

# Quantifying and Identifying Factors Influencing Length Changes in Popular Freshwater Fishes Preserved in Ice

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**Abstract:** Fish length data are important for assessing sportfish populations and establishing and enforcing length-based harvest regulations. Evidence suggests that fish length can change after preservation in ice. These changes can impact comparison of live-caught and post-catch length measurements and therefore angler compliance to regulations, a concern raised by state law enforcement personnel. Similarly, length changes may skew length-based analyses done by fisheries managers. We evaluated TL changes of largemouth bass (*Micropterus salmoides*), catfishes (Ictaluridae spp.), black crappie (*Pomoxis nigromaculatus*), and sunfishes (*Lepomis* spp.) collected in Florida and preserved in coolers of ice for intervals of 3–6 h, 24 h, and 36 h. Our results indicate mean percent shrinkage ranged 0.43%–1.58% among time intervals and fish groups but significantly differed by group and time interval. We found measurement variability and ambient water temperature to be insignificant factors to observed length changes. Where length measurement accuracy is important, fisheries managers should be aware of potential shrinkage of fish post mortality, including during preservation in coolers of ice. As a general recommendation for law enforcement, we suggest absolute shrinkage allowances of 6 mm (0.25 inches) for black crappie and sunfishes and 13 mm (0.5 inches) for largemouth bass and catfishes, when assessing angler compliance to length-based fish regulations after preservation in coolers of ice.

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**Key words:** preservation, shrinkage, regulations, repeated measures

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Accurate fish length and weight data are important in fishery management efforts to establish length-based harvest regulations and assess sportfish populations. Furthermore, law enforcement is tasked with assessing compliance of anglers with established length-based regulations. In Florida, largemouth bass (*Micropterus salmoides*) are managed with a statewide general regulation that allows harvest of up to five bass per day, only one of which may exceed 406 mm TL. Several lakes in Florida have exceptions to this general regulation, however, requiring either a minimum or maximum length of 406 mm for largemouth bass harvest. For black crappie (*Pomoxis nigromaculatus*), ictalurid catfishes, and sunfishes (*Lepomis* spp.), statewide regulations generally allow anglers

to harvest any size fish. However, there are special regulations in some systems that require either a 254-mm or 304-mm minimum TL limit for black crappie. It is generally assumed by biologists, anglers, and law enforcement personnel that holding fish in ice creates insignificant changes in length from the time of capture to the time of measurement. It is imperative that accurate size measurements are obtained for biological and enforcement purposes (Page et al. 2004); thus, there is a need to validate this assumption for these important sportfishes.

Anderson and Neumann (1996) recommend that fish be measured in as fresh a condition as possible due to length reduction occurring from rigor mortis, drying, and preservation. Laboratory

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preservatives, which commonly include alcohol, formaldehyde, and freezing, have been shown to influence fish length for some freshwater sportfish species (e.g., bluegill [*Lepomis macrochirus*] and white crappie [*Pomoxis annularis*], Yeh and Hodson 1975; walleye [*Stizostedion vitreum*], Blackwell et al. 2003; peacock bass [*Cichla kelberi*], Berbel-Filho et al. 2013). Although measuring a fish in its freshest state is ideal, harvested fish are often measured after a period of confinement, thereby subjecting the retained fish to possible length change. For example, recreational anglers and fisheries scientists often hold fish in live wells or in ice for extended periods. Although length change for walleye following confinement in live wells has been found to be minimal, length reduction after preservation in ice has been observed (Blackwell et al. 2003). Further, the effect of these preservation techniques and rigor mortis through the normal dying process can vary by species, ambient water temperature (i.e., temperature of the waterbody from which the fish was taken), length of preservation time, and size of fish (e.g., Stroud 2001, Blackwell et al. 2003, Morison et al. 2003, Morison 2004).

The impact of fish length changes to scientific study and length-based harvest regulations in Florida is currently unknown, and there is a lack of such data in the literature on the fish groups investigated in this study. In the case of freshwater sportfish, an angler may not consider postmortem changes when deciding to harvest, but the changes to the physical condition after post-harvest confinement and preservation may make the fish noncompliant to length-based regulations (Page et al. 2004). Further, if substantial length change occurs, population assessments that involve length data may be skewed. Because fish species and size may influence susceptibility of a preserved fish to length change, we investigated length reduction, or shrinkage, of four fish groups (black crappie, ictalurid catfishes, largemouth bass, and sunfishes) collected on various lakes and rivers throughout Florida and across different length classes. Our study objectives were to 1) determine if shrinkage occurs for fish preserved in ice and quantify it by species group, 2) assess effect of reader variability, i.e., variability among replicate TL measurements of a fish by the same reader, 3) determine if ambient water temperature influences shrinkage, and 4) based on these results, provide recommendations to law enforcement, fish biologists, and managers.

## Methods

### Sampling

Largemouth bass, black crappie, six species of ictalurid catfishes, and four species of sunfishes were obtained intermittently during sampling for other existing projects. These projects used

either boom-mounted boat electrofishers or open water lake trawls between July 2017 and January 2020 on 17 lakes and 6 rivers throughout Florida. On all sampling days, a surface water temperature reading was taken at 0.5 m depth.

### Shrinkage Measurements

Fish examined consisted of a range of sizes and species which are frequently targeted by anglers for harvest and fisheries scientists for studies. Initial TL measurements were taken immediately after trawl pulls were hauled on board or within 1 h of residing in live wells when they were collected by boat electrofishing. Maximum TL was measured on a fish measuring board consisting of a scale delineated in mm following the procedure given in Anderson and Neumann (1996), which is prescribed as the standard procedure for measuring freshwater fish by the Florida Fish and Wildlife Conservation Commission (Bonvechio 2017). Fish were held with the mouth closed and with light pressure against the headpiece of the measuring board while also pinching the caudal fin dorsoventrally to attain the maximum length possible.

After measurement, each fish was placed in an insulated cooler of cubed ice where they were kept for various time intervals. Each fish was marked with either a tournament-style cull clip or a unique set of fin clips to identify each individual throughout ice preservation treatment periods. Fish were distributed in a single layer on a bed of cubed ice in a cooler. Each layer of fish was then covered with at least three inches of top ice, assuring that each fish experienced similar, maximum cooling. If the cooler was large enough, two layers of fish were preserved with top ice. We used larger or multiple coolers if large specimens or quantities were obtained. Storage time intervals were defined as 3–6 h, 24 h, and 36 h, hereafter referred to as Day-1 TL, Day-2 TL, and Day-3 TL, respectively. Day-1 TL represented a typical amount of time period used for fishing trips or sampling trips. At the end of each storage time interval, fish were removed from ice and immediately measured by the same investigator using the same measuring board to ensure no measurement bias due to equipment, but not every fish was measured in each time interval. After each Day-1 TL and Day-2 TL measurement fish were returned to the coolers until the next interval. For the entire investigation period, fish were kept well-iced through addition of fresh ice when needed and excess water was not allowed to accumulate by leaving the cooler drain plug removed.

To examine single reader variability for a subset of fish, one measurer performed three replicates of TL measurements for largemouth bass ( $n=12$ ), black crappie ( $n=8$ ), and sunfishes ( $n=12$ ) within 1 h after a single interval of storage in ice. We in-

serted a dart tag with a unique number into fish in this part of the investigation to ensure each one could be identified separate from the time interval analyses. The measurer was unaware of the dart tag information and was unable to identify specific individuals. The investigator measured the maximum TL of individual fish, then they were placed back in ice as previously described. The entire group was re-measured by the same investigator, with fish being chosen at random from the cooler by another researcher.

### Statistical Analysis

All analyses were performed with percent shrinkage data. Percent change in length was calculated using the formula  $= (TL_y - TL_i) / TL_i * 100$  where  $TL_y$  is the measured TL at time  $y$  and  $TL_i$  is the initial TL. Due to the net loss of length over time, percent change in length values associated with shrinkage are negative. Thus, to express values as percent shrinkage, we multiplied percent change in length values by  $-1$ . For fish used in the reader variability assessment, percent shrinkage was calculated for the second and third reads.

For the ice storage and reader variability analyses, we conducted two-way repeated measures ANOVAs with individual fish as the subject using the MIXED procedure and REPEATED statement in SAS Enterprise Guide 7.1 (SAS Institute 2016), which can handle unbalanced data such as in our study. In analyzing the effect of fish group (black crappie, catfishes, largemouth bass, sunfishes), and ice storage time (Day-1 TL, Day-2 TL, and Day-3 TL) on mean percent shrinkage, we included both factors as fixed effects and added a group\*time interaction. The analysis of single reader variability in percent shrinkage included fish group (black crappie, largemouth bass, sunfishes) and read number (2, 3) as main effects and a group\*read interaction. In each analysis, means were compared using the LSMEANS procedure. Shapiro-Wilks test of normality and Levene's test of homogeneity of variance were conducted to ensure test assumptions were satisfied. For the analysis of reader variability, we  $\ln(x+1)$  transformed the percent shrinkage data to satisfy test assumptions.

An additional two-way ANOVA was completed on a subset of data to look at the effects of fish groups and ambient water temperature on mean percent shrinkage. Water temperature was categorized as either cool ( $<20$  °C), warm ( $20$ – $27$  °C), or hot ( $>27$  °C). Catfishes were not included in the analysis because they were only collected in the hot water temperature category. Initial percent shrinkage (i.e., Day-1 TL values) was the response variable, fish group and temperature category were the fixed treatment effects, and a fish group\*temperature category interaction term was included in the model. For this analysis, we used the MIXED procedure (SAS Institute 2016) along with the LSMEANS procedure.

Test assumptions were checked as previously mentioned. For all analyses,  $P < 0.05$  was considered significant.

### Results

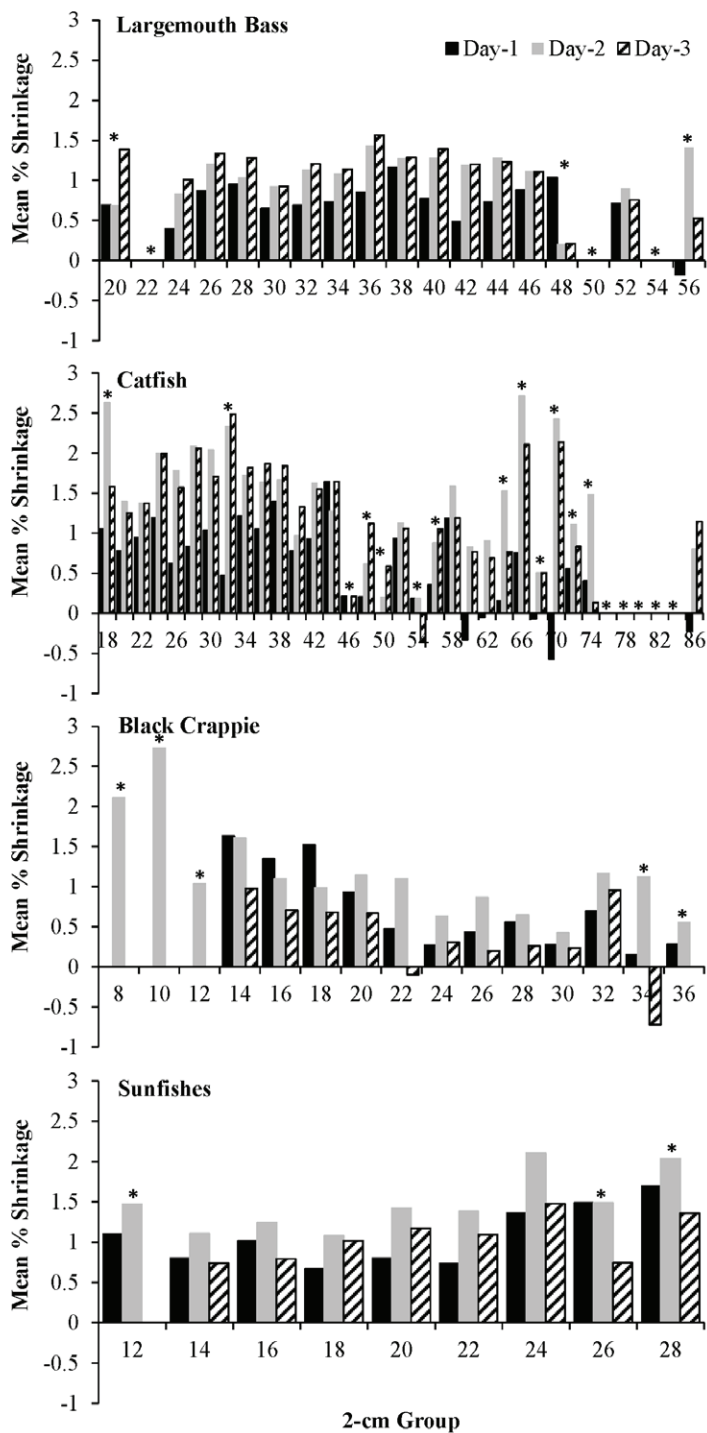
A total of 595 fish of various sizes were measured: largemouth bass, 216–568 mm TL ( $n = 144$ ); catfishes, 190–875 mm ( $n = 105$ ); black crappie, 95–360 mm ( $n = 212$ ); and sunfishes, 13–295 mm ( $n = 134$ ). In the analysis of single reader variability using the subset of 32 fish from the largemouth bass, black crappie, and sunfish groups, mean percent shrinkage was similar among groups and among reads, and no interaction was detected (Table 1, 2). Mean percent shrinkage varied among sizes of fish but was generally below 2% (Figure 1). In all, 94% of all length observations indicated no change or a reduction in TL regardless of fish group or time interval. Observed increases were minimal, with 99% of all fish showing no more than 2 mm of lengthening.

**Table 1.** Mean (SD), range (minimum to maximum), and sample size ( $n$ ) for percent (%) and absolute shrinkage (mm) from the initial read to subsequent reads (Read 2 and Read 3) for each fish group.

Read			Largemouth bass	Black crappie	Sunfishes
Read 2	Percent shrinkage	Mean	-0.08 (0.90)	-0.11 (0.49)	-0.19 (0.51)
		Range	-0.40 to 0.40	-0.98 to -0.05	-0.96 to 0.40
	Absolute shrinkage	Mean	-0.08 (0.90)	-0.25 (1.04)	-0.42 (1.00)
		Range	-2 to 1	-2 to 1	-2 to 1
		$n$	12	8	12
	Read 3	Percent shrinkage	Mean	-0.12 (0.46)	-0.12 (1.01)
Range			-1.21 to 0.62	-1.46 to 1.58	-1.44 to 1.17
Absolute shrinkage		Mean	-0.33 (1.37)	-0.38 (2.33)	-0.42 (1.62)
		Range	-3 to 2	-4 to 3	-3 to 2
		$n$	12	8	12

**Table 2.** Results of two-way analyses of variance performed to test for differences among fish groups, time on ice, ambient water temperature, and measurement reads. Provided are results of the Type III test of fixed effects, including the numerator (Num) and denominator (Den) df,  $F$ -value, and  $P$ -value for each.

Analysis	Effect	Num df	Den df	$F$	$P$
Time on ice	Group	3	575	32.29	<0.001
	Time	2	879	39.76	<0.001
	Group*Time	6	879	9.08	<0.001
Temperature	Group	2	405	1.64	0.196
	Temp	2	405	6.45	0.002
	Group*Temp	4	405	3.94	0.004
Read	Group	2	29	0.20	0.817
	Read	1	29	0.04	0.845
	Group*Read	2	29	0.10	0.906



**Figure 1.** Mean percent shrinkage by 2-cm size bin for each fish species or group. An asterisk indicates less than three fish were included for a particular size bin for at least one of the time periods.

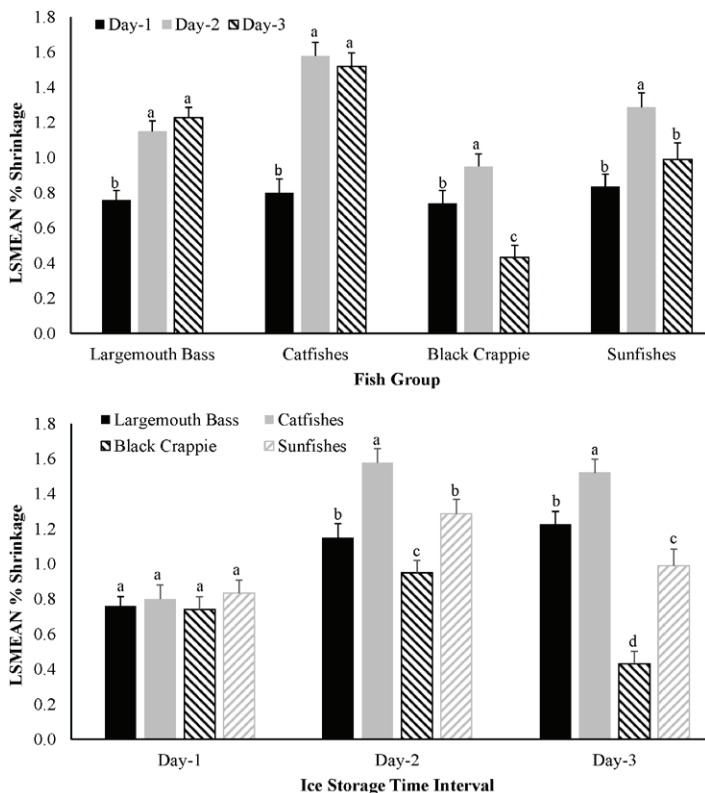
### Effects of Ice Storage

A total of 579 individual fish representing all four groups were included in the analysis to evaluate the effect of time on percent shrinkage (Table 3). Mean percent shrinkage values among time intervals ranged from 0.76%–1.22%, 0.8%–1.58%, 0.43%–0.96%, and 0.83%–1.29% for largemouth bass, catfishes, black crappie, and sunfishes, respectively. Repeated measures ANOVA revealed a significant fish group\*ice storage time interaction (Table 2; Figure 2). Mean percent shrinkage recorded for Day-1 TL was similar among fish groups (LSMEANS,  $|t|=0.18$  to  $0.89$ ,  $df=879$ ,  $P\geq 0.375$ ); however, differences were observed in Day-2 TL and Day-3 TL. Catfishes had the largest mean percent shrinkage (LSMEANS,  $|t|=2.69$  to  $9.81$ ,  $df=879$ ,  $P\leq 0.007$ ) and black crappie had the least (LSMEANS,  $|t|=2.23$  to  $9.81$ ,  $df=879$ ,  $P<0.001$ ) for both Day-2 TL and Day-3 TL. Mean percent shrinkage was similar between sunfishes and largemouth bass for Day-2 TL (LSMEANS,  $|t|=1.34$ ,  $df=879$ ,  $P=0.182$ ), but largemouth bass had a significantly higher mean percent shrinkage than sunfishes on Day-3 TL (LSMEANS,  $|t|=2.16$ ,  $df=879$ ,  $P=0.031$ ).

Within fish groups, Day-1 TL exhibited the lowest mean percent shrinkage for largemouth bass and catfishes ( $|t|=3.92$  to  $6.97$ ,  $df=879$ ,  $P<0.001$ ) (Table 3). Mean percent shrinkage during Days -2 TL and -3 TL was similar for largemouth bass and catfishes ( $|t|=0.52$  to  $0.75$ ,  $df=879$ ,  $P\geq 0.455$ ). Sunfishes exhibited the largest mean percent shrinkage during Day-2 TL ( $|t|=2.72$  and  $4.29$ ,  $df=879$ ,  $P<0.007$ ), but was similar between Day-1 TL and Day-3

**Table 3.** Mean (SD), range (minimum to maximum), and sample size ( $n$ ) for percent (%) and absolute shrinkage (mm) for each ice storage time interval and fish group. Size range and number of fish included in the analysis for each group are also provided.

Time interval		Largemouth bass				Catfishes	Black crappie	Sunfishes
		Mean	Range	Mean	Range	Mean	Range	Mean
Day-1	Percent shrinkage	Mean	0.76 (0.62)	0.80 (0.82)	0.74 (0.78)	0.83 (0.80)		
		Range	-0.71 to 2.58	-1.97 to 2.48	-1.28 to 3.05	-1.37 to 3.41		
	Absolute shrinkage	Mean	2.69 (2.27)	2.76 (3.32)	1.59 (1.70)	1.58 (1.55)		
	Range	-3 to 9	-12 to 11	-4 to 5	-3 to 6			
	$n$	130	105	117	118			
Day-2	Percent shrinkage	Mean	1.15 (0.67)	1.58 (0.80)	0.96 (1.00)	1.29 (0.89)		
		Range	-0.38 to 3.73	-0.33 to 3.64	-2.16 to 4.00	-1.19 to 3.72		
	Absolute shrinkage	Mean	4.10 (2.56)	5.75 (3.37)	2.19 (2.38)	2.47 (1.79)		
	Range	-1 to 14	-2 to 18	-5 to 10	-2 to 8			
	$n$	134	105	208	118			
Day-3	Percent shrinkage	Mean	1.22 (0.64)	1.52 (0.78)	0.43 (0.72)	0.99 (0.97)		
		Range	0.00 to 3.73	-0.36 to 3.58	-1.36 to 2.73	-1.37 to 6.01		
	Absolute shrinkage	Mean	4.30 (2.37)	5.51 (3.07)	0.95 (1.72)	1.91 (1.87)		
	Range	0 to 14	-2 to 15	-3 to 9	-3 to 11			
	$n$	116	105	108	102			
All Fish	Initial TL (mm)	Range	216 to 568	190 to 875	95 to 360	136 to 295		
	$n$		144	105	212	118		

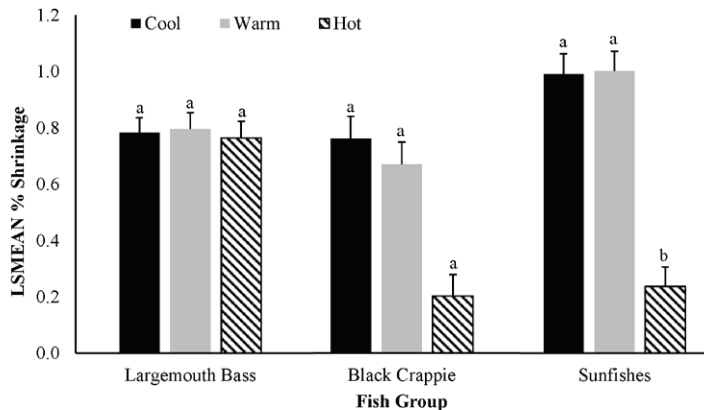


**Figure 2.** Mean percent shrinkage as a function of fish group (top panel) and ice storage time interval (bottom panel). Error bars represent standard error of the least squares mean and different letters signify a significant difference ( $P < 0.001$ ) within either the fish group (top panel) or ice storage time interval group (bottom panel).

TL ( $|t| = 1.42$ ,  $df = 879$ ,  $P = 0.156$ ). For black crappie, Day-2 TL exhibited the highest mean percent shrinkage, followed by Day-1 TL and Day-3 TL ( $|t| = 2.24$  to  $5.40$ ,  $df = 879$ ,  $P \leq 0.025$ ). However, not every fish exhibited shrinkage, as noted by negative values in Table 3, and overall shrinkage never exceeded 18 mm for any fish in any part of the study.

### Ambient Temperature Effects

A total of 144 sunfishes, black crappie, and largemouth bass were included in the analysis to assess the relationship between ambient water temperature and mean percent shrinkage values (Figure 3). The fish group\*temperature category interaction term was significant (Table 2). No significant differences among water temperature categories were detected for either largemouth bass or black crappie ( $|t| = 0.06$  to  $1.84$ ,  $df = 405$ ,  $P = 0.067$  to  $0.950$ ). However, sunfishes collected in hot water temperatures exhibited a smaller reduction in mean percent shrinkage than those collected in the other water temperature categories ( $|t| = 5.15$  to  $5.26$ ,  $df = 405$ ,  $P < 0.001$ ). Black crappie appeared to show a similar pattern to sunfishes, but low sample size ( $n = 6$ ) for the hot water category limited our ability to detect changes (Figure 3).



**Figure 3.** Mean percent shrinkage as a function of fish group and water temperature category. Error bars represent standard error of the least squares mean and different letters signify a significant difference ( $P < 0.05$ ) within fish group.

### Discussion

Our results indicate that the TL of commonly angled Florida freshwater fishes can minimally change after preservation in coolers of ice across a range of time periods from 3 to 36 h. There were rare cases where individual fish length increased (i.e., exhibited lengthening rather than shrinkage), but nearly all these measurements indicated no more than 2 mm of lengthening, which was within the level of reader error observed in this study. Our study provides evidence that mean percent shrinkage values vary by fish group and time interval. When preserved in ice, all fish groups had similar mean percent shrinkage on Day-1 TL, but with time, catfishes consistently displayed greater mean percent shrinkage and black crappie displayed the least. The observed minimal fish TL change after preservation in ice was in line with other reported values in the literature. For example, Lux (1960) observed a mean TL reduction of 1.5% in yellowtail flounder (*Limanda ferruginea*) after 54 h preserved in ice. Single reader variability did not account for the length reduction observed in our results, as differences in measurements by an individual reader were very small and tended to result in longer length values, not shorter. Thus, we believe fish shrinkage was a result of post mortality effects which include the effect of storage in ice.

We found little relation between mean percent shrinkage and ambient water temperature. Although sunfishes and black crappie displayed a smaller reduction in mean percent shrinkage in hot waters compared to those in other water temperature categories, no differences were detected for the other fish groups. Blackwell et al. (2003) identified the greatest decrease in length occurred for walleye collected from colder ambient water temperatures (10 °C). Our inferences were limited because our data had a narrow am-

bient temperature range that consisted of relatively warmer water (>16 °C). Ambient water temperature where fish were collected does not appear to influence TL in Florida waters, but it may be a factor in colder climates, and therefore may warrant further study.

Although we observed fish shrinkage at all time intervals, it was notable that black crappie and sunfishes regained some length between the Day-2 TL and Day-3 TL measurements. Sunfishes and black crappie are both deeper-bodied fishes than largemouth bass and catfishes, which could explain the difference in mean percent shrinkage trends among the fish groups. Size, condition, and species of fish could influence when and how long a fish experiences rigor mortis (Stroud 2001) and could also explain the length increases primarily observed in black crappie and sunfishes between Day-2 TL and Day-3 TL. Rigor mortis has been credited to length changes after a variety of preservation methods including storage in ice (Natsume 1995, Anderson and Neumann 1996); however, this is not consistent across studies (e.g., juvenile Australian snapper [*Pagrus auratus*]; Morison 2004), and could instead be due to other physiological changes associated with death including a drop in blood pressure and fluid loss (Morison 2004). Identifying other specific factors influencing fish shrinkage, such as the effects of body shape and rigor mortis, were outside the scope of this study but may warrant future research.

Careful measurement can minimize reader error, as shown by our single reader results. Although this side investigation had a limited sample size, other studies have also found reader error to be insignificant (Morison et al. 2003, Page et al. 2004, Chesnes et al. 2009). Still, fish placement on the measuring board, equipment condition, and the amount of time spent getting an accurate measurement have been shown to affect an angler's length reading and cause measuring error (Bunch et al. 2013), necessitating the need for appropriate training. Anglers are ultimately responsible for complying with length-based regulations. Nevertheless, law enforcement should be aware of angler measurement error caused by lack of attention to accuracy such as rounding to the nearest half or whole inch or using measuring equipment in inconsistent condition (Page et al. 2004). When measured by biologists, as in this study, reader variability is negligible, but it is important to consider that angler measurement error may be greater.

We used percent shrinkage, rather than absolute shrinkage, so we could compare shrinkage across fish groups with different size ranges. Our data did not reveal a clear relationship between percent shrinkage levels and fish size, although mean percent shrinkage was generally less than 2% regardless of species group or size. Because fish were obtained opportunistically in this study, not all sizes were equally represented, and sample sizes were limited especially at the ends of the size range (Figure 1). Additional samples

could help to elucidate possible length-related effects on percent shrinkage, keeping in mind that any effect would be minimal.

Overall, our results confirm evidence of fish length change, namely percent shrinkage, following death and preservation in ice. Mean percent shrinkage ranged from 0.43% to 1.58% after storage in ice up to 36 h. Although these levels appear minimal, maximum absolute shrinkage was as high as 18 mm TL, for a catfish with an initial TL of 646 mm. However, large ( $\geq 10$  mm TL) shrinkage values were relatively rare, with only 22 fish (3.7%) exhibiting this level of shrinkage at some point in the 36-h period. Fisheries managers should note this phenomenon of fish shrinkage after preservation in ice and its potential to bias fish length measurements, especially in larger specimens, and also be aware that most shrinkage happens within 3–6 hours with the maximum typically reached at 24 h after preservation in ice.

Percent shrinkage allowances may be cumbersome when enforcing length limits. Therefore, as a general recommendation for law enforcement, we suggest absolute shrinkage allowances of 6 mm (0.25 inches) for black crappie and sunfishes and 13 mm (0.5 inches) for largemouth bass and catfishes, when assessing angler compliance to length-based fish harvest regulations after preservation in coolers. These recommendations encompass absolute shrinkage levels we observed for at least 90% of fish during the first three days in ice. For the latter two groups, this would provide a larger allowance than is necessary for smaller fish (i.e., fish <279 mm TL), based on our data, but may be preferable from a law enforcement perspective. Blackwell et al. (2003) made a similar recommendation to law enforcement for angler measurement of walleye, stating a leeway of 6 mm (0.24 in) is sufficient in most situations. Percent shrinkage did not seem to be caused by reader measurement error, but law enforcement should be aware of angler measurement errors due to rounding and inconsistent measuring equipment.

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