Fisheries Research 161 (2015) 1–7

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

X-ray imaging as a time-saving, non-invasive technique for diet analysis

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ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form 20 May 2014
Accepted 25 May 2014
Handling Editor George A. Rose
Available online 9 July 2014

Keywords:
Dietary patterns
Digital radiography
Fish
Nonlethal
Stomach-content analysis

ABSTRACT

Dietary patterns of animals have a long-recognized importance in ecology and evolution, with numerous and diverse applications. While many methods of diet assessment exist, the most common method of direct diet examination for most small vertebrates is stomach-content analysis, using labor-intensive surgical removal of the gut following death. Methods that can reduce the time required to collect diet information without necessarily sacrificing specimens could prove invaluable for a range of applications. We evaluated digital X-ray imaging as a non-invasive method for examination of stomach contents of small fishes. Based on both a feeding experiment and examination of field-collected preserved specimens, we found that digital radiography consistently revealed the presence of moderate- to high-density prey items in the stomach, such as small arthropods. Moreover, X-ray imaging allowed for rapid identification of some particular prey items such as detritus, dipteran larvae, ostracods, hard-shelled molluscs, and small fish. However, this method failed to detect some low-density prey items present in some stomachs, and could not be used for precise taxonomic identifications in most cases. Overall, we found that digital X-ray images can be quickly acquired from anesthetized or preserved animals, permit rapid identification of certain prey items, and facilitate digital data archives. Future studies that employ this method should first "ground-truth" the radiological signatures of prey items observed within a given study using stomach-content analysis, which then permits rapid data collection strictly using X-ray images. This method can provide information useful for determining the inclusion of certain prey items in diets, even quantifying particular taxonomic groups of prey (% occurrence, % by number). Thus our results indicate that for certain study goals, X-ray radiography may provide a time reducing, non-invasive technique for diet analysis of small vertebrates.

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1. Introduction

The measurement and understanding of dietary patterns in animals has central importance in ecology, evolutionary biology, conservation, and management. Diet analysis comprises a long-standing tool for addressing a range of questions, such as community assembly, trophic relationships among species, habitat use, management of threatened, game, or commercially harvested species, and resource competition's role in driving major ecological and evolutionary patterns (e.g. Bolnick et al., 2003; Collar et al., 2009; Morin, 2011; Odum, 1983; Polis and Winemiller, 1996; Schluter, 2000; Schoener, 1971). A number of methods exist for assessing animal diets, such as visual observations of feeding, morphological and molecular identification of prey taxa in feces and stomachs, stable isotope analysis, and lipid analysis (e.g. Hyslop, 1980; Peterson and Fry, 1987; Valentini et al., 2009). For small vertebrates, especially fishes, amphibians, and reptiles, morphological examination of stomach contents is the most commonly employed technique for direct diet analysis. Nonlethal techniques, such as stomach flushing using tubes or gastric lavage, is sometimes possible for larger individuals (e.g. Giles, 1980; Light et al., 1983), but post-mortem dissection represents the most common approach. In fisheries research and management, stomach dissections are regularly used for the analysis of diet.

Typically, the stomach/intestines are surgically removed from freshly-killed or preserved specimens, partially digested prey items extracted, and taxonomic identification of prey accomplished using microscopic examination. Once prey items have been identified, a range of approaches can be used for statistical analysis of diet (review of methods are beyond the scope of this paper, see Cortes, 1997; Hyslop, 1980). This method requires the death of the specimen and is time and labor intensive, and requires specialized training to process and identify the contents. Thus,

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http://dx.doi.org/10.1016/j.fishres.2014.05.015
0165-7836/Published by Elsevier B.V.

alternative methods that can utilize live specimens or reduce the time required to collect diet data would be advantageous, as this would streamline the collection of diet information without sacrificing specimens.

Here we examined digital X-ray radiography as a rapid, non-invasive method for assessing diet of small fishes. A number of recent advances in digital radiography make this assessment timely: e.g. increases in resolution and magnification permit the detection of very small objects of low density, prices of digital radiography equipment has recently dropped considerably, portability of X-ray units has increased substantially, and many universities already have digital X-ray machines capable of at least moderate resolution of small vertebrates. While previous studies have employed X-ray radiography in the context of animal diets (e.g. rates of feed intake and gastric evacuation; Talbot and Higgins, 1983; McCarthy et al., 1992; Jobling et al., 1993, 2001), no previous study has examined the utility of this technique for identifying diet items of small vertebrates.

In this study, we investigated gut/stomach contents using X-ray imaging in four small fish species (15–80 mm standard length): Eastern mosquitofish (*Gambusia holbrooki*, Girard 1859), Bahamas mosquitofish (*G. hubbsi*, Breeder 1934), Trinidadian guppy (*Poecilia reticulata*, Peters 1859), and Hart's killifish (*Anablepsoides hartii*; formerly *Rivulus hartii*, Boulenger 1890). Collectively, these omnivorous species are known to exhibit a broad diet, including detritus, algae, aquatic and terrestrial insects, crustaceans, molluscs, and even juvenile fishes. We hypothesized that X-ray images would reveal the presence of prey contents in the stomach, and permit the detection and identification of some broad groups of prey taxa based on their dense body parts (e.g. shells, exoskeletons, bones), such as molluscs (e.g. gastropods, bivalves), crustaceans (e.g. ostracods, shrimp), insects (e.g. chironomids, beetles), and vertebrates (e.g. fish, tadpoles).

2. Materials and methods

Our goal was to determine whether X-ray imaging could reveal the presence or absence of prey in stomachs of small fishes, and allow the identification of 5 different types of prey items that vary in density (mass per unit volume): (1) soft homogeneous prey (e.g. algae, detritus), (2) weakly shelled arthropods (e.g. small shrimp, ants), (3) moderate-density arthropods (e.g. ostracods, beetles), (4) hard-bodied prey (e.g. shelled molluscs, crabs) and (5) vertebrates (e.g. small fish, anurans). We took a two-pronged approach to accomplish this: we conducted a feeding experiment with live fish to directly assess the accuracy of diet identification using X-ray imaging, and we examined preserved, wild-caught fish specimens to evaluate the utility of the approach for the examination of natural dietary patterns.

2.1. Feeding experiment

We performed a feeding experiment using 21 live individuals of *G. holbrooki* and 3 individuals of *A. hartii*. All fish were collected from the wild (*G. holbrooki*: North Carolina, USA; *A. hartii*: Trinidad) and housed in 38-L aquaria in common laboratory conditions. Prior to the feeding experiment, fish were placed individually into separate 8-L tanks and starved for 48 h. For *G. holbrooki*, we assigned three adult females at random to each of seven diet treatments: (1) no prey: starved, (2) soft, low-density homogeneous prey: TetraMin Pro flakes, (3) low-density crustaceans: live *Artemia* sp. nauplii, (4) low-density insects: live ants, (5) moderate-density insects: thawed bloodworms (*Chironomus tetans*), (6) hard-shelled prey: live snails (*Physa acuta*), and (7) vertebrate: one live *G. holbrooki* juvenile. We fed two live *P. reticulata* juveniles (3–4 mm SL) to *A. hartii* to test for detection of vertebrate consumption in this species.

Within 1 h of feeding, we X-rayed each individual and saved a digital image. We placed each live fish into a small, moist plastic bag, laid the fish on its side within a petri dish, and set the dish in the X-ray machine to capture a lateral image. We used a custom-built digital X-ray unit comprising a micro-focus X-ray source (Hamamatsu L6731-01) and a digital X-ray detector (PaxScan 2520E) housed in a lead-shielded cabinet, set to 45 kV and 40 μA. Radiation exposure to each fish was low, approximately 25–50 mrem – roughly equal to a human dental X-ray for comparison. Fish were then immediately placed into a recovery tank. Removal from water, X-ray imaging, and return to recovery tank typically only required approximately 30 s. Identification of all stomach contents based on digital X-ray images was conducted blind of fish ID.

Table 1
Collection and sample size information for wild-caught adult specimens examined in this study.

Species	Collection location	*N*
Gambusia holbrooki	Melbourne, Florida, USA	60
	James Island Park, South Carolina, USA	60
Gambusia hubbsi	East Twin blue hole, Andros Island, Bahamas	40
	West Twin blue hole, Andros Island, Bahamas	40
	Hubcap blue hole, Andros Island, Bahamas	40
Poecilia reticulata	Kahala, Oahu, Hawaii, USA	120
Anablepsoides hartii	Arima Valley, Trinidad	23

2.2. Preserved specimens

We examined digital X-ray images (using method and equipment described above) of wild-caught specimens preserved in 70% ethanol to assess the ability to detect natural dietary patterns of preserved small fish with X-ray images. We examined 120 *G. holbrooki*, 120 *G. hubbsi*, 120 *P. reticulata*, and 23 *A. hartii* (see Table 1 for collection and sample size details).

For each preserved specimen, we attempted to determine contents of the stomach based on the X-ray image. After viewing a number of images, six natural categories emerged from our identifications: (1) no prey contents, (2) soft homogeneous prey (e.g. algae, detritus), (3) low-density prey (e.g. branchiopods, ants), (4) moderate-density prey (e.g. ostracods, dipterans), (5) shelled mollusc prey, and (6) vertebrate (fish) prey. To determine the accuracy of diet identification, we dissected three randomly selected specimens of each species from each diet category using the traditional method of surgically removing the gut and identifying the contents under a microscope (Leica S8 APO stereoscope). We then compared the diet classification from X-ray images to that from direct morphological identification.

3. Results

3.1. Feeding experiment

The guts of all six starved fish appeared empty in X-ray images (Fig. 1a and b). We could not detect flakes or *Artemia* sp. nauplii with our X-ray images (Fig. 1c). In two out of the three fish fed ants, small hard parts of prey were visible in their guts in the X-ray Images – presumably reflecting broken pieces of the ants – but these were difficult to identify as ants or even as insects (Fig. 1d). Based on X-ray images, we accurately detected prey items in all fish fed bloodworms (Fig. 1e), *P. acuta* snails (Fig. 1f), newborn *G. holbrooki* (Fig. 1g), and juvenile *P. reticulata* (Fig. 1h). Thus, we consistently could not detect the two lowest density prey types, but could detect the three highest density prey types; ants appeared