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A Simple and Rapid Method to Measure Food Intake in Fish Using Brine Shrimp

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Abstract

Food intake is a critical parameter in studies of energy balance, obesity, and metabolic disease, but previous methods to measure food intake in model fish species such as zebrafish (*Danio rerio*) have been labor intensive or indirect. In this study, we describe a simple and rapid method to measure food intake using freshly hatched brine shrimps as food source, followed by rapid collection using filtration of system water and quantification of collected shrimps using a photometric assay to measure extracted shrimp carotenoid content.

Keywords: zebrafish, *Danio rerio*, food intake, *Artemia*, brine shrimp, carotenoid

Introduction

Zebrafish (*Danio rerio*) are often used to study the molecular mechanisms underlying metabolic disorders.[1–3] Accurate measures of food intake are critical for these studies. Previously described methods have included using video imaging, feeding fish flakes doped with fluorescent dyes to measure internalized fluorescence, or counting the number of newly hatched brine shrimps consumed using a light microscope.[4-6] In this study, we describe a rapid method to sensitively measure food intake in zebrafish by quantifying the typical orange-red color of brine shrimp carotenoids[7,8] in a photometric assay (Fig. 1A).

Materials and Equipment

Complete details of the materials and equipment used in this study, as well as the fish husbandry and rationale for using brine shrimps to measure food intake, are provided in the Supplementary Data of this article. Materials required for this assay include adult zebrafish, *Artemia* brine shrimp cysts, preconditioned system water, Instant Ocean Sea Salts, ~400 mL capacity plastic cups, analytical balance, paper coffee filters, plastic funnels with the inner diameter of the stem orifice of ~5 mm, and high-performance liquid chromatography (HPLC) solvent grade hexane and methanol.

Generation of Brine Shrimp Solution

In total, 270 g of Instant Ocean Sea Salts is dissolved in 12 L of distilled water and (28°C) agitated with bubble aeration. Then, 50 mL of freeze-dried shrimp cysts are added to the water and allowed to grow 24 h. Shrimp solution for feeding is generated

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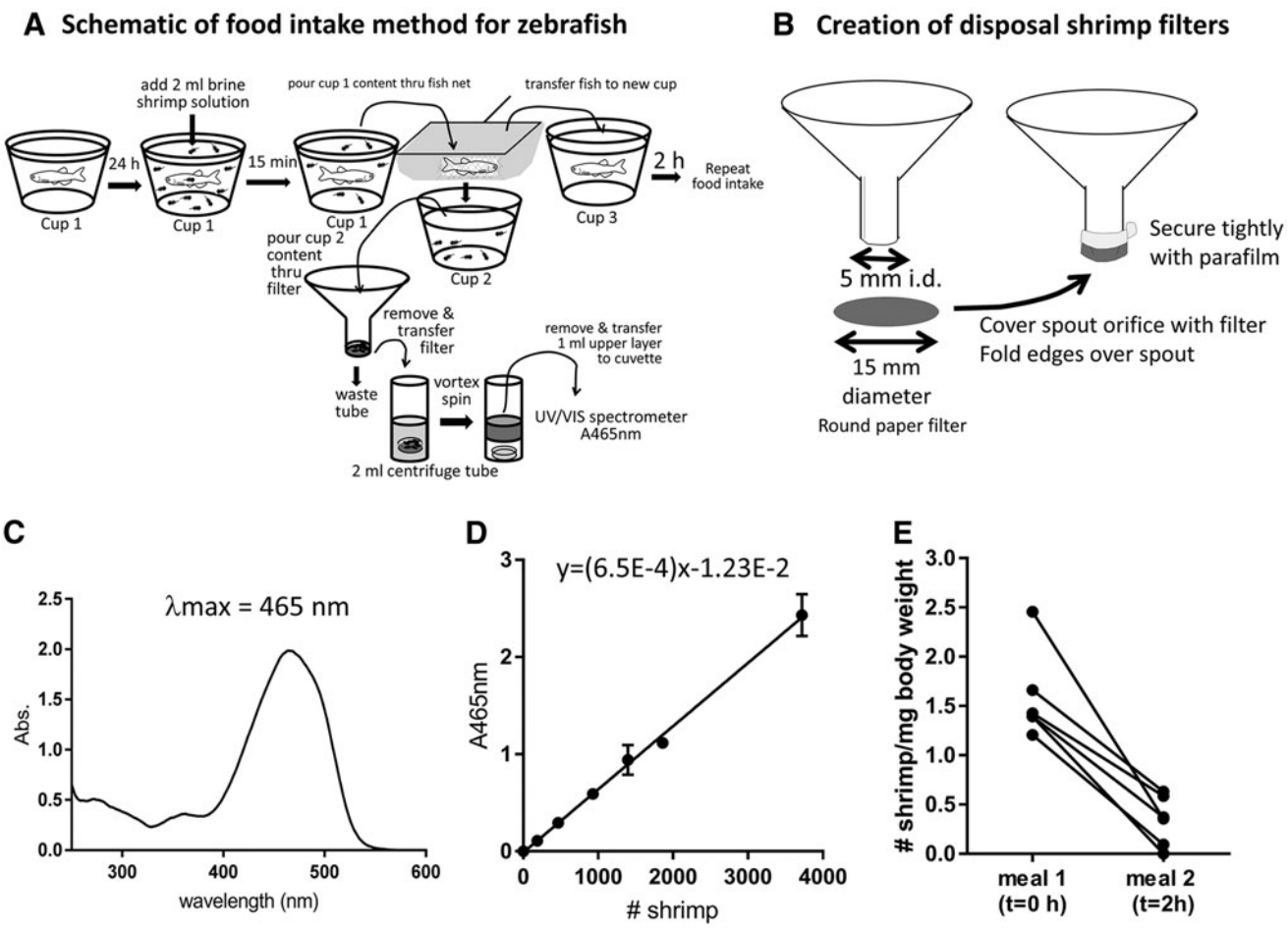

FIG. 1. **(A)** Schematic for measurement of zebrafish food intake. **(B)** Schematic for creation of disposable shrimp collection filters. Fifteen millimeter round circles are cut from paper coffee filters and attached to the stem orifice of a plastic funnel (5 mm inner diameter) using parafilm to secure the filter. **(C)** UV/vis spectrum of extracted carotenoids from brine shrimps. **(D)** External calibration curve measuring A465 nm versus number of input brine shrimps. **(E)** Normalized food intake (number of brine shrimps per milligram body weight) of six 24-h fasted male fish for meal 1 (time = 0 h postfast) and meal 2 (time = 2 h postfast).

by harvesting resulting nauplii using a small mesh filter, washing, and diluting in 3 L of water. Air is bubbled into the shrimp solution, until they are fed to the fish, to maximize viability.

Generation of External Calibration Curve

The concentration of brine shrimps in the shrimp solution was determined by manually counting triplicate 100 μL aliquots under a light microscope (186 ± 6 shrimps). Triplicate 100, 250, 500, 750, 1000, and 2000 μL aliquots of this shrimp solution, calculated to represent 186, 465, 930, 1395, 1860, and 3720 shrimps, respectively, were then used to create a calibration curve for all subsequent experiments. Each aliquot was added to 150 mL of system water and the shrimps were collected as follows: 150 mm diameter rounds cut from disposable paper coffee filter were placed over the bottom of plastic funnel spouts and the edges of the filter secured to the sides of the spout using parafilm (Fig. 1B). Solutions containing shrimps were poured through the filter and any residual water on the filter was pushed through using a syringe plunger. After removing the parafilm, the paper filter with the captured shrimps was transferred to a 2 mL polypropylene centrifuge tube. The funnel spout interior was washed with 0.5 mL methanol to ensure that all shrimps were transferred to the 2 mL tube.

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Then, 1 ml hexane was added to the tube with filter, the tube was capped and vortexed rigorously for >3 min, 0.5 mL water was added, the solution vortexed for 1 min, and tubes were centrifuged for 20 min at 1500 *g*. The upper phase (~ 1 mL) was transferred to a UV/vis spectrometry cuvette and the λ_{max} (465 nm) was established by scanning from 200 to 600 nm (Fig. 1C). The A465 nm was then determined for each sample. If absorbance was >1.0, the solution was quantitatively diluted and final total absorbance calculated using this dilution factor. Slope of the calibration curve was calculated using GraphPad Prism 7.04 (Fig. 1D). More discussion about the external calibration curve is provided in the Supplementary Data of this article.

Measurement of Food Intake and Satiety

Wild-type zebrafish (six males) were weighed and then fasted for 24 h in individual cups holding 150 mL of system water (Fig. 1A). Each fish was fed 2 mL of freshly prepared shrimp solution for 15 min followed by adding another 1 mL of the shrimp solution to the same cup for another 15 min (total 30 min). Number of input shrimps were quantified by adding the same volume of shrimp solution to three cups without fish. After 30 min, the contents of the cup (including the fish) were poured through a fish net into a collection cup. The mesh of the fish net was sufficiently wide to allow brine shrimp to pass through unimpeded. The fish was transferred to a new plastic cup containing 150 mL of fresh system water.

The shrimps in the collection cup were then quantified by funnel filtration and extraction of carotenoids as mentioned, with number of shrimps calculated based on the calibration curve as number of shrimps = (A465 nm +0.012) × 1537. Shrimps eaten = average shrimps in cup without fish − shrimps remaining in the cup after feeding. Number of shrimps eaten were normalized to fish body weight (Fig. 1E) (see Supplementary Data for the rationale for this normalization). The relative amount of fish eaten in this meal was considered a measurement of satiation. To measure the effect of the initial meal on satiety, 2 h after start of meal 1, the fish were again fed 2 mL of shrimp solution for 15 min and another 1 mL of shrimp solution for a total of 30 min and food intake was again determined (see Supplementary Data for our rationale for measuring both satiation and satiety.). On average, food intake for meal 2 was 21% of meal 1.

Authors' Contributions

Z.M. designed and performed the experiments, analyzed the data, and wrote an initial draft of the article. H.H.T. refined the procedures and performed experiments. C.S. conceived the use of carotenoid absorbance to quantitate shrimps. S.S.D. conceived the project, assisted in design of experiments and analysis of the data, and revised the article.

Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Data

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