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Effects of three frozen storage methods on wet weight of fish

Derek P. Crane[a,b,*], Christina C. Killourhy[c], Mark D. Clapsadl[d]

[a] School of Biological Sciences, Lake Superior State University, 650 West Easterday Avenue, Sault Ste. Marie, 49783 MI, USA
[b] Current affiliation: Department of Biology, Coastal Carolina University, 111 Chanticleer Drive East, Conway, 29526 SC, USA
[c] School of Biological Sciences, Lake Superior State University, 650 West Easterday Avenue, Sault Ste. Marie, 49783 MI, USA
[d] Buffalo State College, Great Lakes Center, 1300 Elmwood Avenue, Buffalo, 14222 NY, USA

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ABSTRACT

Wet weight is one of the most common biological descriptors collected for fisheries research. Freezing is frequently used to preserve fish prior to processing in a lab, and wet weights are often collected for thawed fish. Freezing may affect the wet weight of fish and result in biased values for weight based metrics. Our objectives were to determine the effects of three frozen storage methods, size class of fish, and duration of storage on the changes in wet weight of emerald shiner (*Notropis atherinoides*) and rudd (*Scardinius erythrophthalmus*), and how failure to account for changes in wet weight affects percent dry weight estimates. Two size classes of emerald shiner and three size classes of rudd were collected from the upper Niagara River, New York. Fish were weighed immediately after being euthanized and then glazed with water, frozen in water, or vacuum-sealed and stored at −25 °C for 3 or 6 months. After measuring post-thaw wet weight, fish were dried to a constant weight and percent dry weight was calculated based on prestorage wet weight and post-thaw wet weight. Then, we compared species specific prestorage wet weights to post-thaw wet weights for each storage method. Analysis of variance was used to investigate the effects of storage method, size class, and duration stored on wet weights of each species. Prestorage and post-thaw percent dry weights were compared to illustrate the effects of frozen storage on a commonly used weight-based metric. Emerald shiner and rudd wet weights differed from initial weights for all storage methods. Percent change in wet weight of emerald shiner was affected by interactions among storage method, size class, and duration stored, while percent change in wet weight of rudd was affected by the interaction between fish size class and storage method. Overall, storage method and size class had the greatest effects on changes in wet weight following frozen storage. Post-thaw wet weight increased from initial wet weight for fish frozen in water and decreased for vacuum-sealed and glazed fish, and smaller fish generally experienced greater changes than larger fish of the same species. Percent dry weight estimates based on post-thaw wet weights differed proportionally by −10.6–29.8% from estimates based on prestorage wet weights. Frozen storage can substantially affect weight based metrics such as percent dry weight and should be accounted for if weight is not measured prior to freezing.

1. Introduction

Wet weight is one of the most common biological descriptors of fish and is frequently used in calculating a variety of metrics (e.g., body condition, percent dry weight, gonadosomatic index). Accurate, unbiased measurements of wet weight are important because measurement errors or biases in wet weight data can be compounded when calculating weight based metrics (Engel, 1974). Measurement error and bias in wet weight data can arise from a variety of issues such as balance inaccuracy, type of weighing instrument, condition of the fish (live or dead), variable amounts of residual water on fish, and movement of the vessel if fish are weighed at sea (Gutreuter and Krzoska, 1994). If fish are weighed post-mortem, time since death, and care and handling of the carcass may affect the accuracy of wet weight compared to live wet weight. For example, carcasses exposed to warm air temperatures may experience substantial water loss. Conversely, fish left in freshwater post-mortem may absorb water and gain weight following cessation of osmoregulation.

Preservation can also affect fish wet weight and wet weight based metrics (Jennings et al., 2013). For example, fish are frequently frozen shortly after being collected and wet weights are

* Corresponding author at: Department of Biology, Coastal Carolina University; 111 Chanticleer Drive East, Conway, SC 29526.
E-mail addresses: dcrane@coastal.edu (D.P. Crane), ckillourhy@lssu.edu (C.C. Killourhy), clapsamd@buffalostate.edu (M.D. Clapsadl).

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measured post-thawing in studies of energy content (e.g., Bryan et al., 1996; Pothoven et al., 2014; Ruetz et al., 2009; Tirelli et al., 2006). Energy content of fishes is usually reported on a wet weight basis using percent dry weight of a sample to convert dry weight energy density to wet weight energy density. Therefore, changes in wet weight due to freezing would affect estimates of percent dry weight and subsequently energy density of fishes.

We used three common frozen storage methods (vacuum-sealing, glazing, frozen in water) for fish to investigate the effects of freezing on wet weight and percent dry weight estimates. Vacuum-sealing, glazing, and freezing in water are meant to protect fish tissue from degradation by biological, chemical, and physical processes such as bacterial growth, oxidation, and desiccation (Kolbe and Kramer, 2007). Differences in freezing and thawing times and handling associated with each method likely affect if and how fish tissues absorb or lose water. Our objectives were to quantify the effects of vacuum-sealing, glazing, and freezing in water on wet weight, determine if effects of freezing on wet weight are dependent on fish size or duration frozen, and provide guidance for handling and storage of fishes.

2. Methods

2.1. Collection and processing

Emerald shiners (*Notropis atherinoides*) and Rudd (*Scardinius erythrophthalmus*) were collected in nearshore areas of the upper Niagara River from 26 August to 12 September 2014. Adult rudd were collected by electrofishing, while smaller fish including age-0 rudd and emerald shiners were collected by seining. Emerald shiners were divided into two size classes, age-0 (mean TL = 40 mm) or yearling-and-older (YAO; mean TL = 76 mm), based on an obvious size difference between age-0 and YAO fish. Three size classes of rudd were collected: age-0 (<65 mm), 100–150 mm, and 200–250 mm. All specimens were kept alive until processing to insure that measured wet weights were as close as possible live wet weights.

Fish were euthanized in an ice slurry for 15–30 min to minimize the time between death and processing and to promote faster freezing by cooling their bodies. Total length was measured to the nearest mm for adult emerald shiners and all rudd. Due to the fragility of age-0 emerald shiners total lengths were not measured for each individual. Instead, the total lengths of 30 age-0 emerald shiners were measured as a representative subsample. Prior to weighing samples, balances were checked for accuracy with calibration weights. Each fish was patted with a paper towel to remove excess water prior to weighing. Age-0 emerald shiners, YAO emerald shiners, and age-0 rudd were weighed to the nearest 0.0001 g. Age-0 emerald shiners were weighed and frozen in aggregates of 15 due to their small size. Adult rudd were weighed to the nearest 0.01 g.

2.2. Preservation treatments

Specimens from each species and size class were randomly assigned to one of six preservation treatments and frozen at about −25 °C in chest freezers immediately after being measured and weighed. Fish were processed and initially frozen at the SUNY Buffalo State Great Lakes Center and then transferred in coolers to a nearby facility for storage. Specimens did not thaw during the transfer and we assumed that the short duration (<30 min) required to transfer specimens did not affect study results. Temperatures in the freezers during the freezing and storage process were not measured, but post-experiment measurements indicated that temperatures within the freezers ranged from −22 to −27 °C. Preservation treatments were based on a factorial treatment design, with preservation method (vacuum-sealed, glazed, water), duration of storage (3 months, 6 months) and size class as factors. Actual storage durations were generally within one week of assigned durations. However, storage durations were slightly longer than the assigned durations for 200–250 mm rudd due to capacity limitations in the drying ovens; rudd in the 3-month treatment were stored for 3.5 months and rudd in the 6-month treatment were stored up to 7 months. Although age-0 emerald shiners were frozen in groups, individuals were separated within each bag to minimize contact between fish. In subsequent analyses, values for groups of age-0 emerald shiners were divided by 15 to get a value for a single fish (i.e., one value representing the weight of a single fish was used for each group). YAO emerald shiners were frozen individually. Fifteen replicates were frozen for each storage method and time treatment for age-0 emerald shiner, YAO emerald shiner, and <65 mm rudd. Due to limited abundance of 100–150 mm and 200–250 mm rudd, only eight replicates for each storage method and time treatment were frozen. Four replicates from the glazed fish treatment (one each of age-0 rudd, 200–250 mm rudd, age-0 emerald shiner, and YAO emerald shiner) were removed from the analyses due to data recording errors. Specimens preserved in water were placed in a freezer bag and filled with enough near-freezing water to cover the fish. All excess air was removed from the bag before sealing. Vacuum-sealed specimens were placed in 0.1016 mm thick plastic bags and vacuum-sealed using the moist setting on a Cabela's® Commercial-grade Vacuum Sealer (Cabela's, Sidney, Nebraska). Glazed specimens were placed on wax paper in the freezer and allowed to freeze overnight. The next day, specimens were dipped in a bucket of ice water and immediately returned to the freezer to allow the coating of water to freeze on the fish. After the coating of water was frozen, the process was repeated to allow a second coating of water to freeze onto the fish. Finally, each specimen was placed in a plastic freezer bag and all excess air was removed before sealing.

2.3. Thawing

Specimens were thawed under cold running water in their sealed bag. Once fish were fully thawed, they were removed from their package, patted with a paper towel to remove excess water, and final wet weights were recorded. Fish were then dried at 60 °C (Lantry and O'Gorman, 2007), and a final dry weight was recorded when constant mass was observed for two consecutive days (±0.005 g for age-0 emerald shiner, YAO emerald shiner, and age-0 rudd; ±0.01 g for 100–150 mm and 200–250 mm rudd).

2.4. Statistical analyses

Preliminary analyses indicated that changes in wet weight following freezing were related to fish size. Therefore, we analyzed data for emerald shiner and rudd separately because they differ substantially in body size. Because the effects of freezing were size dependent, we used analysis of variance (ANOVA; type III sum of squares) to test the assumption that prestorage wet weights did not differ among treatments. Separate ANOVAs were run for each species and size class. Initial wet weight was the response variable and freezing method, time, and their interaction were the predictor variables. Prestorage wet weights of rudd did not differ among treatments (Table 1). However, prestorage wet weights differed among the three storage treatments for age-0 emerald shiner (Table 1). Post-hoc pairwise comparisons (Dunnett's modified Tukey–Kramer; R package DTK) indicated that the average initial wet weight of age-0 emerald shiners frozen in water was about 0.16 g greater than the prestorage wet weights of glazed and vacuum-sealed age-0 emerald shiners. It is unlikely that the slightly