:  $R^2 = 0.70$ ,  $P < 0.001$ ,  $N = 35$ ).

Crayfish and Fish Counts in White Ibis Boluses

Fish were more abundant in boluses from the Alley North colony ( $U = 189$ ,  $P < 0.05$ ) while crayfish were more abundant in diets of birds found in New Colony 3 (rostrum estimate  $U = 88.5$ ,  $P < 0.01$ , Fig. 3, Table 2). All three estimates of crayfish indicated the same basic differences in crayfish abundance. Rostrum and chelae-based estimates of crayfish were highly correlated ( $r = 0.97$ ,  $P < 0.001$ ) while correlations between gastrolith estimates and the other two estimates were somewhat weaker ( $r$  values = 0.77-0.78,  $P < 0.001$ ). Chelae-based estimates indicated

an eleven-fold difference between colonies in mean crayfish per bolus, rostrums indicated a seven-fold difference, and gastroliths indicated a six-fold difference.

Only 13% of fish counted from the boluses were identified based on heads and bodies; most were recognized by extracting otolith pairs. Brief inspection of otolith shapes and fish heads indicated that the diet composition was a mixture of small-bodied species including Eastern Mosquitofish (*Gambusia holbrooki*) and flagfish (*Jordanella floridae*) as well as larger-bodied species of sunfish (*Lepomis* spp.). Two of the boluses from New Colony 3 did not contain any crayfish or fish and seemed to reflect terrestrial diets (mole crickets: Gryllotalpidae and cockroaches: *Pycnoscelus* sp.).

Crayfish Size-structure

Mean size of crayfish in all ibis boluses was estimated from rostrum sizes at 18.7 mm CL  $\pm$  0.4 SE (appr. 3.3 cm total length). The size-structure of crayfish found in the boluses from New Colony 3 indicated over-representation of size classes  $>20$  mm CL (Chesson's  $\alpha$  values = 0.37, Fig. 4) and under-representation of the smallest size class (10-15 mm,  $\alpha = 0.04$ ;  $\chi^2 = 43.0$ ,  $P < 0.005$ ).

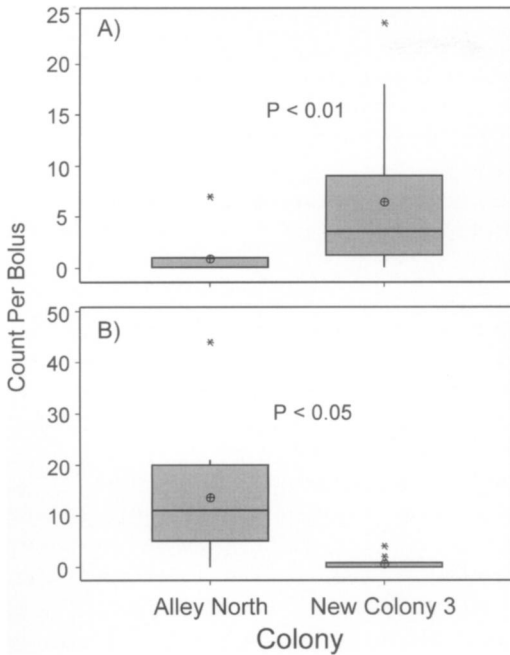
DISCUSSION

Gastrolith-based estimates of crayfish required several steps that made them less attractive candidates than other approaches for quantifying the number of crayfish in boluses. Because gastroliths are dynamic hard parts, only a fraction of a crayfish population bears gastroliths at any one time (Rao *et al.* 1977; Pavey and Fielder 1989; this study); estimating the number of crayfish consumed

**Table 1. Proportion of field-caught *Procambarus fallax* and *Procambarus alleni* with gastroliths. Crayfish were collected from various regions of the Everglades (including ENP and Water Conservation areas) in the dry seasons (Feb-May) of 2005 and 2006. The sample sizes dissected for each size class are listed in parentheses.**

	Size class (mm carapace length)					
	<12	12-14	14-16	16-18	18-20	>20
<i>P. fallax</i>	0.26 (19)	0.52 (21)	0.31 (22)	0.26 (19)	0.33 (12)	0.21 (14)
<i>P. alleni</i>	0.19 (54)	0.15 (99)	0.05 (95)	0.06 (80)	0.06 (53)	0.03 (200)





**Figure 3.** Median and interquartile ranges of A) crayfish and B) fish counts in boluses of White Ibis chicks found in Everglades colonies Alley North (WCA 3; N = 11) and New Colony 3 (Lox; N = 12) in May 2006. Counts were based on crayfish rostrums and fish otolith pairs plus intact heads/bodies. Differences between colonies for both prey types were determined using non-parametric Wilcoxon tests. Means and outliers are indicated with the box plots.

therefore required a separate estimation of that frequency from field caught crayfish as in this study. When we investigated gastrolith presence in field-caught crayfish we found that the two species of *Procambarus* in the Everglades did not bear gastroliths with the same frequencies and both frequencies were <40%. Further, for *P. alleni*, the probability of having a gastrolith differed markedly with size. Using gastroliths alone to estimate the number of crayfish in a bolus that did not contain gastroliths was impossible. When extrapolations were used to estimate the total number of crayfish eaten by the entire group of chicks sampled from a colony the numbers became similar to those of the chelae and rostrum-based estimates. Further studies might investigate these issues for other species of crayfish in other systems, but given all of the caveats and the dynamic nature of the gastroliths we caution against unqualified

use of gastroliths for quantifying crayfish in bird diets.

Estimates of crayfish from rostrum counts and chelae pairs were highly correlated with one another suggesting either one could be used to count crayfish in the diets of wading birds. Because some crayfish have less than two chelae, but all crayfish normally have a rostrum, estimates from rostrum counts require one less assumption. Consequently, we suggest using rostrums to estimate crayfish consumption in boluses for their simplicity and accuracy. The absolute accuracy of these estimates could only be tested by feeding crayfish to ibises and recovering the shredded parts. Sizes of rostrums can also be used to estimate the size-structure of the consumed crayfish. The size of crayfish consumed by ibis at New Colony 3 suggests they were selectively feeding on the larger size-classes. The average length of crayfish eaten (3.3 cm) by birds in this study was much larger than the 2.2 cm average previously reported for White Ibis feeding in the Everglades (Kushlan 1979). Selection of larger crayfish could result from active selection for energetically favorable sizes, similar to the selective behavior of other wading birds like storks and herons feeding on fish (Ogden *et al.* 1976; Trexler *et al.* 1994). However, White Ibis are tactile foragers and could be missing smaller animals such that the observed selectivity is passive. Grant (1981, and references therein) suggests probing shorebirds selectively feed on infaunal amphipods by missing small individuals. The mechanism responsible for this pattern will require more investigation. We know of no differences in crayfish behavior with size that should otherwise produce this pattern.

Most studies of ibis suggest they feed primarily on crustaceans and especially crayfish (Kushlan and Bildstein 1992). While the New Colony 3 had a diet dominated by crayfish, the Alley North colony in the Everglades had large numbers of fish and few crayfish. The small sample sizes in this study could possibly represent a biased sample of the diets of these birds, and there may be diet shifts during the nesting season as well (e.g., Bildstein *et al.* 1990). However, the differences in local wetland crayfish standing stocks are consistent

**Table 2.** Estimates of crayfish consumed by the group within each colony (all boluses combined) and median # per bolus in 23 White Ibis boluses collected in May 2006. The number of boluses collected per colony (*N*) is listed at the top of each column. When adjusting the gastrolith counts based on the fraction of gastrolith-bearing crayfish in the wild population it was not possible to make bolus-level estimates of the number of crayfish (see text). Wilcoxon two-sample test statistics are included.

	Sum		Median		<i>U</i>	<i>P</i>
	Alley North	New Colony 3	Alley North	New Colony 3		
	<i>N</i> = 11	<i>N</i> = 12	<i>N</i> = 11	<i>N</i> = 12		
Chelae	6	74	0.0	3.5	85.5	0.002
Rostrums	10	77	0.0	3.5	88.5	0.005
Gastroliths	3	21	0.0	1.0	94.5	0.015
Gastroliths extrapolated	10	71	na	na	na	na

with the differences in crayfish use. The Lox wetlands around New Colony 3 had crayfish standing stocks of 0.5 to >1 g (dry mass)/m<sup>2</sup> preceding and during the nesting season while the WCA wetlands near the Alley North Colony (WCA 3A) had average crayfish standing stocks generally <0.2 g/m<sup>2</sup> (Anonymous 2008; N. Dorn and J. Volin, unpublished data). The different prey bases suggest crayfish may be heavily used in areas where they are more abundant. Kushlan (1979) suggested that fish are never selectively consumed and are only used substantially when they become highly concentrated. If his contention is correct our observations suggests that at the same time in May, the Alley North colony found high concentrations of fish in some part of the ecosystem while the New Colony 3 birds were feeding on a steady diet of crayfish in an area with relatively deeper water and/or

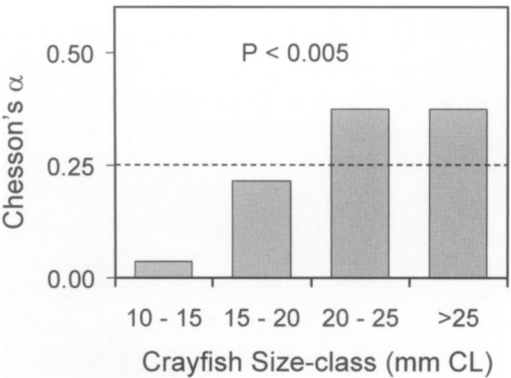
fewer fish. The relative success of these colonies cannot be evaluated at this time, but future studies of the success of these colonies might include diet information over more growing seasons, especially during and after particularly strong droughts like those associated with pulsed breeding success (Frederick and Ogden 2001). Combining nesting success with carefully quantified diet studies and habitat use could provide new information about the value of different diet types and how they relate to nesting success.

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**Figure 4.** Size-selective feeding of White Ibis on *Procambarus* crayfish as measured by Chesson's  $\alpha$ . The dashed line indicates neutral association ( $\alpha = 0.25$ ). The listed P-value is from a Chi-square goodness-of-fit test.

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