Native Cypriniform Fish Larvae of the Gila River Basin

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Morphological Descriptions, Comparisons, and Computer-interactive Keys

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ABSTRACT

Use of collections of fish larvae and young-of-the-year juveniles to help document spawning sites and seasons or assess larval production, transport, distribution, nursery habitat, survival, and other aspects of early life history, requires diagnostic criteria to accurately identify target species from morphologically similar taxa. To facilitate identification of the larvae and early juveniles of most native cypriniform fishes in the Gila River Basin, developmental series of reared and collected desert sucker (*Catostomus clarkii*), Sonora sucker ©. *insignis*), longfin dace (*Agosia chrysogaster*), spikedace (*Meda fugida*), and loach minnow (*Rhinichthys cobitis*) were illustrated and described to detail differences in morphology, meristics, pigmentation, and size relative to developmental state. Comparable illustrations and data were extracted from existing descriptions of flannelmouth sucker ©. *latipinnis*), razorback sucker (*Xyrauchen texanus*), bonytail (*Gila elegans*), roundtail chub (*G. robusta*), Colorado pikeminnow (*Ptychocheilus lucius*), speckled dace ®. *osculus*), and non-native cyprinids common carp (*Cyprinus carpio*), red shiner (*Cyprinella lutrensis*), and fathead minnow (*Pimephales promelas*). For the cyprinids, extracted data were supplemented with original observations and, for roundtail chub and speckled dace, illustrations of protolarvae. The results are documented in detailed descriptive species accounts, comparative summary tables, and computer-interactive keys. A computer-interactive key and a pictorial guide to families of Gila River Basin larvae were also prepared using data from previously published keys and descriptions.

INTRODUCTION

Importance of Early Life History Investigations and Identification

(Modified from Snyder and Muth 2004)

For most fishes, larval and early (young-of-the-year) juvenile development includes a few to several life-history phases that are morphologically and ecologically distinct from each other, as well as later juveniles and adults (Snyder 1990; such phases do not necessarily correspond with the morphologically based developmental intervals defined below). Accordingly, knowledge of fish early life history is often essential for better understanding aquatic ecosystems and communities and more effectively monitoring, protecting, or managing fish populations and habitat. Such knowledge is particularly valuable in assessing environmental impacts and recovering endangered species.

The collection and study of fish eggs, larvae, and early juveniles should be an integral part of holistic fish and aquatic ecology investigations. Densities and spatial and temporal distribution of these life stages are indicative of spawning grounds and seasons, larval production, nursery habitat, behavior, and potential year-class strength. A single specimen is proof of at least some reproductive success. Even in baseline surveys to determine presence and relative abundance of fishes, larval-fish collections can sometimes provide information on species that are difficult to collect or observe as adults because of gear selectivity, behavior, habitat, or low abundance.

Research or monitoring based on collections of fish larvae usually requires accurate identification of collected specimens. Inland fishery managers and researchers often exclude potentially critical larval-fish investigations specifically because they haven't done it before or because adequate descriptions of larvae, taxonomic criteria, and keys for identification are not available. Although the inventory of such information is gradually increasing, much descriptive and taxonomic research is piecemeal, uncoordinated, and often "a labor of love."

Of approximately 800 species of freshwater and anadromous fishes in the United States and Canada (Lee et al. 1980, Robins, et al. 1991), less than 25% have been adequately described as larvae for identification purposes (Snyder 1996, extrapolated from 15% reported by Snyder 1976a). In a relatively comprehensive listing of regional larval-fish guides, keys, and comparative descriptions by Simon (1986), only about 80 of 230 citations (35%) pertain to freshwater species. Kelso and Rutherford (1996) listed 18 regionally oriented larval-fish identification manuals for or including North American freshwater species (some for the same regions and all incomplete in coverage at the species level). Not included in the list were guides by Sturm (1988), Snyder and Muth (1988, 1990–probably treated as comparative descriptions rather than regional guides), and most recently, Simon and Wallus (2004) and Snyder and Muth (2004). No guides to North American freshwater fish larvae were published between 1994 and 2004.

This Guide and Prior Descriptions

The purpose of this guide is to document the early morphological development of most native cypriniform fishes in the Gila River Basin (Figure 1) and better facilitate identification of fish larvae collected in the basin. The well-illustrated species accounts, comparative summary tables, and computer-interactive keys provided herein should be particularly beneficial to

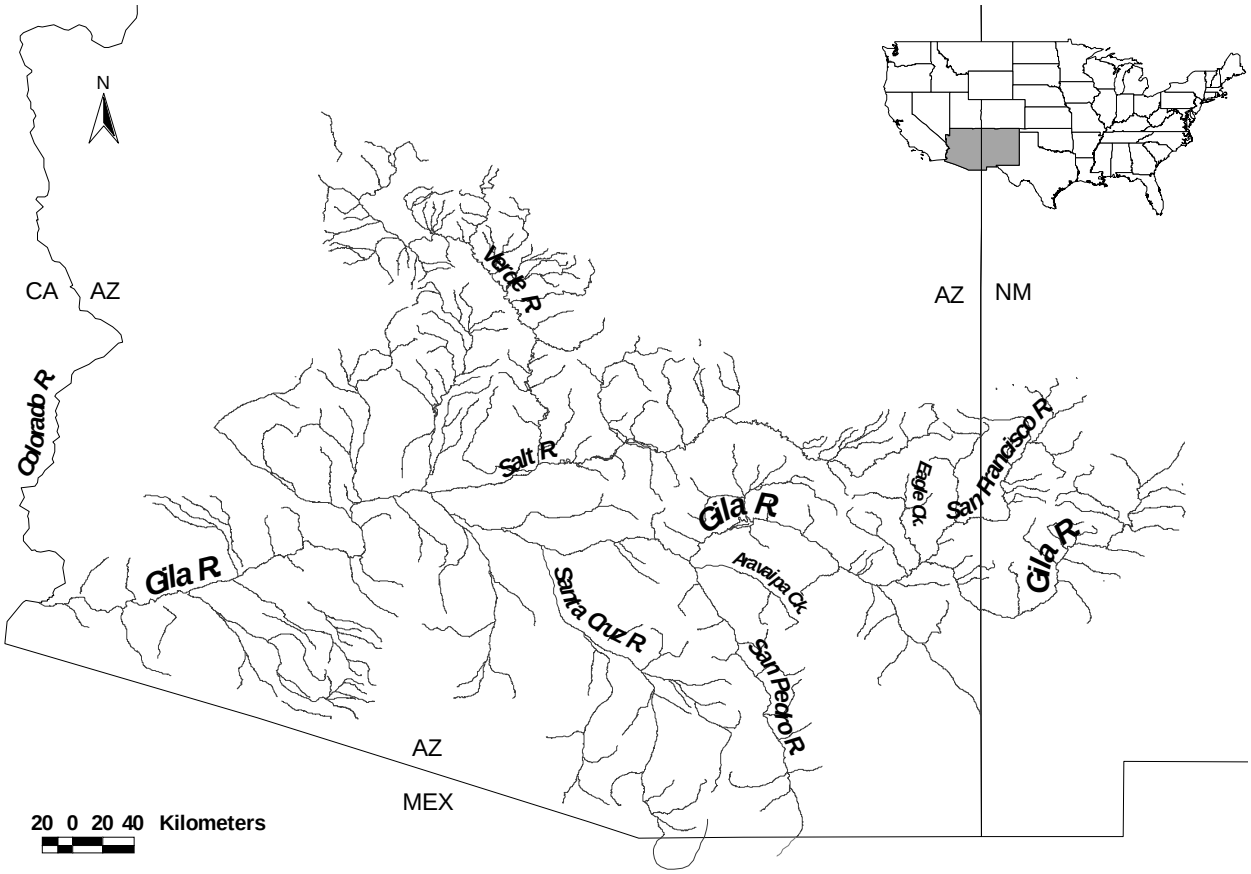

Fig. 1. The Gila River Basin of Arizona, New Mexico, and Mexico.

scientists working in reaches such as Aravaipa Creek and Eagle Creek in Arizona and the upper Gila River Basin in New Mexico, which are known to support mostly intact faunas. In these ways, it contributes to our knowledge of threatened, endangered, and other native fishes in the basin and should help facilitate their conservation or recovery.

Species coverage includes four native catostomids, seven native cyprinids, and, for comparison, three common non-native cyprinids (Table 1). Separate computer-interactive keys were prepared for the covered catostomids and cyprinids. All species in the Gila River Basin are covered at the family level in a third computer-interactive key and in an appended pictorial guide derived mostly from Wallus et al. (1990).

This guide continues a quarter century of work by the Larval Fish Laboratory (LFL) on early life stages of Southwestern fishes. Its format, including the descriptive species accounts, comparative summary tables, and computer-interactive keys, is modeled after Snyder and Muth (2004) except that new species accounts include as few as five rather than eight three-view illustrations of larvae and early juveniles, and osteological features were not included in any account. All descriptive data and illustrations herein for larvae and early juveniles of desert sucker, Sonora sucker, longfin dace, spikedace, and loach minnow are original, as are one drawing of a protolarval roundtail chub, another of a speckled dace, and a composite photograph

Table 1. List of native and most established non-native fishes in the Gila River Basin. Basin status as native (N), native-extirpated (NE), native-extirpated with recent attempts to reintroduce (NER), or native-formerly extirpated but successfully reintroduced (NR) is indicated parenthetically, as is listing status for the U. S. Department of Interior (USDI, E = endangered, T = threatened), State of Arizona (AZ, special concern = SC), State of New Mexico (NM, E = endangered, T = threatened), and Republic of Mexico (Mex, E = endangered, T = threatened, R = rare).[a] Asterisks indicate species covered herein. Current common and scientific names follow Nelson et al. (2004).

tenpounders (Elopidae)
- machete – *Elops affinis* (NE)[b]

herrings (Clupeidae)
- threadfin shad – *Dorosoma petenense*

minnows (Cyprinidae)
- * longfin dace – *Agosia chrysogaster* (N, Mex = E)
- * bonytail – *Gila elegans* (NE, USDI = E, AZ = SC, Mex = E)
- Gila chub – *Gila intermedia* (N, NM = E, AZ = SC, Mex = E)
- headwater chub – *Gila nigra* (N, AZ = SC)
- * roundtail chub – *Gila robusta* (N, NM = E, AZ = SC, Mex = R)
- * spikedace – *Meda fulgida* (N, USDI = T, NM = T, AZ = SC)
- woundfin – *Plagopterus argentissimus* (NE, USDI = E, AZ = SC)
- * Colorado pikeminnow, formerly Colorado squawfish[c] – *Ptychocheilus lucius* (NER, USDI = E (experimental, non-essential), NM = E, AZ = SC, Mex = E)
- * loach minnow – *Rhinichthys cobitis*, formerly *Tiaroga cobitis*[c] (N, USDI = T, NM = T, AZ = SC, Mex = E)
- * speckled dace – *Rhinichthys osculus* (N, Mex = E)
- goldfish – *Carassius auratus*
- grass carp – *Ctenopharyngodon idella*
- * red shiner – *Cyprinella lutrensis*, formerly *Notropis lutrensis*
- * common carp – *Cyprinus carpio*
- * fathead minnow – *Pimephales promelas*

suckers (Catostomidae)
- * desert sucker – *Catostomus clarkii*, formerly *Pantosteus clarki*[c] (N)
- * Sonora sucker – *Catostomus insignis* (N, Mex = E)
- * flannelmouth sucker – *Catostomus latipinnis* (NE)
- Rio Grande sucker – *Catostomus plebeius*, formerly *Pantosteus plebeius*[c]
- * razorback sucker – *Xyrauchen texanus* (NER, USDI = E, AZ = SC, Mex = E)
- smallmouth buffalo – *Ictiobus bubalus*
- bigmouth buffalo – *Ictiobus cyprinellus*
- black buffalo – *Ictiobus niger*

catfishes (Ictaluridae)
- black bullhead – *Ameiurus melas*, formerly *Ictalurus melas*
- yellow bullhead – *Ameiurus natalis*, formerly *Ictalurus natalis*
- channel catfish – *Ictalurus punctatus*
- flathead catfish – *Pylodictis olivaris*

(continued)

Table 1. Continued.

trouts (Salmonidae)
- Gila trout – *Oncorhynchus gilae*, formerly *Salmo gilae* (N, USDI = E, NM = E, AZ = SC)
- Apache trout – *Oncorhynchus gilae apache,* formerly *Oncorhynchus apache* and *Salmo apache* (N, USDI = T, AZ = SC),
- rainbow trout – *Oncorhynchus mykiss*, formerly *Salmo gairdneri*
- brown trout – *Salmo trutta*
- brook trout – *Salvelinus fontinalis*

mullets (Mugilidae)
- striped mullet – *Mugil cephalus* (N) [b]

pupfishes (Cyprinodontidae)
- Santa Cruz pupfish – *Cyprinodon arcuatus* (NE, species extinct)
- desert pupfish – *Cyprinodon macularius* (NR, USDI = E, AZ = SC, Mex = E)

livebearers (Poeciliidae)
- Gila topminnow – *Poeciliopsis occidentalis* (N, USDI = E, NM = T, AZ = SC, Mex = T)
- western mosquitofish – *Gambusia affinis*
- sailfin molly – *Poecilia latipinna*

temperate basses (Moronidae)
- white bass – *Morone chrysops*
- yellow bass – *Morone mississippiensis*
- striped bass – *Morone saxatilis*

sunfishes (Centrarchidae)
- rock bass – *Ambloplites rupestris*
- green sunfish – *Lepomis cyanellus*
- redear sunfish – *Lepomis microlophus*
- bluegill – *Lepomis macrochirus*
- smallmouth bass – *Micropterus dolomieu*
- largemouth Bass – *Micropterus salmoides*
- white crappie – *Pomoxis annularis*
- black crappie – *Pomoxis nigromaculatus*

perches (Percidae)
- yellow perch – *Perca flavescens*
- walleye – *Sander vitreus*, formerly *Stizostedion vitreum*

cichlids (Cichlidae)
- blue tilapia – *Oreochromis aureus*, formerly *Tilapia aurea*
- Mozambique tilapia – *Oreochromis mossambicus*, formerly *Tilapia mossambica*
- redbelly tilapia – *Tilapia zillii*

[a] Sources: Arizona Game and Fish Department (2000-2003); Desert Fishes Team 2003, 2004; Minckley 1973; Minckley and DeMarais 2000; Minckley et al. 1991; Minckley et al. 2002; Propst 1999; Secretaŕia de Desarrollo Social 1994; Voeltz 2002.

[b] Euryhaline, estuarine- or marine-spawning species that historically ranged up the Gila River.

[c] Change in name not universally accepted–former name still used by some biologists (e.g., Minckley 1973, Simons and Mayden 1999, and Desert Fishes Team 2003 and 2004).

of a juvenile red shiner. However, some of the illustrations and data for the suckers were prepared earlier for descriptions in a report by Bestgen (1989). Species accounts for flannelmouth sucker and razorback sucker were replicated with little modification from Snyder and Muth (1990, 2004), but the illustrations and much of the data in those accounts were first published by Snyder (1981). Most descriptive information and illustrations provided herein for the remaining species were originally published by Snyder et al. (1977), Snyder (1981), or Muth (1990), except all illustrations of common carp and most of Colorado pikeminnow and red shiner, and some of fathead minnow.

Winn and Miller (1954) published the earliest comparisons of and key to larvae for native cyprinid (minnow) and catostomid fishes in the American southwest. Their illustrated descriptions and key for the Lower Colorado River Basin below Lake Mead covered all native species included herein except bonytail and Colorado pikeminnow, but it was limited to mesolarval stages (developmental intervals defined below) with single lateral- and dorsal-view photographs for each species. Although not described or illustrated, Colorado pikeminnow was included in their discussion and key as similar to roundtail chub. The illustrations of roundtail chub were attributed to subspecies ***intermedia***, which is now recognized as a separate species, the Gila chub. Tabulated data for the onset of selected developmental events in roundtail chub were noted as being based on both subspecies ***robusta*** and ***intermedia***. Also, ***Pantosteus*** larvae, some of which were illustrated as desert sucker and some as bluehead sucker (***Catostomus discobolus***), have since been recognized as desert sucker by Smith (1966). Winn and Miller's (1954) data and illustrations generally match well with those herein, but we found some exceptions to their diagnostic criteria. We recommend that biologists working with fish larvae in the Gila River Basin and elsewhere in the Lower Colorado River Basin be familiar with this landmark work and use it as a supplement to this guide.

Few other authors have published descriptive information on the early life stages of native species covered in this guide. Minckley and Gustafson (1982) chronicled early development of razorback sucker, but their illustrations are sketchy and include only lateral views. Douglas (1952) published photographs of a razorback sucker protolarva (or recently transformed mesolarva) without yolk and a 10-cm specimen labeled as a juvenile razorback sucker, but, as noted by Winn and Miller (1954), the subject of the latter photograph is actually an adult speckled dace (***Rhinichthys osculus***). In the process of documenting hybridization among several catostomids, Hubbs et al. (1943) and Hubbs and Hubbs (1947) published descriptive information for young-of-the-year juveniles (and some larvae) of flannelmouth sucker. Seethaler (1978) described and illustrated very well the early development of Colorado pikeminnow.

In contrast to native species, the larvae and early juveniles of the three non-native cyprinids covered herein are all widely distributed elsewhere in the United States (and for common carp and fathead minnow, in Canada) and have been well described by other authors and covered in other guides. In addition to Snyder (1981), and Snyder et al. (1977) for fathead minnow, early life stages of all three species have been included in identification manuals by Wang (1986) and Holland-Bartels et al. (1990); and common carp and fathead minnow by Fish (1932), Hogue et al. (1976), and Heufelder and Fuiman (1982). Common carp have also been described or included in many other publications, including Balon (1958), Bragensky (1960), Mansueti and Hardy (1967), May and Gasaway (1967), Nakamura (1969), Taber (1969), Lippson and Moran (1974), Loos et al. (1979), Jones et al. (1978), Wang and Kernehan (1979), and McGowan (1988). Fathead minnow have also been described by Buynak and Mohr (1979), red

shiner by Saksena (1962) and Taber (1969), and both species by Perry (1979) and Perry and Menzel (1979).

Many cypriniform fishes not covered by this guide have been described. We recommend Fuiman (1982) and Kay et al. (1994) for the buffalo fishes, Snyder (1998) for Rio Grande sucker, Soin and Sukhanova (1972) and Conner et al. (1980) for grass carp, and Jones et al. (1978) and Heufelder and Fuiman (1982) for goldfish. Gila chub (except as combined with roundtail chub in Winn and Miller 1954), headwater chub, and woundfin remain undescribed as larvae.

Systematics, Distribution, and Status of the Fish

Native Catostomidae in the Gila River Basin belong to subfamily Catostominae and tribe Catostomini. *Xyrauchen* is a monotypic genus. Among the *Catostomus* species, desert sucker belongs to subgenus *Pantosteus*, a distinctive group known as "mountain suckers" and treated as a separate genus prior to study by Smith (1966); others belong to subgenus *Catostomus*, the "valley suckers" (Smith 1987).

Native Cyprinidae belong to the subfamily Leuciscinae, and are arranged in tribes Plagopterini and Leuciscini (Hubbs 1955). Genera in the tribe Plagopterini consist of *Lepidomeda*, *Meda,* and *Plagopterus*, all of the Colorado River Basin, and are characterized by spine-like modifications of anterior fin rays in dorsal and pelvic fins (Miller and Hubbs 1960). *Meda* (endemic to the Gila River Basin) and *Plagopterus* are monotypic genera. All other native cyprinids in the Gila River Basin are in tribe Leuciscini; *Agosia* is a monotypic genus. Loach minnow *Rhinichthys cobitis* was formerly placed in the monotypic genus *Tiaroga,* a name that is no longer recognized by Nelson et al. (2004). Among the non-native Cyprinidae considered herein, carp (*Cyprinus carpio*) belongs to subfamily Cyprininae, and the others to Leuciscinae, tribe Leuciscini.

Identification of organisms, particularly poorly known larval and early juvenile life stages of fishes, is often aided by knowledge of the species that may occur in the sampling area. Although reporting distribution of native fishes in the Gila River Basin is beyond the scope of this project, general information is available in Minckley (1973). More up-to-date information on status and distribution of native fishes in the Gila River basin is available in reports by the Desert Fishes Team (Desert Fishes Team 2003, 2004) and documented at www.gf.state.az.us/w_c/edits/hdms_abstracts_fish.shtml, the Arizona Game and Fish Department website. The Desert Fishes Team reports summarize efforts to repatriate native fishes in some stream reaches and also report on the general plight of fishes in the Gila River Basin. Chief among the reasons for reduced distribution and abundance of native fishes is negative effects of introduced fishes and over-development of water resources in this arid environment, particularly at lower elevations.

A Combined Developmental Interval Terminology

(Reprinted from Snyder and Muth 2004, modified from Snyder and Muth 1988 and 1990)

It is often convenient and desirable to divide the ontogeny of fish into specifically defined intervals. If the intervals selected are used by many biologists as a frame of reference, such division can facilitate communication and comparison of independent results. The largest intervals, periods (e.g., embryonic, larval, juvenile, and adult), are often subdivided into phases and sometimes into steps (Balon 1975a and 1984); the word "stage," although commonly used as

a synonym for period or phase (e.g., Kendall et al. 1984), should be reserved for instantaneous states of development.

The larval phase terminologies most commonly used in recent years, particularly for descriptive purposes, are those defined by Hardy et al. (1978–yolk-sac larva, larva, prejuvenile; modified from Mansueti and Hardy 1967), Ahlstrom et al. (1976–preflexion, flexion, postflexion; expanded upon by Kendall et al. 1984), and Snyder (1976b and 1981–protolarva, mesolarva, metalarva). Definitions for all three terminologies were presented by Snyder (1983) and Kelso and Rutherford (1996). During a workshop on standardization of such terminologies, held as part of the Seventh Annual Larval Fish Conference (Colorado State University, January 16, 1983), it became obvious that these are not competing terminologies, as they often are treated, but rather complementary options with subdivisions or phases defined for different purposes. As such, it is possible to utilize all three terminologies simultaneously to: (1) facilitate comparative descriptions and preparation of keys based on fish in similar states of development with respect to morphogenesis of finfold and fins; (2) segregate, for fishes with homocercal tails, morphometric data based on standard length measured to the end of the notochord prior to and during notochord flexion from those measured to the posterior margin of the hypural plates following notochord flexion; and (3) approximate transition from at least partially endogenous nutrition (utilization of yolk material) to fully exogenous nutrition (dependence on ingested food) based on presence or absence of yolk material.

The combined terminology presented below and utilized herein effectively integrates principal subdivisions and functions of the three component terminologies. In doing so, Ahlstrom's "preflexion-flexion-postflexion" terminology is treated, for fishes with homocercal tails, as a subset of Snyder's mesolarva phase. Since notochord flexion in the caudal region usually begins when the first caudal-fin rays appear and is essentially complete when all principal caudal-fin rays are well defined, and since presence of fin rays can be more precisely observed than the beginning or end of actual notochord flexion, fin rays are used as transition criteria. As a result, all protolarvae are preflexion larvae, and all metalarvae are postflexion larvae. Although most fish pass sequentially through all phase subdivisions designated, some pass pertinent points of transition prior to hatching or birth and begin the larval period in a later phase or possibly skip the period entirely.

The definition for the end of the larval period is necessarily a compromise deleting all requirements (some taxon-specific, others difficult to determine precisely) except acquisition of the full complement of fin spines and rays in all fins and loss of all finfold (last remnants are usually part of the preanal finfold). Provision for taxon-specific prejuvenile (or transitional) phases are also deleted. In some cases, finfold persists through the endpoint for such special intervals, which are then effectively included in the larval period.

Timing of complete yolk absorption varies from well before notochord flexion and initial fin ray formation, as in most fishes with pelagic larvae, to postflexion stages after all or most of the fin rays are formed, as in many salmonids. Accordingly, the interval during which fish larvae bear yolk should not be represented generally as a separate phase preceding phases based on fin formation as it has been treated by Kendall et al. (1984). The Hardy et al. terminology effectively distinguishes between larvae with and without yolk by modifying the period name with the adjective "yolk-sac" when yolk material is present. Any period or phase name of the combined terminology can be similarly modified to indicate presence or absence of yolk material (e.g., yolk-bearing larva, yolk-sac metalarva, postflexion mesolarva with yolk, protolarva without yolk).

Larva: Period of fish development between hatching or birth and (1) acquisition of adult complement of fin spines and rays (principal and rudimentary) in all fins, and (2) loss beyond recognition of all finfold not retained by the adult.

Protolarva: Phase of larval development characterized by absence of dorsal-, anal-, and caudal-fin spines and rays. (Standard length measured to end of notochord.)

Mesolarva: Phase of larval development characterized by presence of at least one dorsal, anal, or caudal-fin spine or ray but either lacking the adult complement of principal soft rays in at least one median (dorsal, anal, or caudal) fin or lacking pelvic-fin buds or pelvic fins (if present in adult). (Standard length measured to end of notochord or, when sufficiently developed, axial skeleton.)

Preflexion Mesolarva: Among fishes with homocercal tails, subphase of mesolarval development characterized by absence of caudal-fin rays. (Posterior portion of notochord remains essentially straight and standard length measured to end of notochord. When first median-fin ray is a caudal ray, as in most fishes, larva progresses directly from protolarva to flexion mesolarva.)

Flexion Mesolarva: Among fishes with homocercal tails, subphase of mesolarval development characterized by an incomplete adult complement of principal caudal-fin rays. (Posterior portion of notochord flexes upward and standard length measured to end of notochord.)

Postflexion Mesolarva: Among fishes with homocercal tails, subphase of mesolarval development characterized by adult complement of principal caudal-fin rays. (Notochord flexion essentially complete and standard length measured to posterior-most margin of hypural elements or plates.)

Metalarva: Phase of larval development characterized by presence of (1) adult complement of principal soft rays in all median fins and (2) pelvic-fin buds or pelvic fins (if present in adult). (Standard length measured to posterior end of axial skeleton, hypural elements or plates in fishes with homocercal tails.)

Yolk-sac, Yolk-bearing, With Yolk, Without Yolk: Examples of modifiers used with any of the above period or phase designations to indicate presence or absence of yolk material, including oil globules.

The combined terminology is designed to be relatively simple but comprehensive, precise in its transition criteria, applicable to nearly all teleost fishes, and flexible. It can be utilized in part (essentially as one of its component terminologies) or its entirety depending on purposes of the user. For example, if it is necessary to acknowledge only that the fish is a larva and whether it bears yolk, the terms "yolk-sac larva" and "larva without yolk" are all that is needed.

Biologists who formerly utilized one of its component terminologies should have no difficulty in adapting to the combined terminology–essential features and terms of the original terminologies have been retained.

Characteristics Useful in Identification of Cypriniform Fish Larvae

(Modified from Snyder 1981 and Snyder and Muth 1988, 1990, and 2004)

Fishes of the families Cyprinidae (minnows and carps) and Catostomidae (suckers), order Cypriniformes, are closely related and morphologically similar. Together these two families account for 41% of 56 total and 67% of 21 native species in the Gila River Basin (Table 1). Generalizations in the following discussion with respect to the order Cypriniformes refer specifically to North American species in these families. Figures 2 and 3 identify the more obvious morphological features and structures of catostomid (and cyprinid) eggs and larvae.

Identification of fish larvae is in part a process of elimination. Even before examination of a single specimen, the number of candidate species can be substantially reduced by a list of known or likely species based on adult captures in the study area or connected waters. However, there are cases in which the presence of certain species was first documented by collection and identification of larvae. Incidental transport of eggs or larvae from far upstream or distant tributaries also must be considered. Knowledge of spawning seasons, temperatures, habitats, and behavior coupled with information on egg deposition, larval nursery grounds, and larval behavior are also useful in limiting possibilities.

Berry and Richards (1973) noted that "although species of a genus may vary from one geographical area to another, generally the larval forms of closely related species look alike. At the same time, larvae of distantly related forms may be closely similar in gross appearance." Cypriniform larvae as a group are distinctive and generally easy to distinguish from larvae of other families. Beginning workers should become familiar with the general larval characteristics of each family likely to be encountered. The guides and keys cited in Snyder (1983) and Kelso and Rutherford (1996) are most useful in this respect. Auer (1982) is particularly recommended since it covers most families and many non-native species in the Gila River Basin. The pictorial guides to families by Holland-Bartels et al. (1990) and Wallus et al. (1990; also Kay et al. 1994, and Simon and Wallus 2004) and discussions of taxonomic characters by Berry and Richards (1973) and Kendall et al. (1984) are also recommended.

Generally, cypriniform larvae are readily categorized as cyprinids or catostomids. But in the Gila River Basin and elsewhere, if members of the cyprinid subfamily Cyprininae (carps) and the catostomid subfamily Ictiobinae (carpsuckers and buffalofishes) or tribe Erimyzontini (chubsuckers) are present, identification at the family level can be more difficult.

Within their respective families, and especially at the subfamily level, cypriniform larvae are very homogeneous in gross structure and appearance. Accordingly, they may be especially difficult to discriminate at genus or species levels. This is particularly true of Colorado River Basin catostomids. For the latter, specific identification relies on size at which certain developmental events occur, form of the gut, melanistic (brown or black) pigment patterns, osteological characters, and to a limited extent, morphometrics and meristics (especially dorsal-fin-ray counts for metalarvae and juveniles).

There is often a noticeable amount of intra- as well as inter-regional variability in many of the characters to be discussed. This variability necessitates confirmation of identity based on as many diagnostic characters as possible.

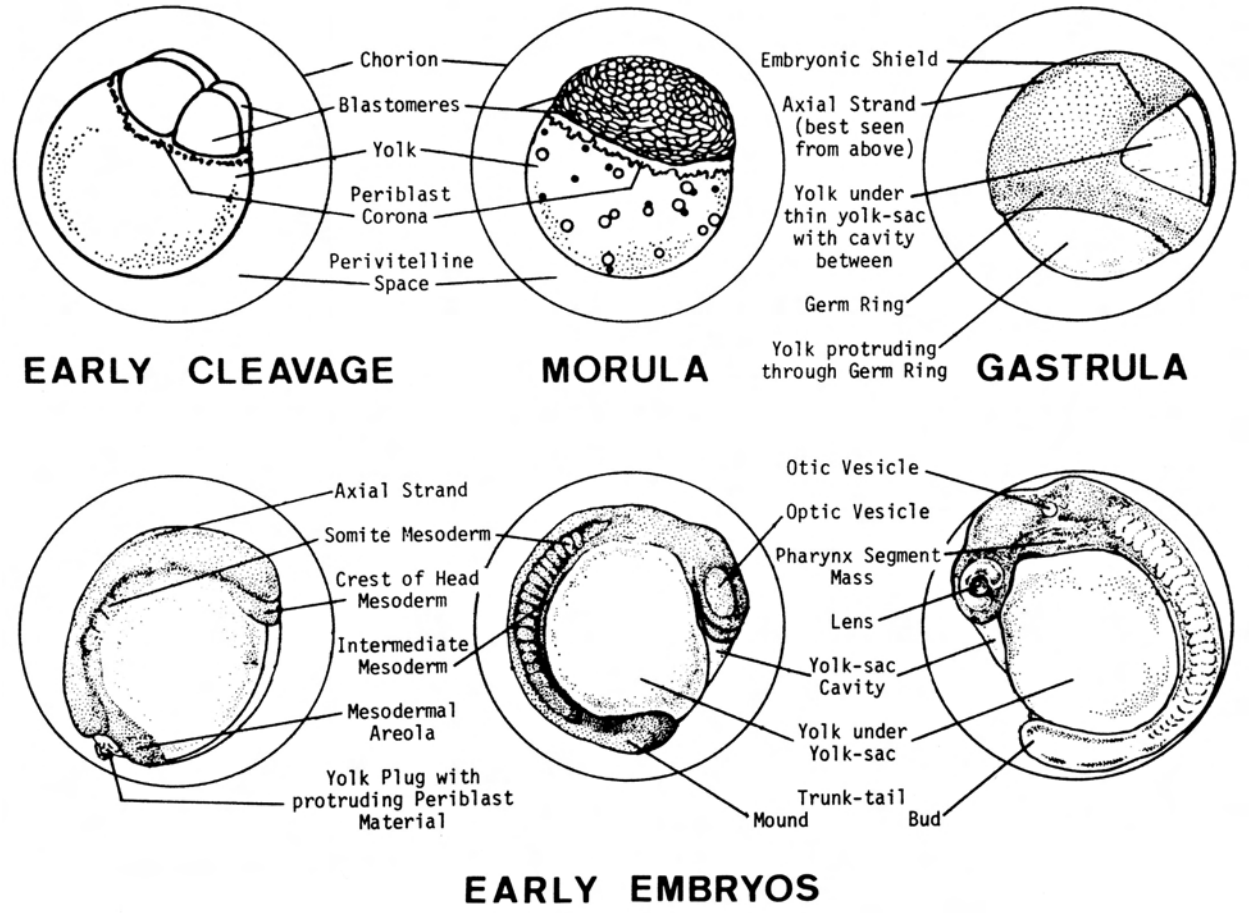

Fig. 2. Selected anatomical features of cypriniform fish eggs and embryos (from Snyder 1981; based on drawings from Long and Ballard 1976).

Myomeres

Myomeres, because they are obvious morphological features and relatively consistent in number and position, are one of the most useful characters available for identification of larvae above (and sometimes at) the species level, especially for protolarvae and mesolarvae. They begin as part of the embryonic somites and are usually formed in their full complement prior to hatching. Throughout the protolarval and much of the mesolarval phase, myomeres are chevron-shaped, but by the metalarval phase they evolve to their typical three-angled adult form. Fish (1932) and many subsequent authors observed that there is a nearly direct, one-to-one correlation between total myomeres and total vertebrae (including Weberian ossicles in cypriniforms). Snyder (1979) and Conner et al. (1980) summarized myomere and vertebral counts for many cypriniform fishes.

The most anterior and most posterior myomeres are frequently difficult to distinguish. The most anterior myomeres are apparent only in the epaxial or dorsal half of the body; the first is often deltoid in shape and is located immediately behind the occiput. The most posterior myomere is defined as lying anterior to the most posterior complete myoseptum. Siefert (1969) describes a "false (partial) myoseptum" posterior to the last complete myoseptum which adds to

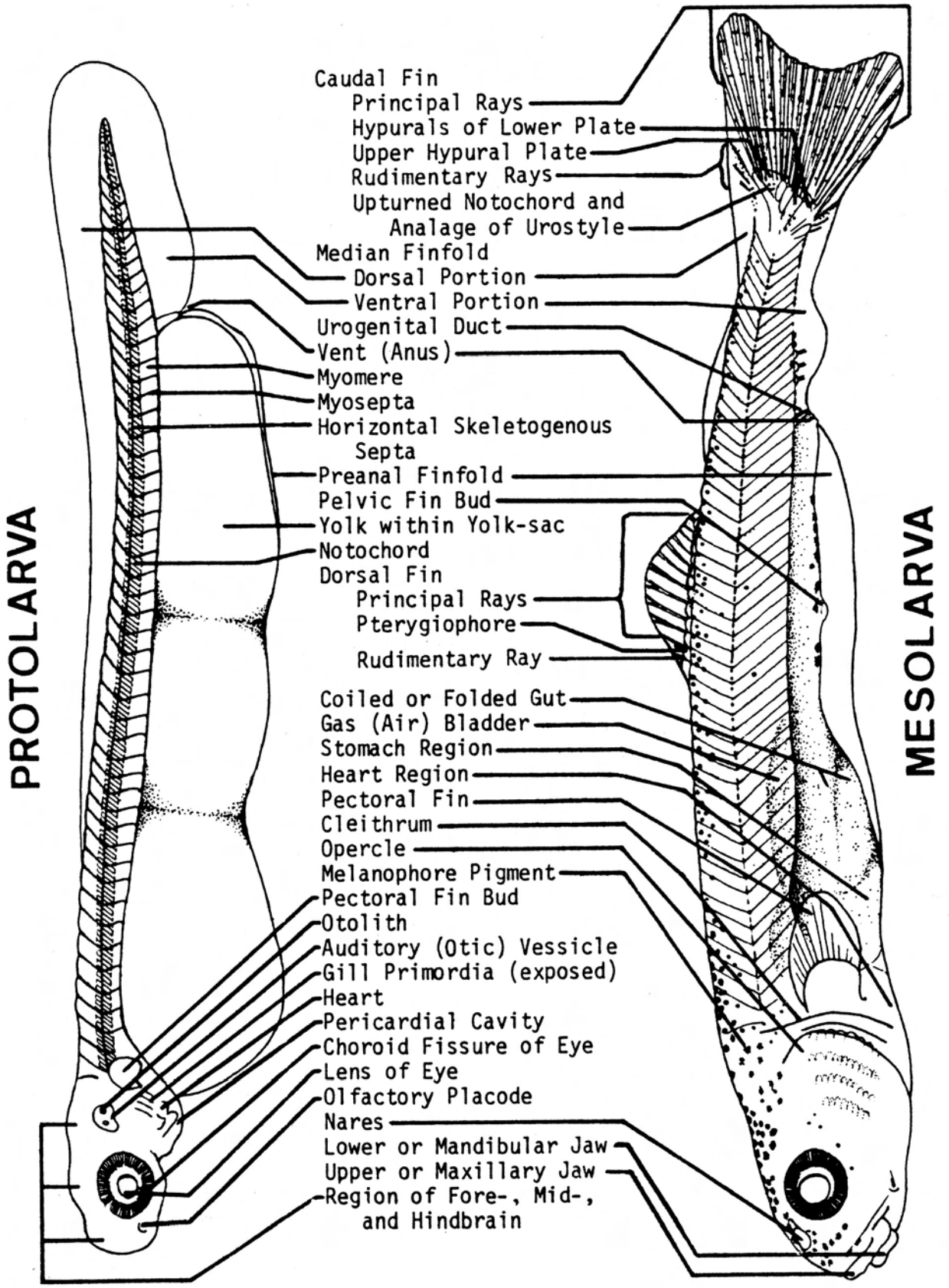

Fig. 3. Selected anatomical features of cypriniform fish larvae (modified from Snyder 1981).

the difficulty of discerning the last myomere. Early in the larval period, myomeres are most readily observed using transmitted light. Polarizing filters, depending on thickness and certain other qualities of the preserved tissues, can dramatically increase contrast between the muscle tissue of myomeres and the myosepta that separate them. Myomeres of some metalarvae and most juveniles are difficult to observe even with polarizing filters; reflected light at a low angle from one side and higher magnification sometimes facilitates observation.

Typical counts used in taxonomic work include total, preanal, and postanal myomeres. Partial counts are frequently used to also reference the location of structures other than the vent or anus. The most generally accepted method of making partial counts was described by Siefert (1969) for distinguishing preanal and postanal myomeres: "postanal myomeres include all [entire] myomeres posterior to an imaginary vertical line drawn through the body at the posterior end of the anus . . . Remaining myomeres, including those bisected by the line, are considered preanal." The technique is equally applicable with other structures or points of reference such as origins of fins or finfolds. The opposite approach was used by Snyder et al. (1977), Snyder and Douglas (1978), Loos and Fuiman (1977) and, according to the last authors, Fish (1932)–only entire myomeres were included in counts anterior to points of reference. Siefert's method is recommended as standard procedure because resulting counts are expected to more nearly approximate the number of vertebrae to the referenced structures.

In the United States and Canada, the range of total myomere (and vertebral) counts for cyprinids, 28 to 52, is slightly larger and nearly includes that for catostomids, 32 to 53. Ranges for preanal and postanal myomere counts also overlap with 19 to 35 and 9 to 22, respectively, for cyprinids and 25 to 42 and 5 (possibly 3) to 14, respectively, for catostomids. Despite the magnitude of overlap in these ranges, proportions of postanal to preanal and preanal to total myomeres will distinguish most cyprinids from catostomids (Snyder 1979). The postanal to preanal myomere proportion is at least 2/5 (often greater than ½) for cyprinids (exclusive of subfamily Cyprininae, the carps) and less (often less than 1/3) for catostomids. Also, the proportion of preanal to total myomeres is 5/7 or less (often less than 2/3) for cyprinids and greater (often greater than 3/4) for catostomids. For cypriniform fishes in the Gila River Basin, exclusive of *Ictiobus* species, the degree of overlap in total and preanal myomere counts is less and larvae with fewer than 44 total or 36 preanal myomeres can only be cyprinids.

Fins and finfolds

Fin-ray meristics and fin positions are among the most useful characters for later mesolarvae and metalarvae, especially among the cyprinids. These data can be determined from older juveniles and adults or gleaned from published descriptions of adults. The sequence and timing of fin development, fin lengths, and basal lengths of the dorsal and anal fins are also useful.

The median finfold, one of the most obvious structures in protolarvae and mesolarvae, is a thin, erect, medial fold of tissue that originates on the dorsal surface, usually well behind the head. It extends posteriorly to and around the end of the notochord, then anteriorly along the ventral surface to the posterior margin of the vent. During the mesolarval phase, the soft-rayed portions of the median fins (dorsal, anal, and caudal) differentiate from this finfold. As the median fins develop, the finfold diminishes and recedes before and between the fins until it is no longer apparent during or near the end of the metalarval phase.

The preanal finfold is a second median fold of tissue that extends forward from the vent. In cypriniform and most other fishes, the preanal finfold is completely separated from the ventral portion of the median finfold by the vent. But in burbot (*Lota lota*), and its marine relatives (Gadidae, codfishes), the preanal finfold is initially continuous with the median finfold and only later are the finfolds entirely separated by the vent (vent initially opens through right side of finfold). The preanal finfold may or may not be present upon hatching, depending upon size and shape of the yolk sac. In cypriniform fishes, it is typically absent or barely apparent upon hatching. As yolk is consumed and the yolk sac decreases in size prior to hatching or during the protolarval phase, a small preanal finfold appears just anterior to the vent. As more yolk is consumed and the larva grows, the preanal finfold enlarges and extends anteriorly. Ultimately, its origin lies anterior to that of the dorsal portion of the median finfold. The preanal finfold remains prominent throughout the mesolarval phase, then slowly diminishes and recedes in a posterior direction during the metalarval phase. It is typically the last finfold to be absorbed or lost.

The caudal fin is the first fin to differentiate from the median finfold in cypriniform and most other fishes with homocercal tails. The portion of the finfold involved first thickens along the ventral side of the posterior end of the notochord and begins to differentiate into the hypural elements of the caudal skeleton. Immediately thereafter, the first caudal-fin rays appear (beginning of flexion mesolarval phase) and the posterior portion of the notochord begins to bend or flex upward. Be careful not to confuse striations or folds in the finfold with developing fin rays. As the fin develops and the notochord continues to flex upward, the hypurals and developing caudal-fin rays, all ventral to the notochord, move to a posterior or terminal position. The first principal rays are medial and subsequent principal rays form progressively above and below. Principal caudal-fin rays articulate with hypural bones of the caudal structure and ultimately include all branched rays plus two adjacent unbranched rays, one above and one below the branched rays. Branching and segmentation of rays can be observed as or shortly after the full complement of principal rays becomes evident and notochord flexion is completed (beginning of postflexion mesolarval phase).

The number of principal caudal-fin rays is typically very stable within major groupings of fish. Cyprinids generally have 19 principal rays (ten based on superior hypurals or hypural plate and nine on inferior hypurals or hypural plate), and catostomids usually have 18 principal rays (nine and nine respectively).

Dorsal and ventral rudimentary rays of the caudal fin (shorter unbranched rays anterior to the outermost principal rays which also remain unbranched in later stages) begin forming sequentially in an anterior direction immediately after all or nearly all principal caudal-fin rays are formed. They are often the last group of fin rays among all fins to form their full adult complement. Accordingly, counts of rudimentary caudal-fin rays are usually ignored in larval fish identification, but they may be of taxonomic value for juveniles and adults.

The dorsal and anal fins, which typically form either simultaneously (many cyprinids) or dorsal first (most catostomids), usually begin development prior to attainment of the full complement of principal caudal-fin rays. Tissue first thickens in vicinity of the future fin, and basal structures or pterygiophores soon become evident. The latter structures permit limited use of dorsal and anal fin position and meristics about midway through the mesolarval phase. Anterior principal fin rays develop first and subsequent rays are added in a posterior direction. The first rudimentary fin rays (shorter unbranched rays anterior to the principal rays) are

frequently evident before all the principal fin rays form. Rudimentary fin rays are added in an anterior direction.

The first or most anterior principal ray in both dorsal and anal fins remains unbranched while all other principal fin rays branch distally as or after ray segmentation becomes evident. The last or most posterior principal ray in each fin is considered to be divided at the base and therefore usually consists of two elements that, except for their close proximity and association with the same pterygiophore, might otherwise be considered as separate fin rays.

Principal dorsal- and anal-fin-ray counts between and within certain genera often vary sufficiently to be of use in identification at the species level, especially anal-fin rays of cyprinids and dorsal-fin rays of catostomids. Positions of dorsal-fin origin (anterior attachment) and insertion (posterior attachment) relative to origin of pelvic fins or fin buds and the vent vary considerably among cyprinids and are useful in identification of genera or species. These position characters are more consistent among catostomids (e.g., dorsal-fin origin is always well in advance of the pelvic fins), especially at subfamily level, and therefore, are of less value in identification.

The pelvic fins begin as buds before or upon transition to the metalarval phase. In cypriniform fishes, they originate in an abdominal position along each side of the preanal finfold. They may erupt shortly after dorsal-and anal-fin development begins or be delayed until just before or shortly after all principal rays are present in the median fins. Pelvic rays begin to form shortly after the buds appear and the adult complement of rays quickly ensues. Among cypriniform fishes, pelvic-ray counts are seldom used diagnostically. However, position of the pelvic fins or fin buds, relative to other structures, and their formation in the sequence of developmental events can be useful in identification, especially among cyprinids.

The pectoral fins typically begin as buds immediately behind the head in the late embryo. However, pectoral buds are not evident in some cypriniform fishes until shortly after hatching. Though strongly striated and occasionally with membranous folds and breaks, they typically remain rayless in cypriniforms until late in the mesolarval phase when most of the principal median-fin rays are present. With the exception of rudimentary caudal-fin rays, the rays of pectoral fins are often the last to establish their full complement. For this reason and because the number of pectoral rays is usually relatively large and difficult to count without excision (especially the smaller ventral rays), pectoral-fin-ray counts are generally of little value in larval fish identification.

Other countable structures

Other structures that may be treated meristically (and in some cases morphologically) include branchiostegals, gill rakers, pharyngeal teeth, and scales. Branchiostegals form early in larval development, but counts are usually constant within major taxon groups. Within the order Cypriniformes, all members of superfamily Cyprinoidea, which includes Cyprinidae and Catostomidae, have three branchiostegals (McAllister 1968). Due to later development, small size or internal location, the other characters are seldom used to diagnose fish larvae. Gill rakers form gradually in postflexion mesolarvae or metalarvae with numbers increasing throughout much of the early portion of the juvenile period. The adult complement of gill rakers on the first gill arch is not achieved in many Catostominae until they reach about 70 mm standard length (Smith 1966). Pharyngeal teeth form relatively early but may not be sufficiently well developed to be readily removed and observed until late in the larval period or early in the juvenile period.

Detailed study of gill rakers and pharyngeal teeth might reveal some useful diagnostic qualities, including size, shape, and number. However, most specimens are more easily identified using external characters. Scales typically become apparent late in the larval period or early in the juvenile period. First scales on cypriniforms typically appear midlaterally on the posterior half of the body and from there spread anteriorly, dorsally, and ventrally toward adult coverage. Scales of large-scaled species are sometimes sufficiently obvious by late in the metalarval phase to distinguish certain species or genera.

Morphology

The shape or form of larvae and specific anatomical structures (e.g., gut, air bladder, yolk sac, and mouth) changes as fish grow and provides some of the most obvious characters for identification, particularly at family and subfamily levels. Within genera, morphological differences among species are usually much more subtle, but may still be of diagnostic value. Much shape or form-related information can be quantified via proportional measurements or morphometrics.

Morphometric data emphasize the relative position and relative size of various body components and dimensions and may be critical to species identification. Such measurements may be allometric, changing in proportion as the fish grow; thus morphometric data should be related to size, at least for protolarvae and mesolarvae. Some morphometric data, particularly body depths and widths, may be directly affected by the condition of individual specimens and volume and form of food items in their digestive tracts. The source of specimens and the preservative in which they are stored also may affect morphometric data. Some measures in wild fish may differ from those of laboratory-reared specimens (e.g., fin lengths). Shrinkage and deformation are notably greater in alcohol than in formalin preservatives.

Morphometric data in this guide are reported as percentages of standard length (% SL). Use of standard length (SL) avoids the allometric influence of caudal fin growth included in percentages based on total length (TL). As explained later (Methods), data can be easily converted to percent TL (% TL) for comparison with other works. Prior to hypural plate formation and completion of notochord flexion (protolarvae and flexion mesolarvae), SL is the length from snout to posterior end of the notochord (notochord length). Thereafter, SL is measured from the anterior margin of the snout to the most posterior margin of the hypural plates (usually the superior plate or hypurals). Use of notochord length for protolarvae and early mesolarvae gives the appearance of greater allometric growth differences than may really exist, at least in comparison with subsequent measures based on the posterior margin of the hypural plates. This undesirable effect is a result of upward bending or flexing of the notochord and the switch from use of end of the notochord to posterior margin of the hypurals as the basis for length measurement. These factors must be taken into account when reviewing morphometric data herein.

In contrast to procedures recommended by Hubbs and Lagler (1958) for larger juveniles and adults, measurements of body length and various parts thereof for fish larvae are generally taken along lines parallel to the horizontal axis of the fish. Exceptions are fin lengths which, in studies conducted for this manual, were measured from origin of the fin base to most distal margin of the fin rays. Typical measures include total, standard, head, snout, eye, and fin lengths, as well as snout-to-vent and snout-to-origin-of-fin (dorsal, anal, and pelvic) lengths.

Snout-to-vent length is measured to the posterior margin of the vent or anus. It is a primary diagnostic character for many species, especially at the family and sometimes subfamily level. In the Gila River Basin, most cyprinid larvae are readily differentiated from catostomid larvae by snout-to-vent lengths less than 72% SL. Exceptions are most larvae of common carp (*Cyprinus carpio*) and occasionally mesolarvae of Colorado pikeminnow (*Ptychocheilus lucius*). The term "preanal length" is often applied to this measure but might be misinterpreted as length to origin of the anal fin. For many fishes, including cypriniforms, the latter measure is approximately the same as snout-to-vent length since the anal fin begins at or near the posterior margin of the vent.

Head length is typically measured to the posterior margin of the operculum in juveniles and adults, but the operculum may be absent or incomplete throughout much of the larval period. Accordingly, many biologists have redefined head length for larvae to be measured to the posterior end of the auditory vesicle or the anterior or posterior margin of the cleithrum, one of the first bones to ossify in fish larvae (Berry and Richards 1973). Unfortunately, the auditory vesicle and cleithrum are not always easy to observe, especially in postflexion mesolarvae and metalarvae. Also, resultant measures to the auditory vesicle are considerably anterior to the eventual posterior margin of the operculum. Snyder et al. (1977) and Snyder and Douglas (1978) measured larval head length to origin (anterior insertion) of the pectoral fin. This measure has distinct advantages over the alternatives–the base of the pectoral fin is readily observed throughout the larval period (except in the few species that hatch prior to pectoral bud formation), it somewhat approximates the position of the cleithrum (part of its supporting structure), and it more nearly approximates the posterior margin of the operculum than does the posterior margin of the auditory vesicle. Accordingly, we recommend this definition of head length (Snyder 1983) and have used it in all our descriptive work. For purposes of consistency, we apply it to juveniles as well as larvae. The measure is most precisely determined while examining the specimen from above or below and, if necessary, holding the fin away from the body.

Body depths and widths are measured in planes perpendicular to the horizontal axis of the fish. Many biologists report these as maximum or minimum measures (e.g., greatest-head depth, greatest-body depth, and least-caudal-peduncle depth). However, for comparative purposes, it seems more logical to specify standard reference points for such measures as was done by Moser and Ahlstrom (1970), Fuiman (1979), and Snyder and Douglas (1978). Five specific locations, four corresponding to specific length measurements, are used herein: (1) immediately posterior to eyes, (2) origin of pectoral fin, (3) origin of dorsal fin, (4) immediately posterior to vent, and (5) at anterior margin of most posterior myomere (along the horizontal myosepta). It is often desirable to approximate position of reference points in larvae prior to formation of the referenced structure (e.g., origin of dorsal fin in protolarvae and flexion mesolarvae based on position in later stages). Neither fins nor finfolds are included in depth measurements herein. As mentioned earlier, care must be used in evaluation of depth and width measures affected by body condition and gut contents (e.g., measures at the origin of the dorsal fin).

Other morphological characters such as position, size, and form of the mouth and gut, and related changes, can be among the more useful characters for identification to the species level. Size of the mouth, as well as its position, its angle of inclination, and the form of specific mouth structures are diagnostic for some cypriniforms, especially in metalarvae. Timing of mouth migration from terminal to inferior position can be especially useful for catostomid metalarvae. Gut-loop length, timing of loop formation, and eventual degree and form of gut

loops, folds, or coils can be diagnostic for the larvae of many fishes. Such characters are especially useful in distinguishing postflexion mesolarvae, metalarvae, and early juveniles of certain catostomids.

Pigmentation

Basic patterns of chromatophore distribution, and changes in these patterns as fish grow are often characteristic at the species level. Used with caution, preferably in combination with other characters, and with an awareness of both intra- and interregional variation, chromatophore distribution and patterns for many fishes are among the most useful characters available for identification. However, in some instances, differences are so subtle or variation so great that use of pigmentation is impractical and may be misleading.

In cypriniform and most other fishes, chromatophores other than melanophores have not been sufficiently studied for identification purposes. Such chromatophores are typically neither as numerous nor as obvious as melanophores and their pigments are difficult to preserve. In contrast, melanin, the amino acid breakdown product responsible for the dark, typically black, appearance of melanophores (Lagler et al. 1977), remains relatively stable in preserved specimens. However, melanin is subject to fading and bleaching if specimens are stored or studied extensively in bright light for long periods of time, stored in highly alkaline preservatives, or subjected to changing concentrations of preservative fluids. To minimize the latter effects, as well as shrinkage and deformation, dilute formalin solutions (3-5%, unbuffered or buffered to near neutral) are strongly recommended over alcohol solutions as storage media. Most of the following discussion refers to chromatophores in general, but in this manual and others for freshwater species in North America, pigmentation typically refers to that of melanophores.

According to Orton (1953), pigment cells originate in the neural crest region (dorsal portion of body and tail) and migrate in amoeboid fashion in waves to their eventual position. The first wave of chromatophores occurs late in the embryonic period or early in the larval period and establishes a relatively fixed basic or primary pattern of chromatophore distribution. In a few species (mostly marine), such cells acquire pigment prior to chromatophore migration and the actual migration can be observed and documented. But in cypriniform and most other freshwater fishes, pigment is not present in chromatophores until after the cells reach their ultimate destination.

For a specific species and developmental stage, pigmental variation in general or specific areas is largely a function of the number of chromatophores exhibiting pigment rather than differences in chromatophore distribution. Chromatophores without pigment cannot contribute to the visible pigmentation pattern. In addition, pigment in chromatophores can be variously displayed from tight, contracted spots, resulting in a relatively light appearance, to widely expanded, reticular networks, resulting in a dark or more strongly pigmented appearance. Differences in environmental conditions and food can significantly affect the presence and displayed form of pigmentation. Accordingly, researchers must be aware that pigmentation of cultured specimens can appear quite different from that of field-collected material.

Pigmentation often changes considerably as larvae and early juveniles grow. Most of the change is due to increased numbers and distribution of chromatophores. Observable pigmentation might also be lost from certain areas through loss of pigment in chromatophores, loss of chromatophores themselves, or, in the case of subsurface or internal chromatophores, by

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