Journal of Fish Biology (2004) **65,** 293–298
doi:10.1111/j.1095-8649.2004.00437.x, available online at http://www.blackwell-synergy.com

Temperate Utah chub form valid otolith annuli in the absence of fluctuating water temperature

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(Received 12 November 2003, Accepted 26 March 2004)

Utah chub *Gila atraria*, a temperate freshwater minnow, formed valid otolith annuli (annual growth rings), even when raised in a constant-temperature desert spring environment. This suggests that factors other than seasonal variation in water temperature control annual otolith marking.

Key words: desert fishes; *Gila atraria*; otolith annuli; Utah chub; water temperature.

In many temperate freshwater fishes, seasonal change in water temperature appears to promote the formation of annual growth rings (annuli) in otoliths (Pannella, 1980; Schramm, 1989; Beckman & Wilson, 1995). In contrast, many species of marine fishes, and several tropical freshwater fishes, form annuli even in the absence of widely fluctuating water temperatures (Yosef & Casselman, 1995). This leads to a basic question regarding the necessity of variable water temperature in annuli formation, particularly in temperate freshwater environments where otolith marking is principally ascribed to temperature variation (Beckman & Wilson, 1995; Bone *et al.*, 1995). In this study, this question was addressed using a natural experiment, where Utah chub *Gila atraria* (Girard) have been isolated in a constant temperature desert spring, but at a temperate latitude in western North America where other factors, such as photoperiod length, resource availability or reproductive cycles, show typical patterns of variation. In this spring environment, Utah chub still form distinct annual rings, suggesting that one of these other factors may have primacy in otolith marking in temperate fish species.

Utah chub evaluated in this study were collected from Big Spring, a 1·5 ha shallow water body on the eastern edge of the North American Great Basin (40° 60′ N; 112°30′ W). The water body receives water from a small springhead in the south and drains northward *c.* 2 km into Great Salt Lake, a highly saline lake that does not support any fish populations. Because flow rate in Big Spring is

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high relative to total water volume, water temperature in the spring was expected to be relatively constant. This assumption was tested by measuring seasonal changes in water temperature over 1 year (August 2002 to July 2003) using a submersible temperature logger (Onset Stowaway TidbiT). Water temperature measurements and fish collections were made near the springhead at a depth of 2 m where Utah chub densities were highest. Periodic temperature measurements were also taken by hand from various points where Utah chub occur and were consistent with the data from the temperature logger, suggesting a spatially uniform temperature throughout the spring system. The degree to which water temperatures remained constant throughout the year was examined by comparing monthly means generated from daily temperature logger measurements. Finally, the annual Big Spring temperature profile was contrasted with that of the adjacent Great Salt Lake, a large lake with seasonal variation in water temperature typical of northern temperate climates. Monthly temperature averages for the Great Salt Lake were calculated from US Geological Survey data gathered between 1960 and 2002 (C. Burden, pers. comm.)

The otolith annuli formation in Utah chub was evaluated from four collections made in 1995 (March, May, September and December). An experimental gillnet (2·5–10 mm stretched mesh) and minnow traps (0·6 cm mesh, 44·5 cm length, 29·9 cm diameter) were used to collect a total of 144 Utah chub; the sampling system ensured collection of all size classes. Fish were killed by an overdose of MS-222 and were preserved frozen. Otoliths (lapilli) were extracted by dissection, stored dry and later thin-sectioned. Mounted sections were immersed in oil for viewing under reflected light and examined using a CCTV camera fitted to a Heerbrugg Wild dissecting microscope (×40). Otoliths were read in random order without prior knowledge of the month of collection. All otolith measurements (0·001 mm) were made using video image analysis software (Mocha release 1.0).

Utah chub otoliths contained repeated zones of opaque and translucent bands that were easily discernable in illuminated thin-sections (Fig. 1). Opaque bands are believed to demark annuli in this species and were used to sort individuals into age-classes. To validate opaque bands as annuli, marginal increment analysis was used (Casselman, 1987). The marginal increment was defined here as the length of the translucent band measured from the distal margin of the outermost opaque band to the otolith edge. If opaque bands form annually, the marginal increment is predicted to increase continually throughout the year and will show a minimal value at only one restricted period when the new opaque band is forming. Utah chub differ in juvenile and adult growth rates (Johnson & Belk, 1999) so the marginal increment analysis was conducted separately for juveniles (age 1–2 years) and adults (age 3–14 years).

Water temperature in Big Spring was found to remain nearly constant through time (Fig. 2), with an overall mean ± S.E. of 17·7 ± 0·3° C. Monthly means ranged from 17·4 to 17·9° C with a gradual increase from November to September and a slight decrease between September and November. Marginal increments were statistically different across the four sampling months for both juveniles ($F_{3,34}$, $P < 0·01$) and adults ($F_{3,99}$, $P < 0·01$). In each group, marginal increments increased throughout the year, with the smallest increments measured in May (Fig. 3). This single marginal increment minimum is consistent

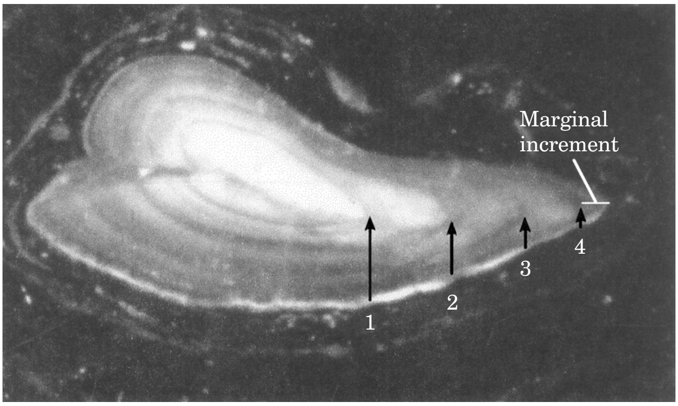

FIG. 1. Illuminated thin-section of a Utah chub otolith showing annuli. Numbers indicate check marks from age 1 to 4 years. The marginal increment for this individual is measured as the distance from the 4 year check mark to the outer margin of the otolith.

with one opaque band forming per year thus indirectly validating opaque bands as annuli. These results, coupled with a decreasing marginal increment in March (for young fish), suggest that opaque bands begin to form sometime during the winter and are completely formed by May. Based on validated annuli counts, individuals included in this analysis were found to range in age from 1 to 14 years.

Relative to other water bodies at this latitude, Big Spring is remarkably stenothermic (Fig. 2). The most substantial decrease in mean monthly water temperature in Big Spring was a decrease of 0·3° C between October and November. This decline, however, was less than a single S.D. in water temperature observed within either of these months. Hence, although it is possible that such fine scale changes in temperature could affect otolith marking in this species, the observed variability in the temperature data suggests that this is not

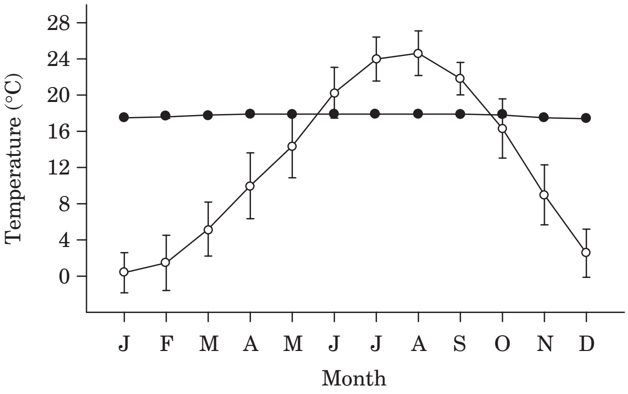

FIG. 2. Mean ± S.E. monthly water temperatures in Big Spring (●) and the Great Salt Lake (○). Big Spring is fed by a natural spring, resulting in fairly constant temperature environment. In contrast, the Great Salt Lake shows seasonal fluctuation in water temperature, a profile typical of lakes at this latitude. The S.E. for the Big Spring means are smaller than the symbol.

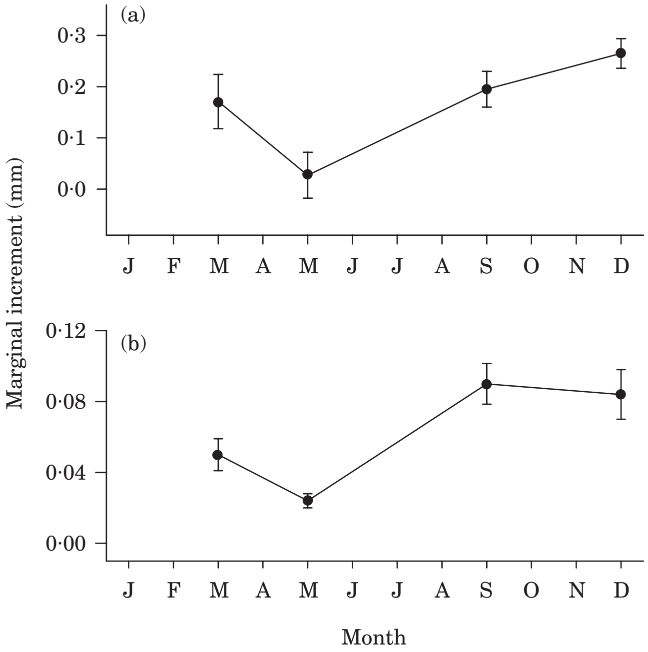

FIG. 3. Mean marginal increment lengths (least squared means ± S.E. from the ANOVA; see text) for Utah chub from four collections made over 1 year. Juvenile (1–2 years) (a) and adult fish (3–14 years) (b) were evaluated separately. Sample sizes of individuals evaluated in each period (March, May, September and December) were $n = 16$, 5, 6 and 11 for juveniles and $n = 12$, 16, 66 and 9 for adults.

likely. What then could be responsible for the clear pattern of annuli formation observed in Utah chub? Three additional possibilities exist: (1) annuli formation in Utah chub could occur as a consequence of the single annual reproductive bout characteristic of this species (Johnson & Belk, 1999). Utah chub in Big Spring spawn from late April to early May and females invest *c.* 10% of their total body mass in gonad tissue and gametes during peak reproductive activity; males invest *c.* 2·5% of their total body mass in gonad tissue and gametes (J.B. Johnson, unpubl. data). Such investment in reproduction could result in physiological changes that in turn affect the chemical composition of materials laid down on otoliths (Pannella, 1974; Sturm & Salter, 1990). This explanation however is limited to reproducing adult fish, and cannot account for annual marks found in juvenile Utah chub evaluated in this study; (2) a second explanation is that annuli form in response to seasonal declines in resource availability. Utah chub, and other temperate fishes in relatively warm, constant environments, face an unusual metabolic challenge when photosynthetically active radiation and primary productivity decline during winter months while temperature-dependent metabolic demands persist throughout the year (Pauly, 1998). Other studies have shown that changes in resource availability can result in annuli formation (Schramm, 1989; Radtke & Fey, 1996) and suggest that if persistent winter food stress occurs, it could be related to otolith marking in Utah chub. Similar annual shifts in resource availability between wet and dry seasons apparently account for otolith marks in some tropical freshwater fishes (Warburton, 1978; Yosef & Casselman, 1995); (3) a third possibility that has received much less attention is that annuli formation is a by-product of circannual processes that are under endogenous physiological control. Opaque zones in otoliths form when crystal deposition on the outer surface of the otolith changes from mostly calcium carbonate to mostly organic material (Watabe *et al.*, 1982). Seasonal changes in photoperiod could entrain annual biological cycles including those related to growth, reproduction or somatic maintenance that could in turn affect the composition of material deposited on otoliths (Schramm, 1989). Support for this idea exists in the fact that daily growth ring deposition on otoliths, although potentially modified by exogenous factors (Neilson & Geen, 1984), occurs primarily as a diel circadian process (Campana & Neilson, 1985). Whether annuli formation in fishes is under similar control remains to be tested. Such an explanation, however, could account for annual marks in both juvenile and adult fishes, as observed in this study.

Utah chub from a stenothermic desert spring do in fact form valid annual growth rings. This finding sheds light on the view that seasonal variation in water temperature is a primary determinant of annuli formation in temperate freshwater fishes. The results demonstrate the utility of otolith studies in desert spring fishes, and add yet another piece to the puzzle of how environmental cues interact with endogenous factors to produce annual growth rings in fishes.

We are grateful to Utah Division of Wildlife Resources who issued collecting permits, to C. Burden with USGS Water Resources for the Great Salt Lake temperature data, and to M.J. Baker who helped with winter fish collections.

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