2-2004

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Matt E. Roberts

James E. Wetzel II

Ronald C. Brooks

James E. Garvey
*Southern Illinois University Carbondale*

### Recommended Citation

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MATT E. ROBERTS, JAMES E. WETZEL II, RONALD C. BROOKS,
AND JAMES E. GARVEY*

*Fisheries and Illinois Aquaculture Center, Department of Zoology,
Southern Illinois University, Carbondale, Illinois 62901-6511, USA*

*Abstract.*—To generate reliable age information for fishes, we must assess the robustness of techniques to environmental variation. We quantified daily ring deposition within the sagittal otoliths of known-age larval and juvenile redspotted sunfish *Lepomis miniatus* reared in ponds. We also determined how daily light : dark cycles mimicking natural conditions, reduced daylight, or constant daylight affected ring deposition in fish housed in aquaria. Additionally, we quantified how constant temperature (26°C), low daily variability in temperature (26±1°C), and high daily variability (26±2°C) affected production of daily rings. In pond-reared fish, the first increment was produced by the evening of hatch, and increment deposition occurred with 24-h periodicity through 119 d. Independent age estimates from two readers closely agreed. Photocycle and temperature variation did not affect periodicity of increment formation or the precision and accuracy of age estimates. Age and growth data can be reliably determined in this species to assess recruitment dynamics and develop management and conservation strategies.

Many models used in fisheries management and conservation rely on accurate estimates of age. For young fishes, estimates of daily age provide insight into growth and survival (Campana 2001). Increment frequency can be used to determine spawn and hatch dates, whereas increment width is related to growth. Although many structures provide age information, otoliths typically yield the most accurate estimates of daily growth (Pannella 1971; Campana and Neilson 1985; Jones 1986).

Campana and Neilson (1982) hypothesized that increment formation in otoliths is controlled by an endogenous circadian rhythm. However, formation of daily increments does not occur in all species (Geffen 1982), and environmental factors such as photoperiod and temperature can affect deposition (Taubert and Coble 1977; Gauldie and Radtke 1990). Daily increments are sometimes produced regardless of variation in these environmental factors (Campana and Neilson 1982; Geffen 1982; Neilson and Geen 1982).

Increments must be produced with 24-h periodicity before age estimates can be used for a particular species (Geffen 1982; Jones 1986). For example, Taubert and Coble (1977) found that increments in green sunfish *Lepomis cyanellus*, bluegills *L. macrochirus*, and pumpkinseeds *L. gibbosus* were produced daily when natural photoperiod and temperature regimes during spawning were simulated. Daily increment formation ceased in green sunfish exposed to winter conditions (Taubert and Coble 1977).

Daily increment formation in the redspotted sunfish *L. miniatus*, formerly considered a subspecies of the spotted sunfish *L. punctatus*, has not been investigated. This species is widely distributed throughout the south-central USA and is threatened in Illinois. Because it resides in small streams and ditches (Pflieger 1997), it is subject to variation in environmental conditions during early life. A validation of daily ring formation would provide valuable data for conservation and management of this species. An assessment of how increment deposition responds to environmental variation would garner general insight into aging reliability for field-caught individuals. Our objectives were to determine whether increments form with daily periodicity in the redspotted sunfish, and to determine the effects of photoperiod and temperature variation on increment deposition.

## Methods

*Age validation.*—To validate ages estimated by otoliths, adult broodfish were taken from St. James Ditch in Missouri and placed in 0.04-ha experimental ponds at the Touch of Nature Research Facility, Southern Illinois University, Carbondale. During June 2000, we inspected the ponds housing broodfish for evidence of nesting. When nests with eggs were found, eggs were collected and distributed in buckets to one of six 0.04-ha ponds according to spawn date, thus creating a single daily age-class in each pond. Eggs and embryos were held in buckets within each pond and released after swim-up. Larvae were then seined every 7–14 d

during July–September 2000 (total duration = 119 d). Three individuals were usually collected from each pond on each sampling date. Each larval fish was measured (total length, mm) and preserved in 70% ethanol.

To increase the number of larvae for our analysis, we repeated the experiment during July–August 2001. Because only one pond was available at this time, we used four cages within the pond to follow individual daily cohorts through time. Eggs were transferred from each nest to a small, floating foam container and placed into an individual cage. After hatch and swim-up, larvae were released into the cage. Cages were constructed of 300-$\mu$m Nitex mesh connected between two halves of an 18.9-L bucket. Generally, two individuals were sampled from each cage weekly and processed as described above. Temperatures were recorded daily.

*Effects of photocycle.*—To determine how photocycle affects daily ring deposition, larvae were produced indoors during late summer 2001. Two male redspotted sunfish were housed in separate tanks connected to aquaria containing females. An artificial nest, constructed of the bottom of an 18.9-L bucket filled with gravel, was placed in each tank. A single ripe female was then placed into a tank with a male. When spawning occurred, 30 eggs were transferred to each of nine mason jars. Each jar was then placed into one of nine 37.9-L aquaria.

The photocycle experiment commenced on 24 August 2003, when larvae hatched in aquarium jars, and continued for 9 d. Each of three treatments included three of the 37.9-L aquaria containing 30 larvae. The first treatment (14 h light : 10 h dark; average incidental intensity = 81 microEinsteins [$\mu$E]) simulated the daily photocycle that larval redspotted sunfish typically experience under natural conditions. To test how the absence of a light/dark cycle affected increment formation, we exposed larvae to constant 24-h light each day in treatment 2. The effect of light intensity was explored in treatment 3, which included the same light/dark period as the control, but used a reduced wattage bulb during the light period (6.7 $\mu$E). Aquaria were partitioned with black plastic. All larvae were fed nauplii of brine shrimp *Artemia* spp. twice daily, starting 3 d after hatch. At the end of the experiment, larvae were collected and processed as described previously.

*Effects of temperature.*—To determine how temperature variation affected ring deposition, we again produced larvae in the laboratory, with the 7-d experiment beginning at larval hatch on 13 December 2001. Larvae were exposed to one of three treatments (three tanks per treatment; 30 larvae per tank): a constant temperature of 26°C (control), high temperature variation (24–28°C), and low temperature variation (25–27°C). Each treatment cycled completely in one 24-h period. This range in daily temperatures is common for many lakes and streams (J. E. Garvey, unpublished data), with greater daily change likely causing high mortality in sunfishes *Lepomis* spp. (Garvey et al. 2002). Temperature was controlled with Visi-Therm heaters operated by timers. Brine shrimp were introduced once during the morning and once during the evening each day starting on day 4. At the experiment's end, larvae were treated as described above.

*Otolith preparation.*—Sagittal otoliths were removed from larvae by the methods described in Sweatman and Kohler (1991). Upon extraction, each otolith was mounted with thermoplastic cement on a glass slide with the sulcus facing up. Ages were estimated by counting each complete daily increment, including both the incremental and discontinuous zones (Mugiya et al. 1981). If necessary, we polished individual otoliths with 3- and 12-$\mu$m aluminum oxide Fibermet discs to reveal central rings near the otolith core.

For ponds, ages were estimated by two independent readers who were unaware of the actual age. If independent counts of the two readers differed by less than 10%, the average of the two became the collaborative estimate. If the independent counts differed by more than 10%, then the two readers collaborated until a final decision was reached. Forty-five otoliths from individual fish stratified among dates and ponds or cages were examined. Only one reader was used for the photocycle and temperature experiments because of high agreement between readers in the validation study. About three to five otoliths per tank from haphazardly chosen larvae were prepared and read for each experiment.

*Data analyses.*—For pond data, we used linear regression to compare reader counts and to evaluate agreement between mean collaborative counts and known larval ages. Because the first discernable ring may be deposited in centrarchids either at hatch (Garvey et al. 2002) or at swim-up (Miller and Storck 1982), we initially defined known age in two ways: days posthatch and days post-swim-up. The null hypotheses that intercepts equaled zero and slopes equaled 1.0 were tested with two-tailed *t*-tests. If the regression model of known
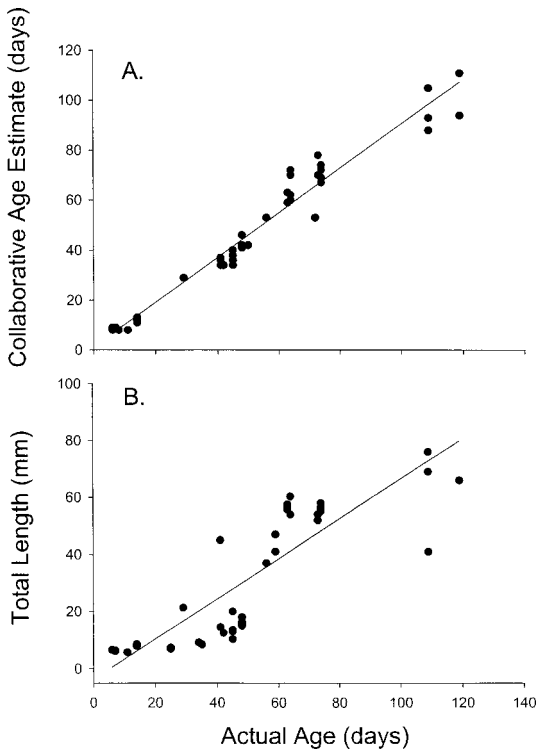
FIGURE 1.—(A) Relationship between days posthatch and mean collaborative age estimates of redspotted sunfish *Lepomis miniatus* larvae reared in experimental ponds at the Touch of Nature (TON) facility, Southern Illinois University, Carbondale, during July–October 2000. (B) Total length expressed as a function of known days posthatch of larval and juvenile redspotted sunfish reared in TON ponds during July–October 2000.
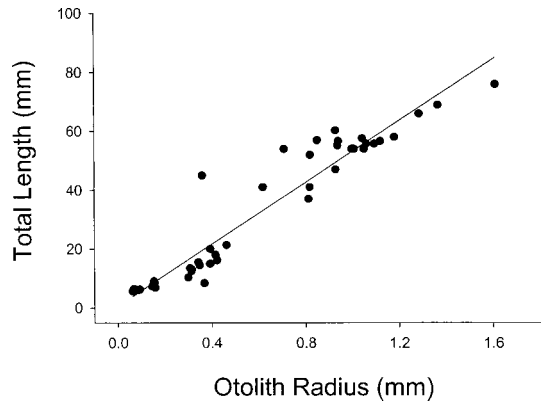


FIGURE 2.—Otolith radius (mm) versus total fish length (mm) of larval and juvenile redspotted sunfish reared in experimental ponds at the Touch of Nature facility, Southern Illinois University, Carbondale, during July–October 2000.

versus estimated age generated an intercept of zero, it likely contained known ages that corresponded to the ages estimated by otoliths (i.e., intercept should occur the day before the first ring on day 0). We plotted the residuals from the regression models to explore how our estimates deviated from predictions at different known ages. The coefficient of variation (CV = $100 \times$ SD/mean) was also determined (Campana 2001). Linear regression was used to determine how otolith size, hatch date, and total length were related. Estimated ages of individual larvae were compared among treatments in the photocycle and temperature experiments with a two-way analysis of variance (ANOVA); individual larvae were used as replicates, and the effect of tanks was nested within each treatment level. All statistics were calculated by use of the general linear models procedure (PROC GLM) in the Statistical Analysis System (SAS), with Type III sums of squares and a significance level of 0.05 (SAS 1990).

## Results

The readers' estimates for the pond validation experiment closely agreed ($y = 0.88x + 1.10$; $N = 44$; adjusted $R = 0.93$), with a mean CV of 10.5%. The final collaborative age estimates and the known days posthatch corresponded well ($y = 0.89x + 2.2$; $N = 42$; adjusted $R = 0.96$; Figure 1A); the intercept did not differ from zero ($t$-test: df = 40, $t = 1.2$, $P = 0.23$) but the slope was less than 1.0 ($t$-test: df = 40, $t = 3.6$, $P = 0.001$). The regression of days post-swim-up versus estimated age was identical to the one for posthatch, except that the intercept in the latter regression changed to 6.7 d, which was significantly different than zero ($t$-test: df = 1, $t = 3.9$, $P = 0.0003$). Residual analysis within the regressions revealed that estimates typically varied 9.9% from predicted values across all ages. However, for some younger larvae (6–15 d of age), deviations reached 22%. Mean daily temperature in ponds was 26°C.

Total length increased with days posthatch (Figure 1B; $y = 0.70x - 3.72$; $R = 0.72$). Otolith radius and total fish length were linearly related (Figure 2; $N = 41$; $y = 52.5x + 0.6$; $R = 0.91$). The slope was positive ($t$-test: df = 1, $t = 2.5$, $P = 0.0001$), whereas the intercept did not differ from zero ($t$-test: df = 1, $t = 1.9$, $P = 0.75$).

Neither treatment nor tank affected the mean estimated ages in the photocycle experiment (Table 1; two-way ANOVA: treatment, $F_{2,35} = 1.56$, $P = 0.22$, tank[treatment], $F_{6,35} = 0.97$, $P = 0.47$)

TABLE 1.—Mean (±SE) estimated age from otoliths of larval redspotted sunfish in the photocycle experiment (23 August through 2 September 2001; expected age = 9 d) and the temperature variation experiment (13–21 December 2001; expected age = 7 d) conducted in aquaria at Southern Illinois University. Photocycle treatments are normal (14 h light : 10 h dark at 81 micro Einsteins [$\mu$E], reduced light (14 h light : 10 h dark at 6.7 $\mu$E), and constant light (24 h light). Temperature treatments are constant (26°C), low variation (26±1°C), and high variation (26±2°C).

| Experiment | Treatment | Aquarium | $N$ | Estimated age (d) | SE |
|---|---|---|---|---|---|
| Photocycle | Normal | 1 | 4 | 9.8 | 0.25 |
| | | 2 | 4 | 9.8 | 0.48 |
| | | 3 | 4 | 9.8 | 0.85 |
| | Reduced | 1 | 4 | 10.5 | 0.87 |
| | | 2 | 4 | 11.8 | 1.18 |
| | | 3 | 5 | 9.8 | 0.20 |
| | Constant | 1 | 4 | 9.8 | 0.75 |
| | | 2 | 4 | 9.5 | 0.29 |
| | | 3 | 3 | 10.7 | 0.88 |
| Temperature | Constant[a] | 1 | 4 | 7.0 | 0.0 |
| | Low variation | 1 | 4 | 7.3 | 0.25 |
| | | 2 | 2 | 7.0 | 0.0 |
| | | 3 | 3 | 7.3 | 0.33 |
| | High variation | 1 | 2 | 7.5 | 0.50 |
| | | 2 | 2 | 7.0 | 0.0 |
| | | 3 | 3 | 7.0 | 0.0 |

[a] Survival in the other two aquaria for this treatment was 0%.

and the temperature variation experiment (Table 1; two-way ANOVA: treatment, $F_{2,35} = 0.36$, $P = 0.70$, tank[treatment], $F_{4,35} = 0.84$, $P = 0.52$). The mean estimated age among all photocycle treatments was 10.2 d, which was similar to the known hatch date of 9 d. Similarly, the mean estimated age among all temperature treatments was 7.1 d, closely matching the known hatch date of 7 d.

## Discussion

We found that daily rings were produced in redspotted sunfish through 119 d. In our regression of estimated versus known age, the small amount (4%) of unexplained variance and the slight tendency to underestimate age (i.e., the slope was somewhat less than 1.0) most likely arose due to our difficulty discerning rings at the edges of otoliths, either because of opaque regions or light refraction (Sweatman and Kohler 1991). In older fish, areas of intersecting daily rings contributed to reader error, similar to three other species of *Lepomis* (Taubert and Coble 1977). In younger fish, error was quite high among some individuals at 6–14 d, likely because it was difficult to discern tightly spaced rings near the core, as occurred for American shad *Alosa sapidissima* (Savoy and Crecco 1987). Because our independent estimates were strongly correlated and variation between readers was typical (Campana 2001), we conclude that the ease of estimating daily age for redspotted sunfish is comparable to that for other age-validated species.

Environmental variation in photocycle or temperature may alter ring deposition in fishes and thus the accuracy and precision of estimates. For example, Taubert and Coble (1977) observed that, in the absence of a 24-h light/dark cycle, daily increment formation in Mozambique tilapia *Tilapia mossambica* was altered to such an extent that there were almost twice as many rings present than what was representative of the actual age. We did not encounter this problem. Larvae exposed to varying photoperiods and temperatures in our experiments produced daily increments in otoliths that corresponded well with actual ages. Hence, daily deposition of rings in redspotted sunfish should be robust to the environmental variation typically present in the field.

Through casual observation, we found that the first daily increments occurred on otoliths by the evening of the day of hatch. This was independently confirmed when we assumed that days post-hatch was the known age in our regression, and found that the first ring occurred on the known hatch date. When we assumed that the swim-up date was the first day of deposition, multiple rings were already present on otoliths on day 0 (i.e., mean intercept = 6.7 d). Hence, the regression incorporating days posthatch appears to capture the true daily age. Knowing this, we can estimate spawning dates, which occurred about 36 h before hatch in our experiments, and swim-up dates, which typically occurred 4–5 d posthatch.

Because fish growth and otolith size correspond-

ed well, our results, coupled with age estimates, can be directly applied to management. For example, reliable back-calculated growth rates and hatch dates can be used to assess the physical and chemical conditions affecting early survival, growth, and, ultimately, population dynamics (DeVries and Frie 1996). We conclude that otoliths in redspotted sunfish generate accurate and precise age information that is robust to environmental variation, thus serving as a useful tool for the management and conservation of this species.

## Acknowledgments

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