growth and increased opacity of overlying tissues. Peritoneal melanophore pigmentation is an obvious character for later stages of some larvae, but in late metalarvae and especially juveniles, dark peritoneal pigmentation can be obscured by overlying muscle or membranes with silvery iridophores (this silvery pigment often dissipates over time in formalin preservative, but is usually retained in alcohol). If internal melanophore pigmentation is obscured by overlying tissues, it can be observed by selective dissection or careful clearing of specimens.

Osteology

When externally visible characters fail to segregate species conclusively, osteological characters may come to the rescue. Although whole-specimen clearing and cartilage- and bone-staining techniques are relatively simple (see Methods in Snyder and Muth 1988, 1990, or 2004), they require much time (a few days, mostly waiting) and a fair amount of attention (monitoring progress and changing fluids). Soft (longwave) X-ray techniques (Tucker and Laroche 1984) may be faster and easier, especially when examining many specimens, but they require appropriate X-ray equipment and a darkroom.

Dunn (1983, 1984) reviewed use of skeletal structures and the utility of developmental osteology in taxonomic studies. Among the first bones to ossify are those associated with feeding, respiration, and orientation (e.g., jaws, bones of the branchial region, cleithrum, and otoliths). The axial skeleton follows with formation of vertebrae and associated bones. Once the axial skeleton is sufficiently established, median- and pelvic-fin supports form, and fins develop. Presence, number, position, and shape of certain bones in many parts of the skeleton can have diagnostic value, even for closely related species. Use of osteological characters for identification of fish larvae has received little attention, but its potential value is great, particularly for confirmation of questionable identities and for species in which external characters are diagnostically inadequate.

METHODS

Specimens Examined

Study specimens for description of desert sucker, Sonora sucker, longfin dace, spikedace, and loach minnow were selected from more than 53,000 as-yet uncatalogued specimens in the LFL Collection that were collected or reared in 1982 through 1984 from the Gila River drainage of southwestern New Mexico. Many of these specimens were identified and all were inventoried for consideration as part of this investigation.

The remaining study specimens for these descriptions were selected from among specimens loaned or contributed by outside sources. These included 16 metalarval and juvenile loach minnow collected from the Gila River Basin in New Mexico (MSB 2544,4692, 4801, 4817), 150 specimens from a recently reared developmental series of longfin dace (MSB 49871), and 72 specimens from a recently reared series of spikedace (MSB 43810) loaned by the Museum of Southwestern Biology (Albuquerque, New Mexico); and 82 specimens from a recently reared series of loach minnow contributed by Michael Childs of the Arizona Game and Fish Department.

Specimens for supplemental study of all previously described cyprinids were selected mostly from reference or study series in the LFL collection. Most of these specimens were either collected in or reared from stock in the Upper Colorado River Basin during the late 1970s to early 1980s A few speckled dace were selected from among 72 specimens contributed by Michael Childs from a developmental series he reared in Arizona in 1998.

Whenever possible, formalin-preserved specimens (usually 3% buffered) were used for analysis to avoid confounding data with the greater shrinkage and deformation effects typical of many alcohol-preserved specimens. However, some data for larger metalarvae and juveniles of loach minnow were from specimens preserved in 70% ethanol.

Specimen Data, Observations, and Illustrations

Developmental series of desert sucker, Sonora sucker, longfin dace, spikedace, and loach minnow were analyzed in detail (124 specimens longfin dace and 79-85 specimens for each of the others, 532 total) for differences in morphology, morphometrics, meristics, pigmentation, and size relative to developmental state. Additional specimens (35 desert sucker, 52 Sonora sucker) were analyzed only to supplement data on pigmentation and selected developmental-state characters.

Most summarized data for bonytail, roundtail chub, Colorado pikeminnow, speckled dace, common carp, red shiner, and fathead minnow species accounts were extracted from Snyder et al. (1977), Snyder (1981), and Muth (1990). Original raw data for the source descriptions were available for all species except bonytail, roundtail chub, and fathead minnow and were re-analyzed for mesolarvae to divide results for flexion and postflexion subdivisions. Morphometric data for fathead minnow were published as percentages of total length and had to be converted by approximate calculation to percentages of standard length.

To supplement existing descriptive data for the seven previously described cyprinids, 17 to 29 specimens each (total of 153) were analyzed for pigmentation patterns and selected developmental-state characters. Of these, three roundtail chub and four speckled dace were fully analyzed to supplement limited existing data for the protolarval phase.

For each newly described species, various measurements, fin-ray counts, and myomere counts (Figure 4) were made on at least two specimens, if available, in each 1-mm-TL interval throughout the larval period of each species. Thereafter, to a length of about 50 mm TL, two or more specimens were similarly processed for each 5-mm interval, if available. Specimens were studied under low-power stereo-zoom microscopes with measuring eyepiece reticles and various combinations of reflected, transmitted, and polarized light. Most measurements were made using multiple digital images of the specimens captured through the microscope and a computer image-analysis and measurement program (Optimas 5.1, Optimas Corp., Seattle, Washington; now owned by Media Cybernetics, Silver Spring, Maryland). Some meristic data were obtained from specimens cleared and stained for skeletal study and from available adults.

Size at apparent onset of selected developmental events was documented for fully analyzed and cursorily examined specimens. Selected events were hatching, attainment of eye pigment, formation of pectoral- and pelvic-fin buds, loss of yolk and preanal finfold, formation of first and last principal fin rays in each of the median fins, formation of first and last fin rays in the paired fins, formation of first and last rudimentary rays of the caudal fin, and initial and complete formation of lateral scales on the body.

Among other characters considered, developmental phase and extent of gut folding were determined for all analyzed and many other specimens. Gut folding was classified as one of five gut phases (Figure 5). Changes in mouth position and size, lower-lip-lobe separation in the

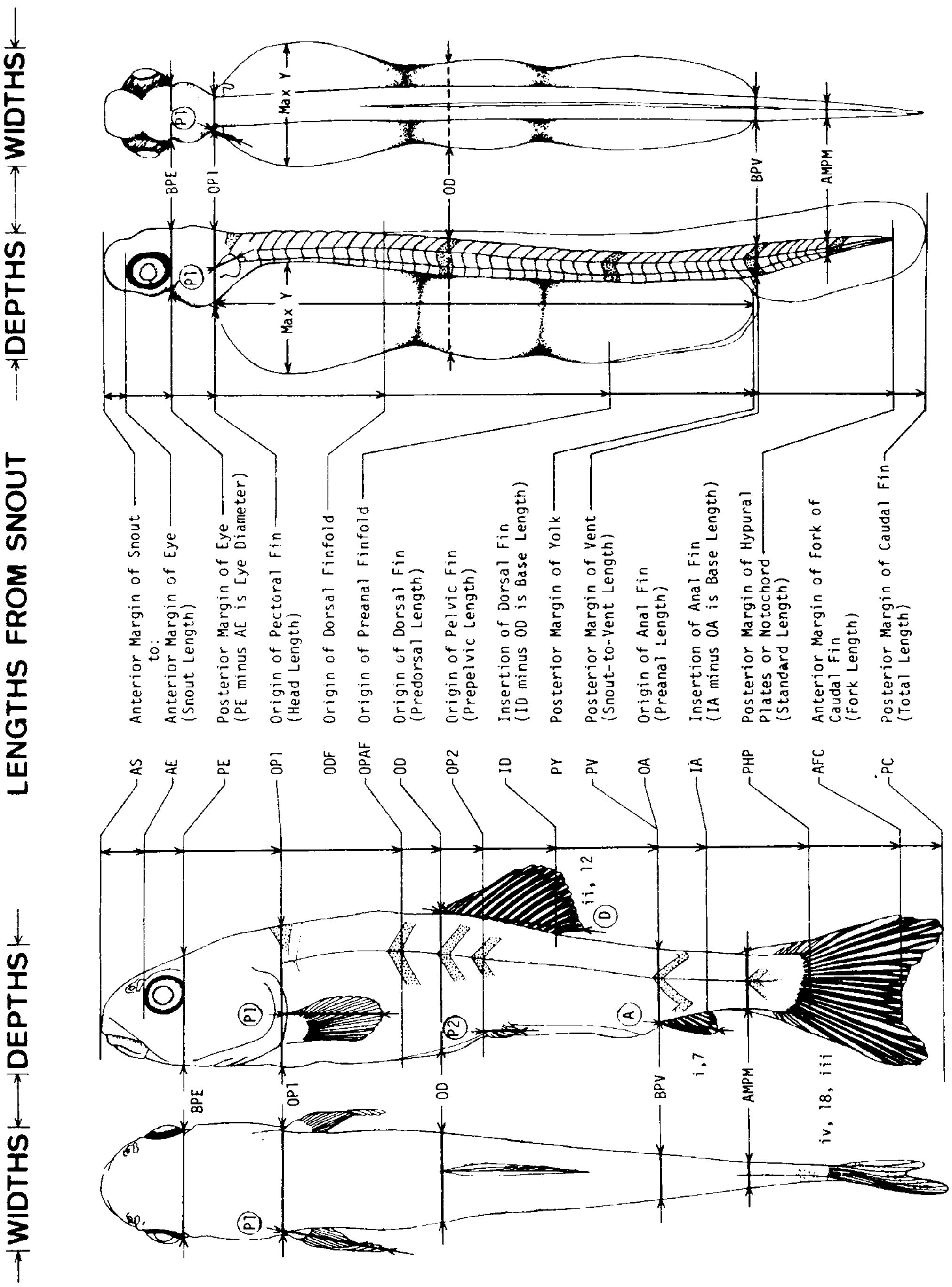

Fig. 4. Measures and counts for larval and early juvenile fishes. Yolk sac and pterygiophores are included in width and depth measures but fins and finfolds are not. "B" in BPE and BPV means immediately behind. AMPM is anterior margin of most posterior myomere. Location of width and depth measures at OD prior to D formation is approximated to that of later larvae. PHP is measured to end of notochord until adult complement of principal caudal-fin rays are observed. Fin lengths (D, A, P1, and P2, encircled) are measured along plane of fin from origin to most distal margin. When reported together, rudimentary median-fin rays (outlined above) are given in lower case Roman numerals, while principal median-fin rays (darkened above) are given in arabic numerals; rudimentary rays are not distinguished in paired fins. Most anterior, most posterior, and last myomeres in counts to specific points of reference are shaded above. (From Snyder 1981.)

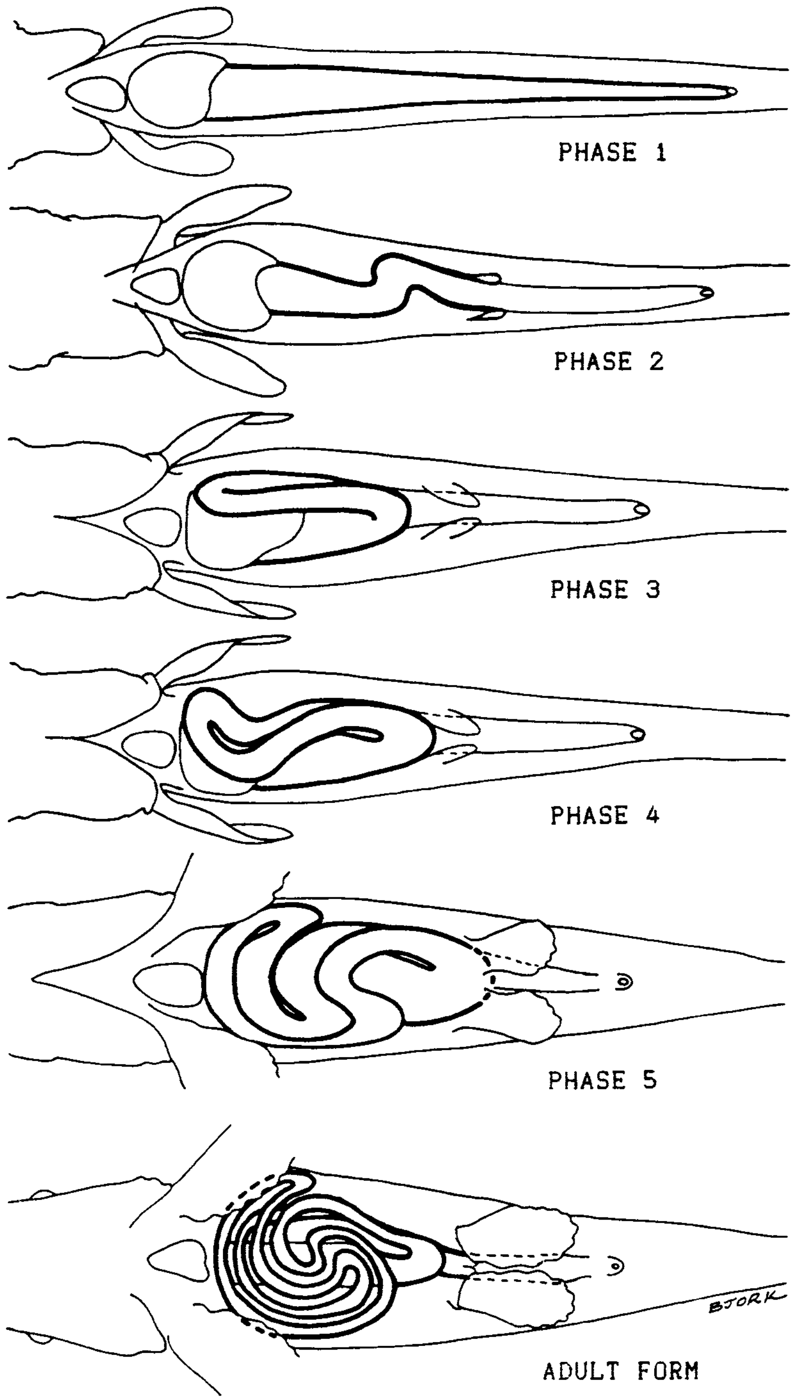

Fig. 5. Phases of gut coil development in catostomid fish larvae and early juveniles with comparison to adult form in ***Catostomus commersonii*** (latter modified from Stewart 1926). Phase 1 – essentially straight gut. Phase 2 – initial loop formation (usually on left side), begins with 90° bend. Phase 3 – full loop, begins with straight loop extending to near anterior end of visceral cavity. Phase 4 – partial fold and crossover, begins with crossing of first limb over ventral midline. Phase 5 – full fold and crossover, begins with both limbs of loop extending fully to opposite (usually right) side, four segments of gut cross nearly perpendicular to the body axis. Later in Phase 5 and in adult form, outer portions of gut folds or coils extend well up both sides of visceral cavity.

catostomids, fin position in the cyprinids, and development of other special structures (e.g., mouth barbels in certain cyprinids) were noted when appropriate. Variation in pigmentation patterns was studied by categorizing observed patterns and character-coding their frequency (see keys to pigment characters at the ends of summary Tables 62 (catostomids) and 69 (cyprinids).

Nineteen new continuous-tone graphite and black-ink drawings were prepared–five each for longfin dace, spikedace, and loach minnow (mid-phase protolarvae, early flexion mesolarvae, and mid-phase postflexion mesolarvae, metalarvae and juveniles), and, to supplement existing illustrations, one stage each for desert sucker (flexion mesolarva), Sonora sucker (flexion mesolarva), roundtail chub (protolarva), and speckled dace (protolarva). Black ink was used only for surface or near-surface pigmentation to distinguish it from deeper pigmentation, other structure, and shading. Each drawing consists of dorsal, lateral, and ventral views. Enlarged digital prints of primary drawing specimens were traced to assure accurate body proportions. Various structures were checked and detail added while drawing specimens were examined under a microscope. If necessary, drawings were idealized (e.g., closed or frayed fins opened and smoothed and curved bodies straightened), and melanophore distribution and other structures were modified to represent a more typical pattern or condition based on secondary drawing specimens.

All morphometric, meristic, and size at onset of selected developmental event data are summarized in species accounts with associated illustrations, maps of recent distribution, and brief descriptions of the adult, reproduction, and early life history (young). The more diagnostically useful of those data are also compared among species in sets of summary tables, along with all pigmentation and special character data. All data, except those in terms of TL, are also used by, and accessible in, the computer-interactive keys.

Computer-Interactive Key

For complex sets of organisms, computer-interactive keys are easier to prepare, update, and expand than traditional printed keys, and much more flexible for the user. Most computer-interactive keys are data sets designed to be used with specific commercial, public-domain, or proprietary host programs (Dallwitz et al. 2000 et seq.). The features and flexibility of several alternative computer-interactive key programs were compared (in part via Dallwitz 2000 et seq.) during preparation of early versions of the key included in Snyder and Muth (2004). Based on this comparison, our prior experience with the DELTA (DEscriptive Language for TAxonomy) suite of programs for taxon description and keys (Dallwitz 1974, 1980, 1993; Dallwitz and Paine 1986), including *Intkey* (Dallwitz et al. 1993 et seq., 1995 et seq.), and successful production of the key for Snyder and Muth (2004), we decided to continue developing our keys for use by the *Intkey* program. The latest versions of *Intkey*, *DELTA Editor* (Dallwitz et al. 1999 et seq.), and associated programs and files can be freely downloaded from the Internet (http://delta-intkey.com/).

DELTA Editor was used to add character data for desert sucker and Sonora sucker to the catostomid data set prepared for Snyder and Muth (2004). Characters were encoded using the DELTA format, a powerful, flexible, and widely accepted method for recording descriptive taxonomic data for computer processing. Output from *DELTA Editor* to the derived files required by *Intkey* was then limited to just the Gila River Basin catostomids. A series of similar data sets and derived files were prepared and progressively refined for the cyprinids, including the three non-native species, and for identification of the larvae of all Gila River Basin fishes at the family level. Because developmental state changes dramatically as fish grow and to better facilitate use of character dependencies (e.g., if yolk is absent, characters such as length of yolk are removed from consideration), it was necessary in the catostomid and cyprinid keys to treat each developmental phase and size interval for a species as separate taxa (e.g., *Catostomus insignis* protolarva, 13 mm SL). Rich-text files of background information, beginning instructions, and other information to be accessed when using *Intkey* were prepared or modified with a word processor. Character lists and natural-language, taxon descriptions were also generated as rich-text files for reference when using the key.

The computer-interactive key to families of Gila River Basin fish larvae is based on characters and character states utilized in previously published family keys by Drewry (1979), Auer (1982), Holland-Bartels, et al. (1990), and Wallus et al. (1990; also Kay et al. 1994 and Simon and Wallus 2004).   Those data were then modified or supplemented as necessary with descriptive data and observations from other publications, particularly for the family Cichlidae (Mironova 1969, Fryer and Iles 1972, Trewavas 1983, McGowan 1988, Morrison et al. 2003; also FishBase at http://www.fishbase.org/home.htm). Mostly to illustrate representative larvae for use with the key, but also as an alternative to it, a pictorial guide to the families of Gila River Basin fish larvae was prepared as an appendix. It consists of pertinent portions of the pictorial family guide with brief lists of distinguishing characteristics published by Wallus et al. (1990) for the Ohio River drainage, but is supplemented with an original section on Cichlidae with drawings of larvae from Fryer and Iles (1972) and McGowan (1988).

Although *Intkey* can make extensive use of taxon and character-state-selection images, preparation and inclusion of such were neither critical for operation of the key nor logistically and budgetarily feasible for this guide (if there is enough interest and support, they could be prepared and incorporated in future versions of the key). Also, such images can require a considerable amount of storage memory and at times a strictly text key may be preferable, especially for the experienced user or when using a slower computer with limited memory. Instead, the user is expected to extensively reference the illustrations and descriptive information provided in the species accounts. However, as examples of how character-state-selection images function, such illustrations were prepared and included in the key for developmental phase and phases of gut development. Images used by *Intkey* were created or modified from scanned files using computer drawing and presentation programs.

Interim and near-final versions of the three keys were subjected to in-house testing. However, based on reviews and feedback from use in routine collection processing, future refinements of the key will likely be implemented and made available for download over the Internet.

RESULTS AND DISCUSSION

Results are divided into three complementary sections–Species Accounts, Comparative Summary Tables, and Computer-Interactive Keys. For identification purposes, users should become familiar with and use all three taxonomic tools. Although all descriptive data in the species accounts and comparative summary tables (except those replicated in terms of total length in the species accounts) comprise the data sets for the keys, results from the keys can be confirmed using the well-illustrated species accounts and comparative summary tables.

Whenever possible, specimen identification should be based on multiple characters.