

Health Assessment, Associated Metrics, and Nematode Parasitism of Rainbow Trout in the Colorado River below Glen Canyon Dam, Arizona

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Abstract.—We captured rainbow trout *Oncorhynchus mykiss* in the Colorado River below Glen Canyon Dam, Arizona, between 1995 and 1997 to develop baseline data for a modified health assessment index (mHAI) and to determine relationships among mHAI, parasite burden, relative condition, mesenteric fat index, and diet. We also examined effects of a simulated flood on these variables during 1996. There were no significant effects of the flood on variables measured in the fish. Total stomach volume increased during spring and summer following the flood and reflected an expected trend. Health of fish, based on the mHAI, remained good throughout the study but declined with increasing burden of the nematode *Truttaedacnitis truttae* and with trout size. Increasing relative condition and greater mesenteric fat levels and stomach volumes of macroinvertebrates were associated with reduced mHAI. Larger trout also ate more *Cladophora glomerata* than did smaller fish, and nematode burden was related negatively to volumes of the alga and macroinvertebrates in stomachs, suggesting relationships among fish age, parasite burden, and diet. We suggest that health assessments for the tailwater rainbow trout fishery at Glen Canyon Dam should include relative condition and indices of mesenteric fat levels, nematode burden, and diet as metrics. Moreover, evaluations of health should address length-specific categories for long-term monitoring of rainbow trout in the tailwater and fish populations in general.

Goede and Barton (1990) developed a necropsy-based method for evaluating individual fish health in order to establish databases for monitoring and detecting the responses of fish to environmental stressors. The necropsy-based technique was modified by Adams et al. (1993) to provide a quantitative health assessment index (HAI) allowing statistical comparisons of data among fish populations. The Goede and Barton (1990) procedure was based primarily on data derived from salmonid fishes, but modifications of the method have been applied to coldwater and warmwater fishes (Adams et al. 1993; Coughlan et al. 1996; Robinson et al. 1998). No consistent approach to assessment of fish health has yet emerged, and distinctions between health and condition are unclear in relation to various health assessment procedures (Goede and Barton 1990; Adams et al. 1993; Coughlan et al. 1996).

Condition indices based on length–weight relationships are widely used to evaluate the physiological well-being of fish populations and effects of environmental variables and management strategies (Bolger and Connolly 1989; Murphy et al. 1991; Anderson and Neumann 1996). However,

the relation of these indices to other aspects of fish population ecology is poorly understood (Murphy et al. 1991). Values of Fulton’s condition factor (K) are length- and species-dependent and may limit comparison of fish of disparate lengths within a species (Murphy et al. 1991). Relative condition (K_r) can be used within a population and is based on local and regional weight–length summaries (Murphy and Willis 1992); relative weight (W_r) is based on the length-specific standard weight representing the species as a whole (Murphy et al. 1991; Anderson and Neumann 1996; Simpkins and Hubert 1996). Coughlan et al. (1996) used relative weight in health assessments, whereas others (Goede and Barton 1990; Sutton et al. 2000) incorporated Fulton’s condition factor. In contrast, a fish condition index was excluded from the HAI developed by Adams et al. (1993). Robinson et al. (1998) found no significant correlation between K and an HAI in seven riverine fishes.

Better condition may be reflected in greater mesenteric fat levels, which reflect long-term feeding and energy deposition (Goede and Barton 1990; Porath and Peters 1997). However, in developing HAIs, Adams et al. (1993) omitted mesenteric fat levels, whereas others (Goede and Barton 1990; Coughlan et al. 1996; Robinson et al. 1998) examined mesenteric fat levels in health assessments. Hematological variables have been incorporated

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into health assessments (Goede and Barton 1990; Adams et al. 1993; Coughlan et al. 1996). However, use of some hematological indices (e.g., hemoglobin and hematocrit) to evaluate fish health is suspect because of lack of measurement precision, distortion associated with sampling procedures and storage conditions, and uncertainty regarding hematological norms (Houston 1997). Hematological values also may vary with age of fish, season, and reproductive status of salmonids (Denton and Yousef 1975; Lane 1979; Wagner et al. 1997). Coughlan et al. (1996) found that blood variables contributed little to health assessments of largemouth bass *Micropterus salmoides*. Robinson et al. (1998) found no correlation between hematological variables and an HAI or condition for five fish species, but hematocrit and an HAI were positively correlated for desert suckers *Catostomus clarki*. Although Sutton et al. (2000) examined serum lysozyme and protein in rainbow trout *Oncorhynchus mykiss*, blood variables were excluded from calculation of an HAI.

The ability of an HAI to represent the health profile of a fish population can be evaluated by comparing it with other measures of fish health or condition collected simultaneously (Adams et al. 1993). Robinson et al. (1998) excluded parasite infection from an HAI in order to determine the relationship between parasites and health of native and nonnative riverine fishes. They found that HAI values increased (poorer health) with greater parasite burden for some species. Condition of round-tail chub *Gila robusta* also declined with increasing parasite burden, suggesting the need for further evaluation of associations among health assessment, parasite burden, and condition indices. Adams et al. (1993) included parasite abundance in their HAI, but Coughlan et al. (1996) suggested that fish parasites should not be used in developing health assessment indices because any effects of bacterial or parasitic infection should lead to changes in relative weight.

Parasite infestation in a riverine system may impact host fishes and limit populations (Anderson 1978; Anderson and May 1978; May and Anderson 1979; Anderson and Gordon 1982). The parasitic nematode *Truttaedacnitis truttae* (Maggenti 1971; Choudhury and Dick 1996) is prevalent in rainbow trout in the tailwater fishery below Glen Canyon Dam, Arizona (GCD), but the ecological implications of nematode infestation are poorly understood (Dick and Choudhury 1995). The nematode attaches to the pyloric caeca of the intestine (Maggenti 1971) and may influence food consumption,

impair growth, and reduce reproductive potential and survival of host fish (Hiscox and Brocksen 1973; Anderson and Gordon 1982). Host age, species, and diet may be key variables influencing the intensity of certain parasitic infections (Amin 1985; Coughlan et al. 1996; Robinson et al. 1998). The life cycle of *T. truttae* in North America is undocumented (Dick and Choudhury 1995) but may include an intermediate copepod host (Hiscox and Brocksen 1973). However, Brouder and Hoffnagle (1997) found no copepods in stomachs of rainbow trout caught below GCD, suggesting occurrence of an alternative intermediate host in the tailwater.

Development of health assessment indices may be useful in monitoring the well-being of free-ranging fish populations. However, little information is available regarding associations between health assessment indices and other measures of fish well-being. The objectives of our study were to collect baseline data on health and parasitism of rainbow trout in a regulated river and to determine possible relations with a modified HAI, fish parasite burden, relative condition, mesenteric fat index, and diet. During the period that we collected baseline data, a simulated flood occurred (Rubin et al. 1998), and a secondary objective was to determine if the flood influenced the above variables.

Study Site

Glen Canyon Dam impounds the Colorado River in northcentral Arizona near the Arizona–Utah border, forming Lake Powell, a 653-km² meromictic reservoir. Hypolimnetic releases from the reservoir are clear and cold (8–10°C; Stevens et al. 1997). The tailwater trout fishery occurs in a reach from the dam to 25.5-km downstream; mean depth of this 341-ha reach (at dam releases of 426 m³/s) is 6.5 m, and mean width is 135.6 m. Nonnative rainbow trout predominate in the tailwater reach, are self-sustaining, and are sympatric with native flannelmouth sucker *Catostomus latipinnis* (McKinney et al. 1999b, 2001) and nonnative common carp *Cyprinus carpio*. Releases from GCD generally were 568–625 m³/s during our study. However, a simulated flood (March 22–April 7, 1996) discharged a peak flow of 1,290 m³/s from the dam for 7 d, which was immediately preceded and followed by 4 d of steady low releases (238 m³/s; Rubin et al. 1998).

Methods

Rainbow trout were collected by electrofishing between dusk and dawn during six periods from

December 1995 to April 1997. We electrofished from an aluminum boat, making a single pass of about 33 min at each of 15 (1995–1996) or 9 (1997) fixed transects (the reduced number still allowed for adequate stock assessment). We used a complex pattern of pulsed DC and applied 215-V and 15-A output to a 30-cm stainless steel anode system (Sharber et al. 1994). Fish greater than 150 mm total length (TL) were subsampled randomly from the total electrofishing catch to exclude young-of-year but include both juveniles (≤ 300 mm) and adults. Fish were held (15–90 min) in open live wells until measured for total length (in millimeters), weighed (grams), and necropsied within 30 min of death by manual concussion. Relative condition ($K_n = W/W$, where W = total wet weight, $W = 10^{-4.6 + 2.856 \cdot \log_{10} TL}$) was computed. The length-specific mean weight (W) equation used in this study was developed for rainbow trout collected in the tailwater during 1984–1990 using the 75th percentile technique (Murphy et al. 1990, 1991).

Stomach contents of trout were identified to the lowest practical taxonomic level and measured (milliliters) using volumetric displacement. Relative stomach volume (Filbert and Hawkins 1995) was computed for total stomach contents (RGVT), total macroinvertebrates (RGVI), and *Cladophora glomerata* (RGVC).

We qualitatively rated characteristics of 10 anatomical features (fins, eyes, opercula, gills, thymus, pseudobranchs, spleen, kidney, liver, and hindgut) following Goede and Barton (1990) and calculated a necropsy-based modified health assessment index (mHAI) using these variables for each fish, following Robinson et al. (1998). The mHAI differed slightly from Robinson et al. (1998) in that skin condition was not assessed. Thus, the maximum attainable index value was 300. We also ranked the quantity of mesenteric fat (MFI; Goede and Barton 1990) for each fish. From postmortem evaluations of each fish, we ranked nematode burden (NEM) ordinality (0 = none, 1 = 1–10, 2 = 11–100, 3 = 101–250, 4 = 251–500, 5 = >500), which we based on previously reported levels of parasite infection (Hiscox and Brocksen 1973; Ingham and Arme 1973; Brown 1986). After preliminary assessments, we excluded measurement of hematological variables because we encountered difficulties with sampling, measurement, and sample handling under field conditions.

We used multivariate analysis of variance (MANOVA) and Tukey's multiple comparisons (Bonferroni corrected) to determine differences

among sampling periods for mHAI, TL, K_n , MFI, NEM, RGVT, RGVI, and RGVC. We evaluated effects of the simulated flood on these variables by comparing March 22–24 (preflood) and April 5–7 (postflood) data. We used chi-square analysis to assess differences in nematode prevalence (frequency of fish with nematodes in stomachs) among sampling periods and Pearson's correlation to assess diet (RGVT, RGVI, and RGVC) changes as fish length increased.

We used forward stepwise multiple linear regression in SPSS (SPSS 1998) to assess relationships among variables. The stepwise method is the most commonly used, especially when the independent variables are correlated (SPSS 1998); this routine removes a correlated independent variable if it diminishes the importance of an already entered variable. In addition, we used Pearson's correlation to assess all pairwise correlations among variables to further assess the appropriateness of the regression models. We performed three regressions, to assess relationships between (1) mHAI and TL, K_n , MFI, NEM, RGVI, RGVC, and RGVT, (2) MFI and TL, K_n , RGVI, RGVC, and RGVT, and (3) NEM and K_n , TL, MFI, RGVT, RGVI, and RGVC. A subset of the data (all data except April 1997) was used in the first two regression analyses because neither mHAI or MFI differed among periods when April 1997 was removed. We used a different data subset for the third (K_n) regression (all except December 1995; K_n in the resulting subset did not differ among periods).

Results

We collected 215 rainbow trout ranging from 172 to 538 mm TL for assessment of health and other variables. Length frequencies and relative nematode burden among all fish followed normal distribution patterns. Differences were detected among sampling periods for seven of the nine variables examined (Table 1). Total RGV increased following the peak flow (March 22–24 versus April 5–7, 1996) and remained high in August 1996, but changes in other variables immediately following the simulated flood were not significant.

Linear correlations between variables typically were weak (< 0.45), except for the correlation between RGVT and RGVC (Table 2). However, five of seven variables were correlated with mHAI, and K_n declined with increasing TL but was positively associated with MFI. Diet composition also changed as fish length increased. Relative stomach volumes of macroinvertebrates did not change, but RGVC and RGVT increased with greater TL. In-

nificantly with NEM, but linear correlations indicated negative relationships of TL and NEM versus relative stomach volumes (Table 2).

Discussion

Fish health, as measured by the mHAI, remained high throughout the study; values never exceeded about 14% of the maximum attainable level and were as low as 6%. Health assessment index values reported elsewhere (Adams et al. 1993; Coughlan et al. 1996; Robinson et al. 1998; Sutton et al. 2000) indicate that the mHAI values we found that were above 75 might be cause for health-related concerns. Several variables differed statistically among sampling periods (mHAI, MFI, TL, NEM, RGVC, RGVI, and RGVT), but clear patterns of change over time generally were not apparent. Only total volume of stomach contents changed (increased) following the simulated flood, probably reflecting seasonal trends in trout diets or availability of algae and macroinvertebrates in the drift (Brittain and Eikeland 1988; Shannon et al. 1996; McKinney et al. 1999a, 1999d). Our results are consistent with conclusions for the total electrofishing sample that the simulated flood had no significant short-term influence on rainbow trout in the tailwater (McKinney et al. 1999c).

Prevalence of infection by *T. truttae* remained high throughout the study. Mean parasite burden was similar to previously reported levels for helminths in rainbow trout (Hiscox and Brocksen 1973; Ingham and Arme 1973; Brown 1986), but more than one-third of fish contained more than 100 nematodes. Most eukaryotic organisms, such as helminths, appear to do little harm to their hosts, unless present in very large numbers (Anderson and May 1978). The number of parasites required to kill a host is difficult to estimate (Crofton 1971), but Hiscox and Brocksen (1973) found that under laboratory conditions as few as 1–7 *T. truttae* per juvenile rainbow trout were associated with increased consumption rate and reduced growth. Hiscox and Brocksen (1973) also suggested that a 50% infection level among hosts associated with 4–5 nematodes per infected fish could depress growth rates of juvenile rainbow trout, but they cautioned against extrapolation of these results to natural populations. They further reported that up to 13 of the nematodes were observed in catchable trout, many fewer than we observed in more than one-third of fish.

Despite a high prevalence of infection, adequate relative condition and low mHAI values indicated that *T. truttae* were not regulating the GCD tail-

water rainbow trout population (McKinney et al. 1999c, 1999d, 2001). However, host mortality generally is dependent on helminth parasite burden in vertebrates (Anderson and May 1978; Anderson and Gordon 1982), and mortality associated with greater nematode burden may contribute to the rarity of large fish (>405 mm) captured by electrofishing in the GCD tailwater (McKinney et al. 1999d, 2001).

Scores of the mHAI increased with fish length and parasite burden, and K_n declined with increasing TL, suggesting that larger trout tended to be in relatively poorer condition and health than smaller fish and that greater nematode burden negatively influenced fish health. It may be that declining trout health and relative condition were related to a shift toward a less nutritious diet as fish increased in size, because larger fish consumed more *C. glomerata* (Weiland and Hayward 1997). However, we found weak positive associations between mesenteric fat levels and trout length and relative condition. The positive relationship between K_n and MFI supports the notion that the ponderal index reflects surplus energy stored primarily as fat and protein (Lemly and Esch 1984; Gutreuter and Childress 1990).

Our results are consistent with those of Hiscox and Brocksen (1973) in that infestation by the parasitic gut nematode *T. truttae* may have influenced health and diet of rainbow trout. Greater nematode burden in our study was associated with reduced relative stomach volumes of *C. glomerata*, macroinvertebrates, and total contents, suggesting that increased parasite load may alter feeding behavior, reduce food capacity of the stomach, affect food assimilation, or interfere with rate of food passage through the intestine (Hiscox and Brocksen 1973; Ingham and Arme 1973). Depression of food intake often is the first indication of fish disease under natural conditions (Néji and de la Noüe 1998). In contrast, Hiscox and Brocksen (1973) found that juvenile rainbow trout inoculated with *T. truttae* increased food consumption. Disparity in food intake in our study may be related to natural versus laboratory conditions or to fish age (length), diet, or high parasite burden (Hiscox and Brocksen 1973; Ingham and Arme 1973; Grove et al. 1978). Amin (1985) also found that increased acanthocephalan burden was associated with greater consumption of invertebrates by older fish.

In general, the relative importance of different metrics potentially included in calculating an HAI is unclear. Adams et al. (1993) suggested that no more than 6 of 14 HAI variables influenced health

of largemouth bass or redbreast sunfish *Lepomis auritus*. Sutton et al. (2000) questioned the merit of evaluating pseudobranchs and thymi, which we assessed in calculating mHAI. In contrast to the suggestion of Coughlan et al. (1996), we found that nematode burden was associated with changes in mHAI but not in K_r . However, our results support the recommendation of Coughlan et al. (1996) that a length-weight metric be incorporated into calculation of an HAI.

Goede and Barton (1990) recognized that health assessment indices are not designed as a substitute for other measurements of health or condition, nor will they meet all requirements of fishery managers. Modifications of the necropsy-based method may be necessary to address specific concerns, such as those related to particular aquatic systems and fishery management objectives (Adams et al. 1993). Relative condition and the mHAI in our study provided generally similar but weakly related indices of trout well-being. We suggest that use of relative condition and indices of nematode burden, mesenteric fat, and diet as metrics in necropsy-based assessments will provide additional useful information to monitor health and the influences of long-term environmental variables on the Lee's Ferry rainbow trout fishery. Evaluations of specific length categories of fish also should provide greater insight into the dynamics of rainbow trout health in the GCD tailwater (and fish populations in general) in relation to environmental factors than assessments of these variables based on total (not length-specific) electrofishing samples (Goede and Barton 1990; Marwitz and Hubert 1997; McKinney et al. 1999d, 2001).

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