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SUSAN M. SHIRLEY
THOMAS C. SHIRLEY

Juneau Center for Fisheries and Ocean Sciences
University of Alaska—Fairbanks
11120 Glacier Highway
Juneau, AK 99801

REEXAMINATION OF THE USE OF OTOLITH NUCLEAR DIMENSIONS TO IDENTIFY JUVENILE ANADROMOUS AND NONANADROMOUS RAINBOW TROUT, *SALMO GAIRDNERI*[1]

Otoliths are a potential source of taxonomic characteristics for identifying stocks of fish (Ihssen et al. 1981). Differences in dimensions of the otolith nucleus have provided a basis for separating winter from summer races of steelhead, anadromous rainbow trout, *Salmo gairdneri*. In addition, otoliths provided data from which to distinguish steelhead from resident nonanadromous forms as well (McKern et al. 1974; Rybock et al. 1975). Neilson et al. (1985) studied the development of sagittal otoliths in resident rainbow trout and steelhead from south-central British Columbia, and identified sources of variability in the size of otolith nuclei. However, they were unable to find morphometric differences between the two forms of trout. They concluded that the usefulness of dimensions of the otolith nucleus for separating steelhead from resident rainbow trout was much more limited than that suggested by Rybock et al. (1975) for rainbow trout in the Deschutes River, Oregon.

The difference in mean length of the otolith nuclei between the rainbow trout studied by Rybock et al. (1975) and those studied by Neilson et al. (1985) suggested either population differences or differences in defining the nuclear boundary. These disparate results, which led to opposite conclusions, limit the usefulness of measurements of otolith nuclei for the racial identification of juvenile rainbow trout until the source of these differences is better understood. Consequently, to determine whether juveniles of the two forms could be distinguished by differences in dimensions of otolith nuclei, we measured the nuclei in sagittae from steelhead and resident rainbow trout collected from the same Deschutes River, OR, locations used by Rybock et al. (1975). We used the definitions proposed by Rybock et al. and by Neilson et al. (1985), and compared our measurements for the two forms with each other and with published values.

Methods

Resident rainbow trout and steelhead were col-

lected from three locations in the Deschutes River, OR. Resident rainbow trout, which were collected from the main stem near the mouth of Nena Creek in March 1985, were mature and smaller (280–450 mm FL) than the steelhead, and, based on analyses of scales and otoliths (McKern et al. 1974), had never entered saltwater. Juvenile progeny of steelhead were collected from Round Butte Hatchery on the Deschutes River in 1984. Wild juvenile rainbow trout (<200 mm FL) of unknown parental origin were collected in 1984 and 1985 from Bakeoven Creek, an important spawning tributary for steelhead in the Deschutes River.

Sagittae removed from rainbow trout were stored in 90% ethanol for up to two months. Before they were viewed, one otolith from each pair was mounted (concave face up) with epoxy on a glass slide. The back of the slide was blackened with indelible ink. The otolith was ground by hand with 600 grit wet sandpaper and periodically inspected under a light microscope at 100× until the microstructure of the nucleus, as described by Neilson et al. (1985), was visible. The otolith was rinsed with 5% HCl for several seconds to remove scratches and improve resolution.

To reduce bias, we coded each slide with a random number and ordered the slides sequentially for viewing. Otoliths were examined with a Zeiss[2] dissecting microscope at 125×. A camera lucida attachment enabled us to use a computer digitizer to measure three dimensions of the otolith. In measuring length and width of the central nucleus, we used the first growth increment encircling all the central primordia, which was the nuclear boundary defined by Neilson et al. (1985). In addition, we measured the maximum length along the longest axis through an area defined by the first metamorphic check, a narrow hyaline ring surrounding an opaque ring with a hyaline center, to replicate the measurements of Rybock et al. (1975).

We used analysis of variance (ANOVA) to test for significant differences in each dimension of the otolith nuclei among groups in our study. Where adequate data were available, we tested for significant differences between groups in our study and similar groups described by Rybock et al. (1975) and Neilson et al. (1985) for mean dimensions of otolith nuclei. Neilson et al. (1985) showed that the mean length of otolith nuclei for

rainbow trout incubated at 6.5°C was significantly less than those for trout incubated at 9.5° or 15.0°C. Because of this discrepancy, we evaluated the potentially confounding effects of incubation temperature on the comparisons of otolith dimensions between our samples and those of Rybock et al. (1975), by testing the hypothesis that water temperatures during 1967–69 were higher than those during 1982–83. We used a paired t-test of average daily water temperatures recorded by the U.S. Geological Survey on the 1st and 15th day of each month from 1 January to 1 August during 1967–69 and 1982–83 (U.S. Department of the Interior Geological Survey 1967, 1968, 1969, 1982, 1983). These dates represent the incubation periods for most of the resident rainbow and steelhead trout sampled in our study and by Rybock et al. (1975). Incubation temperature for steelhead at Round Butte Hatchery is from hatchery records. We estimated spawning and incubation periods for resident rainbow and steelhead trout on the basis of reports of the Oregon Department of Fish and Wildlife (Fessler 1972) and personal observations.

Results

For each dimension, we failed to reject the hypothesis ($\alpha = 0.05$) that rainbow trout collected from different populations for our study had otolith nuclei of the same size (Table 1). Therefore, we concluded that these dimensions could not be used to discriminate between the resident and steelhead forms of rainbow trout sampled in our study.

Water temperatures during 1967–69 were slightly greater than those during 1982–83 ($t = 2.03$, df = 14, $P = 0.03$). Mean difference between the two periods was 0.8°C. Spawning dates for resident rainbow trout and steelhead differ; steelhead spawn from January to April and resi-

TABLE 1.—Means, standard errors (in parentheses), and sample size for three otolith dimensions in resident rainbow trout and steelhead from three Deschutes River populations.

Populations compared	No. of fish	Dimensions of nuclei		
		Nucleus length (mm)	Nucleus width (mm)	Check length (mm)
Resident rainbow trout	44	0.173 (0.006)	0.070 (0.003)	0.323 (0.012)
Hatchery steelhead	30	0.190 (0.006)	0.070 (0.002)	0.349 (0.009)
Suspected wild steelhead	32	0.178 (0.006)	0.069 (0.002)	0.312 (0.007)

dent rainbow trout spawn from May to mid-July (Fessler 1972). Mean water temperature during the period of steelhead egg incubation was 8.4°C for 1967–69 and 7.6°C for 1982–83. Mean water temperature during the period when resident rainbow trout eggs were incubating in the main stem of the river was 12.6°C in 1967–69 and 11.9°C in 1982–83. Incubation temperature for steelhead at Round Butte Hatchery was 10°C and did not vary.

The dimensions of otolith nuclei from resident rainbow trout and steelhead in our study were indistinguishable from those in fish from British Columbia. No significant difference ($\alpha = 0.05$) in mean length of otolith nuclei existed between the British Columbia steelhead incubated at 9.5° or 15°C and suspected wild steelhead from Bakeoven Creek or Round Butte Hatchery steelhead incubated at 10°C. Among resident rainbow trout, the mean length of otolith nuclei for fish from the Deschutes River was also not significantly different from that for fish from British Columbia incubated at 9.5° or 15°C. Because Rybock et al. (1975) did not provide variances, we were unable to test the hypothesis that means from our study coincided with theirs. However, mean length and width of otolith nuclei in our study were 29 and 55% less, respectively, for resident rainbow trout and 49–70% less, respectively, for steelhead than those studied by Rybock et al. (1975).

Discussion

The similarity of our results to those of Neilson et al. (1985), who used similar methods, might be expected for different populations under similar genetic and environmental control. The disparate results of our study and that of Rybock et al. (1975) for the same populations after little genetic change (based on comparisons of unpublished, biochemical genetic data for these populations from 1972 to 1974 and 1984 to 1986) and little environmental change partly reflected the use of different definitions for the nucleus. We defined the nuclear boundary as the first growth ring surrounding all the fused primordia, whereas Rybock et al. (1975) defined the nucleus as the hyaline area in the center of the otolith that is bounded by a metamorphic check formed at hatching; they resolved the check by rendering the otolith with HCl. We also measured the length of the check surrounding the nucleus, as-defined by Rybock et al. (1975), which we found either to correspond with the area enclosed by the

first check or to increase in density of growth increments surrounding both the central and rostral primordia. The close similarity between our estimate for Round Butte Hatchery steelhead (0.349 mm) and the mean calculated by Rybock et al. (1975) for steelhead (0.354 mm) suggested similar checks. It is unclear, however, why values for resident rainbow trout for this dimension and the results of tests to discriminate races differed between the two studies. Rybock (1973) noted that the nuclear check could not be distinguished in 29% of the otoliths and that the use of HCl may have caused the frequent confusion between the metamorphic check and other groups of daily growth rings. The grinding and polishing of otoliths greatly reduce this source of error. Neilson et al. (1985) also discouraged the use of metamorphic checks as boundaries because the causal links between checks and developmental events, such as hatching, have not yet been established.

Neilson et al. (1985) demonstrated that nuclear length increased significantly with increase in incubation temperature from 6.5° to 9.5°C but not from 9.5° to 15°C. Although average water temperatures in the Deschutes River were 0.8°C lower during 1982–83 than in 1967–69, it is unlikely that such differences completely explain the greater estimates of mean length and width of otolith nuclei in the earlier study by Rybock et al. (1975). Rybock et al. (1975) calculated mean nuclear lengths and widths of 0.354 and 0.230 mm for steelhead and 0.243 and 0.154 mm for resident rainbow trout in the Deschutes River. Our estimates were 29–70% less than their estimates for a 0.8°C difference; whereas under controlled conditions in British Columbia, mean nuclear length for resident rainbow trout at 6.5°C was 18% less for resident rainbow trout and 21% less for steelhead than the nuclear length for fish incubated at 9.5°C, a difference of 3°C (Neilson et al. 1985).

Comparisons of otolith nuclear dimensions between resident rainbow trout and steelhead incubated at similar temperatures would establish whether significant differences exist for these measurements between the two races from the Deschutes River. The use of a common definition of nuclear boundaries would allow better comparisons between studies. However, given the disparate results of our study, which were similar to the results of Neilson et al. (1985), and the original study for steelhead and resident rainbow trout in the Deschutes River, as well as our failure to discriminate between races using both nuclear definitions proposed by Neilson et al. (1985)

and Rybock et al. (1975), we believe that population differences do not explain the differences in results between the studies of Rybock et al. (1975) and Neilson et al. (1985). Furthermore, our study provided strong evidence to support the conclusion of Neilson et al. (1985) that the usefulness of measurements of otolith nuclei to identify sympatric juvenile progeny of resident rainbow trout and steelhead reared in the wild may be limited.

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KENNETH P. CURRENS
CARL B. SCHRECK
HIRAM W. LI

Oregon Cooperative Fishery Research Unit
Oregon State University
Corvallis, OR 97331[3]

AGE-SPECIFIC VULNERABILITY OF PACIFIC SARDINE, *SARDINOPS SAGAX*, LARVAE TO PREDATION BY NORTHERN ANCHOVY, *ENGRAULIS MORDAX*

To a large degree interannual variability in recruitment determines the size of pelagic fish populations. Recruitment to the Pacific sardine, *Sardinops sagax*, population off California varies from year to year over several orders of magnitude and is unrelated to spawning stock size (Murphy 1966; MacCall 1979). Variable mortality rates in the first year of life must determine year-class strength, although the sources of this variability are unknown. Mortality rates in the earliest stages are size specific with highest rates in the egg and yolk-sac stage (Ahlstrom 1954; Butler 1987) and may contribute to variability in year-class strength (Smith 1985).

The sources of mortality of sardine larvae have yet to be investigated. In other pelagic larvae, mortality is due to either starvation or predation, and starvation is significant only during the brief period after the onset of feeding (O'Connell 1980; Hewitt et al. 1985; Theilacker 1986; Owen et al. 1987). In sardines, significant mortality occurs during the egg and yolk-sac stages (Ahlstrom 1954) and this mortality can only be due to predation. Variable mortality in older larval and juvenile sardines may also contribute to variability in recruitment, and this mortality, as in other fishes, may also be due to predation (Hunter 1984).

The objective of this paper was to determine the size-specific vulnerability of Pacfiic sardine larvae to predation by adult northern anchovies, *Engraulis mordax*. The vulnerability of cape anchovy and northern anchovy larvae to