Chapter 30

Fundulidae: Topminnows

Robert C. Cashner, Jacob Schaefer, Melvin L. Warren, Jr., Anthony A. Echelle, Fernando Galvez, and Michael J. Ghedotti

The family Fundulidae, the Topminnows (or killifishes), is largely North American in distribution, where it is widespread. As the name Topminnow implies, the 40 or so species of the group typically occur high in the water column of fresh, brackish, and coastal marine waters. The scientific name Fundulidae is derived from the Latin *fundus* meaning bottom, certainly not an intuitive name for Topminnows. Some supposed this name refers to an affinity for shallow waters (Etnier & Starnes 1993) or a behavioral propensity of some of the first described species to burrow in the substrate when startled (Moyle 2002). Fundus may also refer to the bottom of a tidal river in estuarine habitats where members of this group are often dominant. Most Topminnows are relatively small (adults 27–180 mm SL), slender fishes with rounded or truncate caudal fins, upturned mouths, flattened dorsums, and a dorsal fin typically far back on the body (Ghedotti & Davis 2013; Fig 30.1). Many Topminnows inhabit brackish waters, and all tested to date have a greater range of salinity tolerance than freshwater ostariophysans (e.g., Catfishes, Siluriformes; Carps, Loaches, Minnows, and relatives, Cypriniformes) (Griffith 1972, 1974b). A number of the coastal and estuarine forms can tolerate salinities considerably higher than sea water (Nordlie 2000b, 2006). Topminnows are often some of the most visible species in their native habitats. They can be seen near the surface of shallow waters in small groups, typically lying motionless until startled (Godin & Morgan 1985), often near the shore or available structure. The diversity of form and function among the 40 species in this group have made some popular in the aquarium trade (bright coloration, high activity, and small size), and others are model organisms in ecotoxicology (abundance in coastal ecosystems, physiological tolerances) and medical applications (kidney function and osmoregulation).

DIVERSITY AND DISRIBUTION

Diversity

The Topminnows comprise 40 extant, described species in three genera (Tables 30.1 and 30.2) native to the fresh and

Figure 30.1. Two widely distributed and closely related species of *Fundulus*. (*upper*) A male Blackspotted Topminnow, *Fundulus olivaceus*, in the Buffalo River, Arkansas, in May. (*lower*) A male Blackstripe Topminnow, *Fundulus notatus*, from Big Darby Creek, Ohio. The Blackstripe Topminnow generally lacks the intense dorsolateral spotting typically seen in the Blackspotted Topminnow (courtesy of © Isaac Szabo / Engbretson Underwater Photography, *upper*, and Anthony Terceira, *lower*).

brackish waters of North America, the Yucatan Peninsula, Cuba, and Bermuda (Mayden 1992; Ghedotti & Davis 2013). The genus *Leptolucania* is monotypic (Fig. 30.2), and the genus *Lucania* has only three species (Fig. 30.3). The remaining 36 extant (plus one recently extinct) species are assigned to the genus *Fundulus* (Figs. 30.1 and 30.4), which contains four subgenera representing recognized clades (Ghedotti & Davis 2013; Table 30.1). Recognition of species of *Fundulus* has engendered some controversy. Two pairs of coastal brackish water species are named taxa: Striped Killifish (*Fundulus majalis*)-Longnose Killifish (*Fundulus similis*) and Bayou Killifish (*Fundulus pulvereus*)-Marsh Killifish (*Fundulus confluentus*). In each case, recognition of the respective pair as separate species was controversial (Relyea 1965, 1983; Wiley & Mayden 1985; Boschung & Mayden 2004), but the most current work to date supports their recognition as distinct, separate species (see phylogenetic relationships section). Their geographic distinctness and morphological diagnosability indicate they are functioning as separate evolutionary lineages. The two named species on the island of Bermuda, the Bermuda Killifish, *Fundulus bermudae*, and the Lover's Lake Killifish, *Fundulus relictus*, are most likely geographically disjunct populations of the Mummichog, *Fundulus heteroclitus*. Some authors suggested that some of the Bermuda populations are separate species, but works on relationships within Fundulidae did not recover them as separate clades (Able & Felley 1988; Grady et al. 2001; Whitehead 2010; Ghedotti & Davis 2013). Following Ghedotti & Davis (2013), we recognize the Northern Plains Killifish, *Fundulus kansae*, as a separate species from the Plains Killifish, *Fundulus zebrinus* (Kreiser et al. 2001). We also include the Conservationist Killifish, *Fundulus philpisteri*, a species (García-Ramírez et al. 2007) found only in northeastern Mexico. This species is found inland in arid areas and is most likely closely related to the Gulf Killifish, *Fundulus grandis*; however, specimens were unavailable for inclusion in some analyses of Fundulidae (Whitehead 2010; Ghedotti & Davis 2013). Whitehead (2010) recognized *Fundulus saguanus* (Cuban Gulf Killifish),

Figure 30.2. A male Pygmy Killifish, *Leptolucania ommata*, in breeding colors in Lake Hiawatha, Hillsborough County, Florida, in May (courtesy of © 2013, Anthony Terceira).

Figure 30.3. (*upper*) A large male Rainwater Killifish, *Lucania parva*, in Alexander Springs, St. Johns River, Florida, in October. Large males have a black spot at the front of the dorsal fin that is lacking in the female. (*middle* and *lower*) Male Bluefin Killifish, *Lucania goodei*, in the Hillsborough River, Hillsborough County, *middle*, and McBride Slough, Wakulla County, *lower*, Florida. Note the bright iridescent blue on the front of the dorsal and anal fins. The male flares these fins to compete with other males for spawning sites and to court females. The Rainwater and Bluefin Killifishes are among the smallest of North American fishes (adult size 15–70 mm TL). The Rainwater Killifish is largely a brackish water species occurring along the lower, tidally influenced reaches of coastal rivers (Ross 2000; Rohde et al. 2009). Its relative, the Bluefin Killifish, can occur in brackish water but is most common in calm, shallow water of drainage ditches, backwaters, rice fields, and springs in or near dense aquatic vegetation (courtesy of © Isaac Szabo / Engbretson Underwater Photography, *upper*, and John Brill, *middle* and *lower*).

Figure 30.4. A breeding male Northern Studfish, *Fundulus catenatus*, cruises just under the surface in Jimmie Creek, White River drainage, Arkansas, in June. Northern Studfish, which inhabit small to medium size, rocky streams, spawn over shallow gravel beds in slow to still water areas where males establish and guard small territories from about April through July (Pflieger 1997; Page & Burr 2011) (courtesy of © Isaac Szabo / Engbretson Underwater Photography).

but Ghedotti & Davis (2013) noted that the species was originally described and subsequently treated in the literature as a subspecies of *F. grandis* (Lee et al. 1980; Relyea 1983; Miller et al. 2005), a position we adopt here.

Native Distribution

Topminnows are distributed from southern and central Canada through the south and central United States, along the Gulf Coast to the northern Yucatan Peninsula, in northern Cuba, in Bermuda, and along the Pacific Coast in California and Baja California, Mexico. Of the North American species, 37 are native to the United States, nine are native to Mexico, and three are native to Canada (Lee et al. 1980; Page & Burr 1991, 2011; Mayden 1992). The center of diversity for the group is the southeastern United States where 27 species occur, including six species with limited, single-basin distributions.

North America supports 34 extant species of *Fundulus*. Members of *Fundulus* are distributed in eastern North America from southern Canada and the western Great Plains eastward along the Pacific Coast in California and Baja California and along the Gulf Coast of Mexico (Lee et al. 1980; Page & Burr 1991, 2011; Miller et al. 2005; Fig. 30.5). Species of *Fundulus* also are distributed outside of North America in Bermuda, along the northern coast of Cuba, and along the northern Gulf Coast of the Yucatan Peninsula. Substantial variability occurs in the size of the geographic range among species. Some of the more broadly distributed species are the Banded Killifish, *Fundulus diaphanus* (southern Canada and northeastern United States), *F. heteroclitus* (Atlantic Coast from southern Newfoundland to northeastern Florida), and Blackstripe Topminnow, *Fundulus notatus* (central Gulf Coast up through the Great Lakes drainages). Conversely, the Broadstripe Topminnow (*Fundulus euryzonus*), Stippled Studfish (*Fundulus bifax*), Waccamaw Killifish (*Fundulus waccamensis*), Barrens Topminnow (*Fundulus julisia*), and extinct Whiteline Topminnow (†*Fundulus albolineatus*) all have or had fairly limited distributions.

The Topminnows also include the genera *Leptolucania* and *Lucania*. The only representative of *Leptolucania*, the Pygmy Killifish, *L. ommata* (Table 30.1), inhabits lentic, fresh waters in northern and central Florida, southern Georgia and Alabama, and the southeasternmost portion of Mississippi (Page & Burr 1991, 2011; Ross 2001; Boschung & Mayden 2004; Fig. 30.6). The genus *Lucania* comprises three species (Table 30.1) found in fresh waters from Florida, through coastal Georgia, and in the partially endorheic Bolson de Cuatro Ciénegas, Coahuila, Mexico (Lee et al. 1980; Miller et al. 2005; Fig. 30.7). The Rainwater Killifish (Fig. 30.3), *Lucania parva*, occurs along the Atlantic Coast from Cape, Massachusetts, southward in Mexico into Laguna de Tampamachoco, near Tuxpan, Veracruz, in the northeast and Laguna de Terminos, Campeche, in the southeast, a disjunction of 800 air km. The Rainwater Killifish also occurs in the lagoons of Cancun and Isla Cozumel, Mexico (Miller et al. 2005), and native inland populations occur in the Pecos River (Rio Grande drainage), Texas and New Mexico (Hoagstrom 2003; Hoagstrom et al. 2010). In contrast, the Cuatro Ciénegas Killifish, *Lucania interioris*, has a limited distribution, being restricted to the Cuatro Ciénegas basin in Mexico (Hubbs & Miller 1965; Miller et al. 2005).

Non-native Distribution

Remarkably, given the high physicochemical tolerances in *Fundulus*, just seven members have well-established populations outside of their native ranges. Fuller et al. (1999) reported nonindigenous occurrences for 13 Topminnows. The majority of those are not established (reported as extirpated or they are represented by a single individual) or not established as introduced because the putative introduced populations are adjacent to native populations. Introduced populations of *Fundulus zebrinus* and *F. kansae* are now established in localities in California, Nevada,

Table 30.1. Scientific and common names, salinity tolerance, and salinity of commonly occupied habitat for described Topminnows, Fundulidae. Salinity tolerance: M = tolerate ≥ 50% salinity, B = 30–50%, F = <30%, ND = no data. Data compiled from Miller 1955b, 1978; Echelle & Echelle 1972; Griffith 1972, 1974a; Stanley & Fleming 1977; Nordlie 2000b; Ruiz-Campos 2000; Boschung & Mayden 2004; Vega-Cendejas & Hernández de Santillana 2004; Miller et al. 2005; Main et al. 2007; Whitehead 2010; Ghedotti & Davis 2013.

Scientific Name	Common Name	Salinity Tolerance/Habitat
Genus *Fundulus*		
Subgenus *Wileyichthyes*		
Fundulus lima	Baja California Killifish	ND/low salinity habitat
Fundulus parvipinnis	California Killifish	M/high salinity habitat
Subgenus *Plancterus*		
Fundulus kansae	Northern Plains Killifish	M/low salinity habitat
Fundulus zebrinus	Plains Killifish	M/low salinity habitat
Subgenus *Zygonectes*		
Fundulus blairae	Southern Starhead Topminnow	ND/low salinity habitat
Fundulus cingulatus	Banded Topminnow	F/low salinity habitat
Fundulus chrysotus	Golden Topminnow	F + M/low salinity habitat
Fundulus dispar	Northern Starhead Topminnow	ND/low salinity habitat
Fundulus escambiae	Russetfin Topminnow	F/low salinity habitat
Fundulus euryzonus	Broadstripe Topminnow	ND/low salinity habitat
Fundulus lineolatus	Lined Topminnow	F/low salinity habitat
Fundulus luciae	Spotfin Topminnow	M/high salinity habitat
Fundulus notatus	Blackstripe Topminnow	F/low salinity habitat
Fundulus nottii	Bayou Topminnow	F/low salinity habitat
Fundulus olivaceus	Blackstripe Topminnow	F/low salinity habitat
Fundulus rubrifrons	Redface Topminnow	ND/low salinity habitat
Fundulus sciadicus	Plains Topminnow	F/low salinity habitat
Fundulus xenicus	Diamond Killifish	M/high salinity habitat
Subgenus *Fundulus*		
†*Fundulus albolineatus*	Whiteline Topminnow	ND/low salinity habitat
Fundulus bifax	Stippled Studfish	ND/low salinity habitat
Fundulus catenatus	Northern Studfish	F/low salinity habitat
Fundulus confluentus	Marsh Killifish	M//high salinity habitat
Fundulus diaphanus	Banded Killifish	M/low salinity habitat
Fundulus grandis	Gulf Killifish	M/high salinity habitat
Fundulus grandissimus	Giant Killifish	ND/high salinity habitat
Fundulus heteroclitus	Mummichog	M/high salinity habitat
Fundulus jenkinsi	Saltmarsh Topminnow	M/high salinity habitat
Fundulus julisia	Barrens Topminnow	F/low salinity habitat
Fundulus majalis	Striped Killifish	M/high salinity habitat
Fundulus persimilis	Yucatan Killifish	ND/high salinity habitat
Fundulus philpisteri	Conservationist Killifish	ND/ND
Fundulus pulvereus	Bayou Killifish	M/high salinity habitat
Fundulus rathbuni	Speckled Killifish	B/low salinity habitat
Fundulus similis	Longnose Killifish	M/high salinity habitat
Fundulus seminolis	Seminole Killifish	B/low salinity habitat
Fundulus stellifer	Southern Studfish	F/low salinity habitat
Fundulus waccamensis	Waccamaw Killifish	M/low salinity habitat
Genus *Leptolucania*		
Leptolucania ommata	Pygmy Killifish	F/low salinity habitat
Genus *Lucania*		
Lucania goodei	Bluefin Killifish	B/low salinity habitat
Lucania parva	Rainwater Killifish	M/high salinity habitat
Lucania interioris	Cuatro Ciénegas Killifish	ND/low salinity habitat

Table 30.2. Summary of life-history characteristics of genera of fundulids.

	Fundulus	*Leptolucania*	*Lucania*
Number of extant species	35	1	3
1° or 2° fresh water	Peripheral	Peripheral	Peripheral
Maximum size	220 mm TL *F. grandissimus*	30 mm TL	70 mm TL
Maximum age	7 years *F. majalis*	<2 years	2 years
Age and size at first reproduction	Often within first year, 20–40 mm SL	Within first year, 12–15 mm SL	2–4 months, 23 mm SL
Iteroparous versus semelparous	Iteroparous	Iteroparous	Iteroparous
Fecundity estimates from ovarian counts	Range from 100–500	NA	100
Egg deposition sites	Vegetation, rocks, and occasionally sand and gravel	Vegetation	Vegetation
Clutch size	7–50	10	10–50
Range of nesting/spawning dates and temperatures	Maximum in March-May, often extends through September; estuarine forms on spring tide	April-August, some populations all year	January-September, peak in early spring; some populations all year
Habitat of spawning sites; average water depth	Shallow stream margins, tidal pools, marsh margins, typically <1 m	Littoral zone or margins near vegetation, <1 m	Littoral zone or margins near vegetation, <1 m
Incubation period (in days and C); larval type (i.e., protolarva, metalarva, mesolarva) at hatching	6–21 days at 24°C; delayed development in estuarine forms; mesolarvae	10–12 days at 24°C; mesolarvae	5–7 days at 24°C; mesolarvae
Mean size at hatching	4–6 mm TL	4 mm TL	4–5 mm TL
Sex building nest; sex incubating eggs; other care-giving behaviors	None	None	None
Major dietary items	Insects, copepods, amphipods, larger species small fish, shrimp, and crabs	Chironomidae, copepods, and cladocerans	Mosquito larvae, crustaceans, small worms, and mollusks
General year-round habitat	Shallow habitat, typically <1 m, near vegetation or structure	Vegetated and low flow backwaters, swamps, and sloughs	Coastal rivers, springs, estuaries, and lagoons near vegetation
Migratory or diadromous	Some estuarine forms spawn in high marsh habitat	No	No
Imperilment status	Extinct: *F. albolineatus* Endangered: *F. julisia*, *F. lima* Threatened: *F. waccamensis* Vulnerable: *F. bifax*, *F. euryzonus*, *F. grandissimus*, *F. persimilis*	Not imperiled	Endangered *L. interioris*

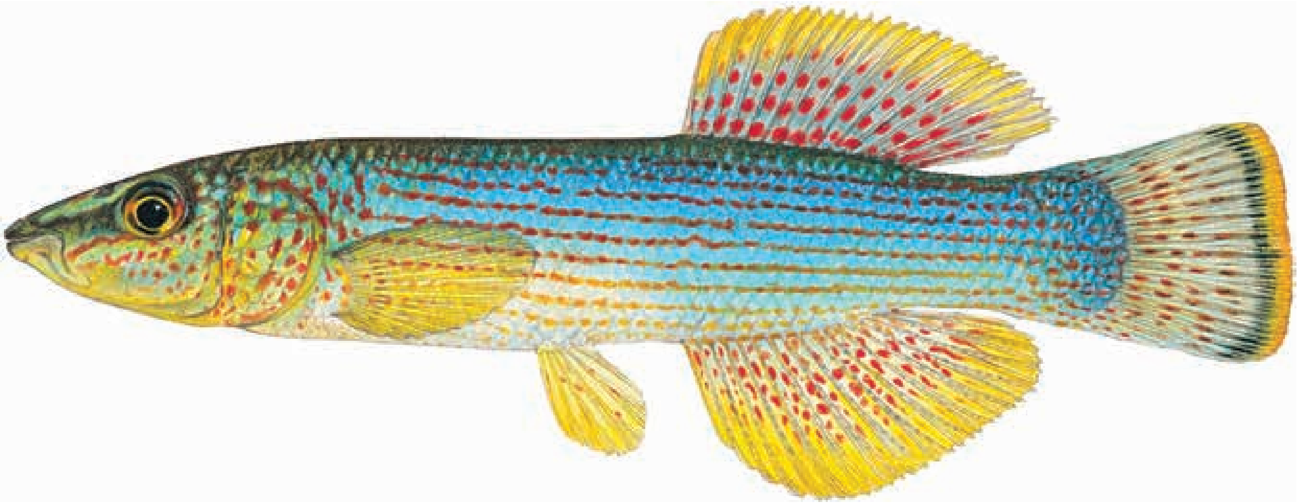

Plate 30.1. Northern Studfish, *Fundulus catenatus.*

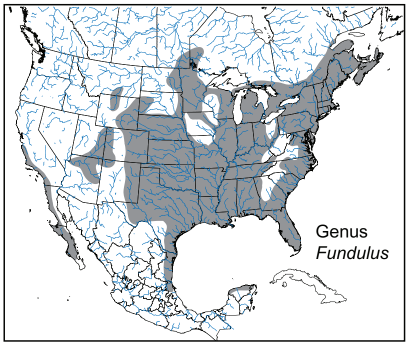

Figure 30.5. Geographic range of *Fundulus* in North America.

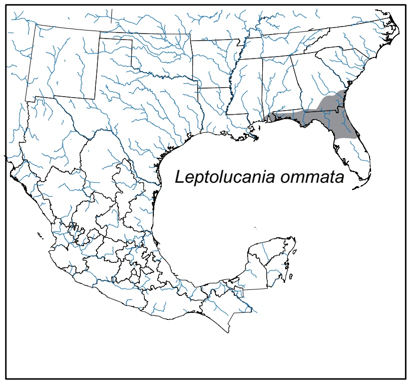

Figure 30.6. Geographic range of the Pygmy Killifish, *Leptolucania ommata.*

Figure 30.7. Geographic range of *Lucania.*

New Jersey, Oregon, Texas, and Utah (Stiles 1978; Lee et al. 1980; Poss & Miller 1983; Deacon & Williams 1984; Hubbs et al. 2008). Using allozyme and mtDNA data, Kreiser et al. (2000) concluded that western Colorado populations resulted from multiple independent introductions. The means of introduction is unknown but baitbucket transfer seems likely. The Mummichog is the most frequently sampled species in salt-marsh habitat along the southern coast of Spain (Gutiérrez-Estrada et al. 1998). Individual Mummichogs were first recorded from the Iberian Peninsula from 1973 to 1976, and the species quickly spread and colonized all salt-marsh habitat between Portugal and the Mediterranean Sea. The rapid range expansion was most likely stopped by the lack of suitable marsh habitat farther east or west (Gutierrez-Estrada et al. 1998). The invading Mummichog populations are from Nova Scotia (Bernardi et al. 1995), though the mechanism of introduction is unknown. Some speculate the species was unintentionally introduced along with the intentional introduction of the Largemouth Bass (*Micropterus salmoides*) and Red Swamp Crayfish (*Procambarus clarki*). A second population of *F. waccamensis* is reported in Phelps Lake, far from Lake Waccamaw where it is endemic (Hubbs & Raney 1946; Rohde et al. 1994; Krabbenhoft et al. 2009). This population may also have come from a baitbucket transfer; however, whether the population actually represents the Waccamaw Killifish or native Banded Killifish is in some doubt.

Non-native, inland populations of the primarily estuarine-marine Gulf Killifish, *Fundulus grandis*, are widespread in the Pecos River (Rio Grande drainage), Texas and New Mexico. The non-native status of these

Plate 30.2. Pygmy Killifish, *Leptolucania ommata* (male *upper*, female *lower*)

Plate 30.3. Bluefin Killifish, *Lucania goodei*.

populations is indicated by absence of the species in historical records from the Rio Grande. These include a 1953–1955 survey of 60 sites on the Rio Grande from the mouth of the Pecos River to the coast (Treviño-Robinson 1959) and a 1977 survey of 33 sites on the Rio Grande upstream from Amistad Reservoir, a Pecos River-Rio Grande impoundment. The first record of the Gulf Killifish from the Rio Grande drainage was made in 1970 from a location downstream of Falcon Reservoir about 140 km upstream from the coast (Hillis et al. 1980). Then, in 1979, the species was taken from three Pecos River localities upstream from Amistad Reservoir, probably reflecting baitbucket transport (Hillis et al. 1980). As late as 1987, the species was uncommon in the Pecos River and largely confined to the most downstream segments (Cheek & Taylor 2015), but by the late 1990s (Hoagstroam 2003) and in 2011 (Cheek & Taylor 2015), it was widespread over about 700 km of the Pecos River downstream of Carlsbad, New Mexico. Now the Gulf Killifish is a dominant species in brackish-water segments of the drainage where it has effectively replaced the native Plains Killifish, probably via competitive displacement (Hoagstroam et al. 2010; Cheek & Taylor 2015).

In 1958, or perhaps earlier, *Lucania parva* appeared in five widely disjunct localities in three western states (Hubbs & Miller 1965). These sites included Yaquina Bay, Oregon, San Francisco Bay, California, pluvial Blue Lake, Utah, and a reservoir in southern California. In its native habitat, the species feeds extensively on the larvae and pupae of salt-marsh mosquitoes (Harrington & Harrington

1961). As a result, the Rainwater Killifish is often introduced in an effort to control mosquito populations. Hubbs & Miller (1965) concluded that the species was not introduced for mosquito control in these western U.S. states. Rather, the presence of the Rainwater Killifish in Utah and southern California was likely because of inadvertent introduction along with gamefishes stocked from the Pecos River, New Mexico, which is well within the native range of the species (Hubbs & Miller 1965). The established populations in Oregon and northern California likely originated from Rainwater Killifish eggs on oysters (*Crassostrea virginica*) from the Atlantic Coast. This association seems plausible because Atlantic Coast oysters are cultivated extensively in San Francisco and Yaquina Bays. The hypothesis is bolstered by similarities in meristic and morphometric data for both introduced and native Atlantic Coast *L. parva* (Hubbs & Miller 1965). Sometime around 1980 the species was introduced by unknown means into Clear Creek, San Saba River drainage, Texas (Hubbs et al. 2008), which still supports a population. Finally, two introduced populations of the Bluefin Killifish, *Lucania goodei*, are near the heavily populated cities of Charleston, South Carolina, and Wilmington, North Carolina (Fuller et al. 1999). Nonindigenous occurrences for six other fundulid species are reported, but the majority of those are not established (reported as extirpated or they are represented by a single individual) or not established as introduced because the putative introduced populations are adjacent to native populations (Fuller et al. 1999).

PHYLOGENETIC RELATIONSHIPS

Disagreement continues over the number of cyprinodontiform families, generic limits within families, intergeneric and interspecific relationships, number of valid taxa, species limits, and historical biogeography (Cashner et al. 1992; Whitehead 2010; Ghedotti & Davis 2013, 2017). A wealth of comparative data exists, and this has fueled some of the disparate hypotheses. Before the rise of modern phylogenetic methods many evolutionary studies of multiple fundulid taxa were published based on morphology (Gosline 1949; Foster 1967b; Farris 1968), ethology (Foster 1967b), karyology (Setzer 1968; Chen 1971), hybridization experiments (Hubbs & Drewry 1959; Setzer 1970), developmental sequences (Hubbs & Burnside 1972), and electrophoresis of serum proteins (Griffith 1968, 1972).

The relationships of Fundulidae to other cyprinodontiform families remain in dispute. In a landmark phylogenetic study based on morphology, Parenti (1981) established the monophyly of the order Cyprinodontiformes (Killifishes) and recognized eight families, including Fundulidae with its modern composition as sister to all other cyprinodontoids (except the genus *Profundulus*) based on two osteological characteristics of the upper jaw. Later authors using sequences from the X-src gene (Meyer & Lydeard 1993) and a reevaluation of the morphological data with families as terminal taxa (Costa 1998b) demonstrated Fundulidae as sister to a clade comprising Profundulidae and Goodeidae with which it shares two characteristics of the upper jaw, now interpreted as derived rather than ancestral. Using 10 protein-coding nuclear genes, Fundulidae were resolved in the clade Ovalentaria and nested within Atherinomorpha (Near et al. 2012b, 2013), a relationship further corroborated in an extended analysis using 21 genetic markers (Betancur-R. et al. 2013ab) that incorporated the Near et al. (2012b, 2013) sequence data. Those efforts included four of the 10 cyprinodontiform families and found the Asian Rivulines, Aplocheilidae, were sister to a clade comprising the fundulids, cyprinodontids (Pupfishes), and poeciliids (Livebearers). The relationships among these families differed between the two 2013 studies with Fundulidae sister to Poeciliidae in Near et. al. (2013) and Fundulidae sister to the Cyprinodontidae in Betancur-R. et al. (2013ab). Later, a combined analysis of four gene sequences (two nuclear, two mitochondrial), morphological data, and behavioral data yielded evidence for Fundulidae as sister to a clade comprising Cyprinodontidae, Profundulidae, and Goodeidae (Ghedotti & Davis 2013).

Relationships within Fundulidae also remain controversial. The initial morphological phylogenetic studies by Parenti (1981) and Wiley (1986) recognized the monophyletic genera *Adinia*, *Leptolucania*, *Lucania*, and *Plancterus* (including *Fundulus zebrinus* and *F. kansae*). Although Parenti (1981) considered an expanded second pharyngobranchial (bony elements of pharynx and gills) as diagnostic of *Fundulus*, Wiley (1986) did not observe this state consistently and found no convincing characters to diagnose *Fundulus* as a natural group. Derived morphological characters supported recognition of four monophyletic groups (subgenera) within *Fundulus*: *Fundulus*, *Fontinus*, *Xenisma*, and *Zygonectes* (Wiley 1986). The phylogenetic position of *F.* (*Plancterus*) *zebrinus* and the Pacific Coast forms, the Baja California Killifish, *Fundulus lima*, and California Killifish, *Fundulus parvipinnis*, were not addressed in these studies. Molecular systematic studies of Fundulidae using allozyme data (Cashner et al. 1992), mitochondrial cytochrome *b* sequences (Bernardi 1997; Grady et al. 2001), and sequences from one mitochondrial and two nuclear genes (Whitehead 2010) substantially disagreed with one another and with the relationships recovered by Wiley (1986); some genera and many subgenera were recovered as non-monophyletic. These authors did not make taxonomic recommendations based on their phylogenies.

A combined analysis used an expanded data set of four gene sequences (two nuclear, two mitochondrial), morphological data, behavioral data, fossil taxa, and a substantial number of non-fundulid taxa for rooting (Ghedotti & Davis 2013, 2017; Fig. 30.8). The analyses yielded evidence for the reciprocal monophyly of *Leptolucania*, *Lucania*, and *Fundulus* (including *F. parvipinnis*, *F. lima*, *F. zebrinus*, and *F. kansae*). The analysis also placed the genus *Adinia* (Diamond Killifish, *Fundulus xenicus*) within *Fundulus* (subgenus *Zygonectes*) and sister to the Spotfin Topminnow, *Fundulus luciae*. The latter was based on the strength of support in the DNA sequence data, the uniquely shared low karyotype number of these two species ($2n=32$, versus the common ancestral karyotype of 48), and three homoplastic (convergent) osteological character states (Ghedotti & Davis 2013).

A phylogenomic study that used RNA sequence data from 16 fundulid and four outgroup species supported recognition of *Fundulus xenicus* as a member of the genus *Fundulus* and suggested new subgeneric relationships (Rodgers et al. 2018). *Fundulus xenicus* was sister to *F. luciae*, and the western coastal species *F. parvipinnis* and *F. lima* formed a clade (subgenus *Wileyichthys*) sister to a clade composed of all other fundulids (i.e., *Lucania*, *Leptolucania*, and *Fundulus*). These relationships suggest that if additional data supports this placement that *Wileyichthys* might be more appropriately recognized at the generic rather than subgeneric level.

FOSSIL RECORD

The fossil record of Topminnows, although not extensive, contains representatives of two extant and six extinct species (Smith et al. 2002). Fossils referred to the extant *F. diaphanus* from North and South Dakota (Newbrey & Ashworth 2004) and the Seminole Killifish, *Fundulus seminolis*, from Florida (Scudder et al. 1995) occur in diatomaceous Pleistocene deposits (about 10,000 years ago). The fossils of *Fundulus* from South Dakota initially were described (Cope 1891) as *Gephyura concentrica* but were later placed in the synonymy of *F. diaphanus* (Uyeno & Miller 1963).

The fossil species †*Fundulus detillae* is known from many specimens from diatomaceous Pliocene deposits of the Ogallala Formation (about 3–5 mya) in Logan County, western Kansas (Hibbard & Dunkle 1942). †*Fundulus sternbergi*, described from the same strata at the same locality (Robertson 1943), is a junior synonym of †*F. detillae* (Smith 1962). Smith (1962) stated that †*F. detillae* is related closely to the extant and sympatric *F. zebrinus*. Griffith (1972), however, suggested that †*F. detillae* was the ancestor of most freshwater *Fundulus* not including *F. zebrinus*. In phylogenetic analyses of the Fundulidae (Ghedotti & Davis 2013, 2017), †*F. detillae* was identified as a member of a clade, including *Fundulus kansae* and *F. zebrinus*, and character-state optimization suggested it likely had the relatively high salinity tolerance exhibited by these two species.

Interestingly, five of the six described fossil species are from the southwestern United States west of the Western Continental Divide, an area where only *Fundulus parvipinnis* exists today. Miller (1945b) described the putatively contemporaneous †*Fundulus curryi* and †*Fundulus eulepis* from uncertain stratigraphic members in Death Valley, California. He also described †*Fundulus davidae* based on a single, nearly complete specimen from uncertain stratigraphic members in the Mohave Desert, northwestern San Bernadino County, California. These three species are likely Pliocene or Pleistocene in age (Miller 1945b; Uyeno & Miller 1963). Another unnamed fossil *Fundulus* sp. is the oldest fossil referred to the family and was collected in sediments >23 mya from a volcanic unit at the old Bullfrog Mine, southeast of Beatty, Nevada (Smith et al. 2002).

†*Fundulus nevadensis*, originally described in the monotypic genus *Parafundulus* (Eastman 1917), is known from a well-characterized member of the Miocene Truckee Formation (about 9.8 mya) near Hazen, Nevada (Jordan 1925; Miller 1945b; LaRivers 1962; Fig. 30.9). These fossils are abundant and are sold on the fossil market along with the co-occurring fossil species †*Gasterosteus doryssus* (a Stickleback, Gasterosteidae) (Jordan 1907b). A total evidence analysis using data from four genes and morphology (Ghedotti & Davis 2017), including three fossil *Fundulus* species recovered †*F. nevadensis* in a clade with *F. kansae*, *F. zebrinus*, *F. lima*, and *F. parvipinnis* (subgenera *Plancterus* and *Wileyichthys*). The recovery of the subgenus *Wileyichthys* as sister to all other Topminnows by RNA-seq genomic (Rodgers et al. 2018), again casts the relationships of this abundant fossil taxon into doubt.

†*Fundulus lariversi* from the Early to Middle Miocene Seibert Tuff (17 mya) of central Nevada (Lugaski 1977) is

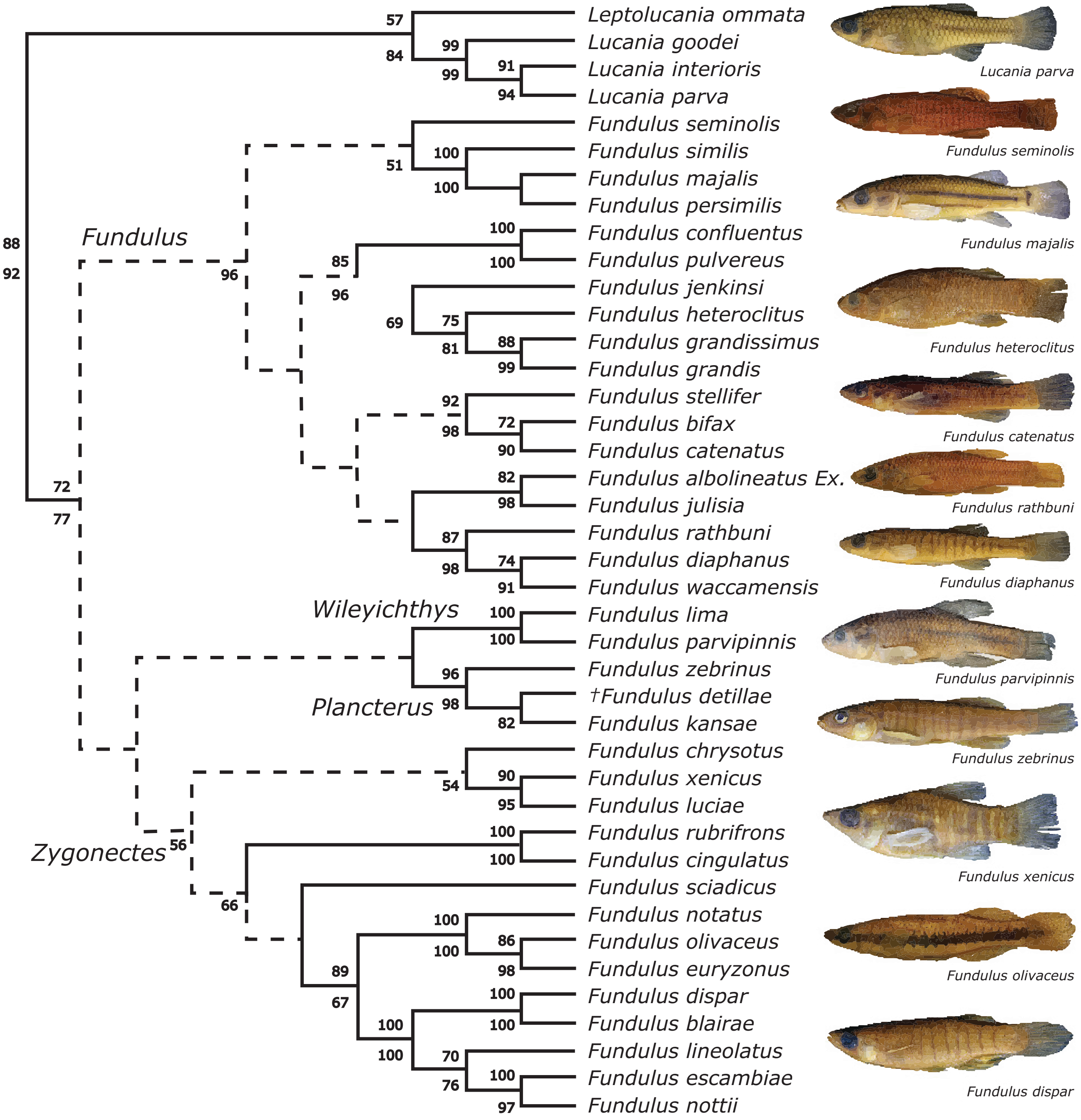

Figure 30.8. Phylogenetic hypothesis of relationships among Topminnows, Fundulidae, based on maximum-parsimony and partitioned mixed-model, maximum-likelihood analysis of available data to date. Numbers above branches are the percentage maximum-parsimony bootstrap support >50, and numbers below, the percentage maximum-likelihood bootstrap support >50. Dashed lines indicate clades present in the maximum-likelihood topology that are not present in all equally most-parsimonious trees in the maximum-parsimony analysis. Subgenera are given at major nodes in the phylogeny (redrawn from Ghedotti & Davis 2013).

the oldest fossil currently named in Fundulidae (Smith et al. 2002). Its relationships provide some information about the origins of the Fundulidae and western fossil fundulid diversity. †*Fundulus lariversi* was suggested as the ancestor of the poolfishes (Lugaski 1977), a group then thought to be closely related to fundulids. However, in a total-evidence phylogenetic analysis (Ghedotti & Davis 2017), this species was nested well within *Fundulus* in a clade with the *F. heteroclitus* group, the *F. confluentus* group, and *F. jenkinsi*. This placement in the context of divergence

Figure 30.9. A fossil specimen of †*Fundulus nevadensis* from the Miocene Truckee Formation (about 9.8 mya), near Hazen, Nevada, where this fossil is abundant (72 mm SL, LACM 17310; courtesy of MJG).

time analyses suggests that the family and the genus *Fundulus* originated in the Eocene or Oligocene (56–23 mya). In addition, the placement of †*F. lariversi* in a clade of eastern North American taxa demonstrates the significant taxonomic diversity present among the western fossil taxa.

The relationships of many western fossils remain unclear. †*Fundulus curryi* was proposed as the ancestor of poolfishes, genus *Empetrichthys* (family Goodeidae) (Miller 1950a). †*Parafundulus erdisi*, the type for the genus *Parafundulus* (Jordan 1924), first was placed in *Fundulus* (Miller 1950a) and later moved to *Empetrichthys* (Uyeno & Miller 1963). Additional fossil collections (≥3) were referred to *Fundulus* in Nevada, which are not currently assigned to a species (Smith et al. 2002). Given past confusion about relationships of western fossil cyprinodontiforms, it would be unsurprising if after further study of some of the fossil taxa currently recognized as *Fundulus* sp. might be assigned to another genus or to the goodeids rather than *Fundulus*.

MORPHOLOGY

Topminnows are morphologically rather typical of cyprinodontiform fishes. They are usually relatively small (adults, 27–180 mm SL) and have soft-rayed fins lacking spines, a single dorsal fin typically placed far back on the body, well-developed jaw teeth, primarily cycloid scales that extend onto the head, a relatively flat dorsal profile from the snout to the dorsal fin, and a rounded or truncate caudal fin (Figs. 30.1–30.4). The cephalic lateralis system is typically closed and discontinuous in Topminnows (described by Gosline 1949). Notably, the middle supraorbital canal is open in *Leptolucania*, *Lucania interioris*, *Fundulus luciae*, and *F. xenicus*, and the mandibular canal is open in *Leptolucania*, *Lucania*, and *F. xenicus* (Gosline 1949; Ghedotti & Davis 2013). Teeth in Topminnows are conical and in multiple rows, except in *Lucania parva* and *L. interioris* in which they have one row (Ghedotti & Davis 2013).

Swimming

Topminnows have swim bladders and can maintain neutral buoyancy while using their pectoral and other median fins for low speed swimming or maneuvering. Topminnows exhibit subcarangiform caudal fin-initiated locomotion for higher speed swimming (i.e., the wave increases quickly in amplitude toward the tail). Topminnows seem to exhibit higher swimming ability (e.g., 30.7 cm/s in *Fundulus notatus* and 43.4 cm/s in *F. zebrinus*) than poeciliids (e.g., 18.6 cm/s in *Gambusia gaigei*, Big Bend Gambusia, and 15.7 cm/s in *Poecilia latipinna*, Sailfin Molly) despite sharing the characteristics of a rounded caudal fin and relatively deep caudal peduncle (Leavy & Bonner 2009). Water velocity is associated with body depth in some Topminnows. In the *F. notatus* species group, individuals in upper reaches of flowing waters have deeper bodies than conspecifics farther downstream (Schaefer et al. 2011a). Either rapid evolution of body shape or ecophenotypic effects on body shape may occur in Topminnows; the Blackstripe Topminnow exhibits a shorter dorsal-fin base in lakes (including reservoirs) than in streams in Illinois (Welsh et al. 2013).

Contact Organs

Many Topminnows have contact organs on scales and fin rays composed of a bony extension of a scale or fin-ray segment covered in skin. The location and extent of contact organ development can vary interspecifically even among closely related species (Fig. 30.10). The contact organs differ structurally from the tubercles commonly seen in cypriniform fishes in lacking a thick keratinous surface. Contact organs presumably are sensory in function and allow the fishes to feel the position of their spawning partner. The organs become more prominent during the breeding season and may be present in both sexes, although male Topminnows typically have longer contact organs than females (Smith et al. 1969; Williams & Etnier 1982). Notably, the contact organs of male *F. lima* are extensively developed with some contact organs almost equaling the length of the scale itself (Myers 1930).

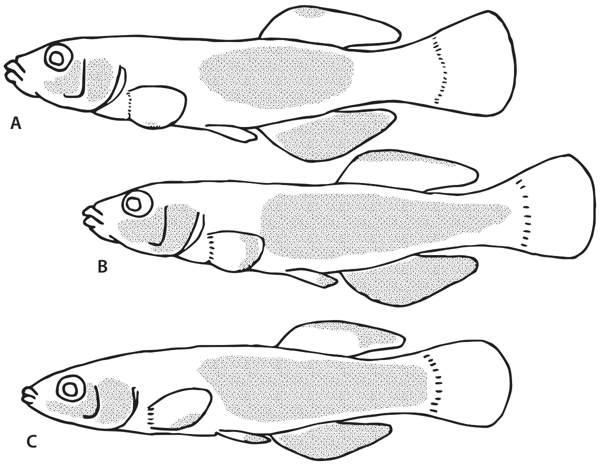

Figure 30.10. Extent of development of contact organs on breeding male Northern Studfish (A and B), *Fundulus catenatus*, and Southern Studfish (C), *Fundulus stellifer.* (A) Male Northern Studfish with usual extent of contact organs; (B) Male Northern Studfish with unusually extensive development (rarely seen); and (C) Male Southern Studfish with usual extent of contact organs (redrawn from Thomerson 1969).

Urogenital Sheath

Topminnows lack distinctive urogenital papillae; however, mature female Topminnows have a urogenital sheath. The sheath is a fleshy integumentary hood or tube that is diagnostic of the family and extends over the urogenital opening and frequently covers the anterior base of the anal fin (Wiley 1986; Ghedotti & Davis 2013; Fig. 30.11). The likely function of this structure is not extensively discussed in the literature but is reasonably assumed to be associated with oviposition (Breder & Rosen 1966). The sheath is most extensive in *Fundulus heteroclitus* and is small in *Leptolucania*, *Lucania*, and members of the *Fundulus* subgenus *Zygonectes*, including *F. xenicus* (Boschung & Mayden 2004; Ghedotti & Davis 2013). The sheath is not always present in mature females of *F. xenicus* and when present is notably small (Ghedotti & Davis 2013).

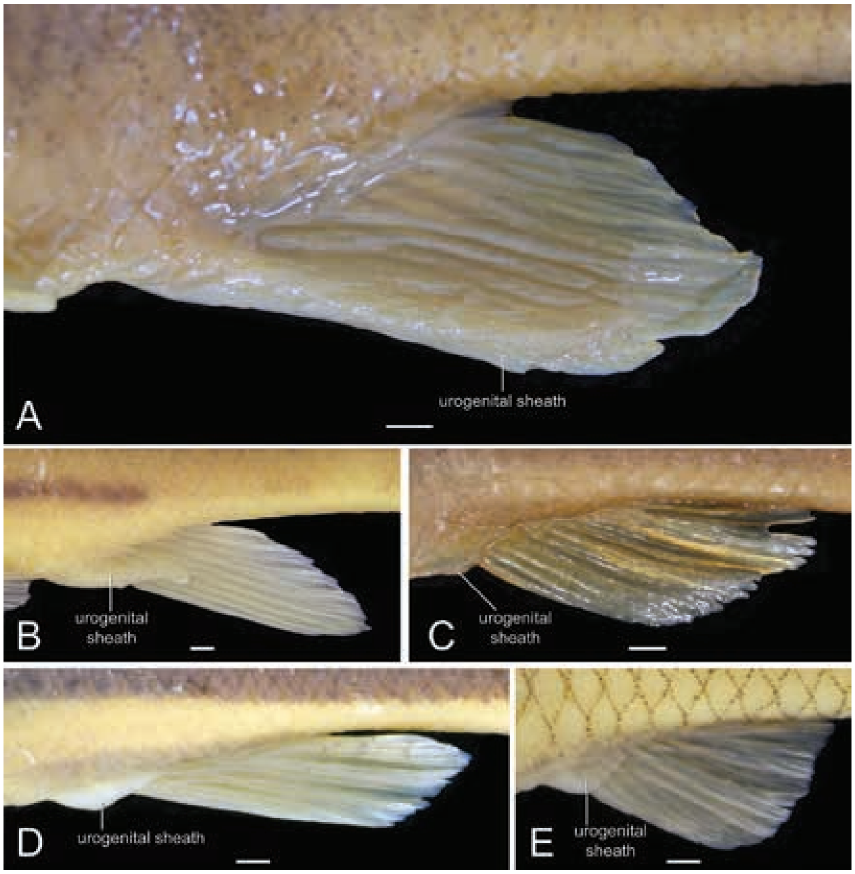

Figure 30.11. Mature female Topminnows, Fundulidae, although lacking distinct urogenital papillae, possess a urogenital sheath that is diagnostic of the family and presumably functions in oviposition. Left lateral views of the urogenital sheath in adult females of the (A) Mummichog, *Fundulus heteroclitus* (FMHH 60131), dotted line indicates margin of urogenital sheath; (B) Striped Killifish, *F. majalis* (FMNH 109220); (C) Blackstripe Topminnow, *F. notatus* (FMNH) 51470; (D) Blackspotted Topminnow, *F. olivaceus* (FMNH 60553); and (E) Rainwater Killifish, *Lucania parva* (FMHH) 113089. Scale bars = 1 mm (from Ghedotti & Davis 2013; used with permission of MJG).

Internal Anatomy

The viscera of Topminnows is similar to that of most other cyprinodontiforms, having a left-displaced atrium of the heart, a simple liver with the primary lobe on the left, a discrete spleen, and an elongate pancreas running along the left surface of the first part of the intestine (Ghedotti et al. 2004). In Topminnows, as in all cyprinodontiform fishes, the stomach is absent and the intestine immediately follows the esophagus (Babkin & Bowie 1928). In most Topminnows the intestine is simple with a single, simple anterior bend (Fig. 30.12A). In *Fundulus parvipinnis* and *F. lima*, however, the intestine has an additional dorsolateral bend (Fig. 30.12B), and in *Fundulus zebrinus* and *F. kansae*, the intestine is more complexly coiled (Parenti 1981; Fig. 30.12C).

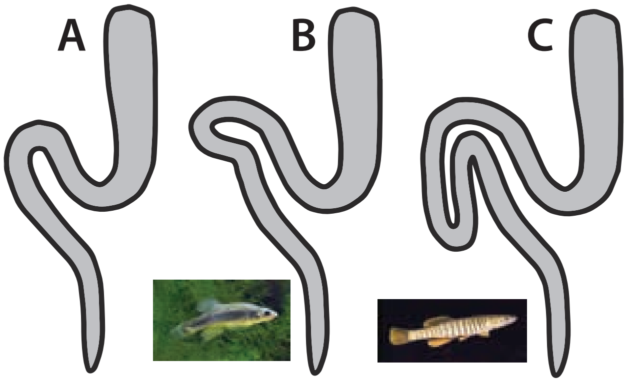

Figure 30.12. Intestinal configurations (ventral view) in Topminnows, Fundulidae. (A) The simplest condition seen in most fundulids is a single anterior bend after which the intestine continues mostly straight posterior to the anus; (B) in the California Killifish (*inset* B), *Fundulus parvipinnis*, and its sister-species, the Baja California Killifish, *F. lima*, the intestine additionally proceeds laterally, forming a right lateral bend; and (C) in the closely related Northern Plains Killifish (*inset* C), *Fundulus kansae*, and Plains Killifish, *F. zebrinus*, the intestine loops laterally as in the former two species but also loops posteriorly, producing a distinct posterior bend (redrawn from Ghedotti & Davis 2013; *inset* B, California Killifish, Marina Del Ray, Los Angeles, California; *inset* C, Northern Plains Killifish, Salt Creek, Missouri River drainage, Cooper County, Missouri; courtesy of John Brill).

Jaw Morphology

The family is diagnosed osteologically by a long, narrow ascending process of the premaxilla (pmx), a ventrally curved ventromedial process of the anterior head of the maxilla (mx), and an open first neural arch (not shown) (Wiley 1986; Ghedotti & Davis 2013; Fig. 30.13). The long premaxillary processes are connected to the ventral process of the anterior maxilla by a pronounced ligament. This arrangement allows the twisting of the maxilla to transfer additional force from lower-jaw lowering to the upper jaw as it is protruded to facilitate a forceps-like picking mode of feeding (Hernandez et al. 2008). Topminnows feed by biting or picking in combination with some minor suction feeding (Hernandez et al. 2008). The adductor mandibulae in Topminnows is divided into distinct lateral (A1), middle (A2 and A2/3), medial (A3 and A2/3), and anterior (Aω) components; the lateral division assists in upper-jaw protrusion, and the other components are involved in jaw retraction and closing (see general description by Hertwig 2008 and more specific coverage across Topminnows by Ghedotti & Davis 2013).

Early Development

Early development is relatively typical for cyprinodontiform fishes and is described in detail for *Fundulus xenicus* (Koenig & Livingston 1976; Cunningham & Balon 1985, 1986), *F. heteroclitus* (Oppenheimer 1937; Armstrong & Child 1965; Fig. 30.14), *Lucania goodei* (Crawford & Balon 1994bc), and *L. parva* (Crawford & Balon 1994ac; Fig. 30.15). Interestingly, the integumentary pigment cells, derived from neural crest tissue, in *F. heteroclitus* retain some degree of amoeboid mobility post-hatching and change position in newly hatched fish. The cells become immobile, however, by adulthood (Gilson 1926). The rate of ossification of cranial skeletal elements during development in *F. xenicus* (and presumably other Topminnows) is affected by environmental conditions that affect the rate of hatching, such as temperature and whether or not the eggs are submerged. As a result, embryos may hatch with differing degrees of cranial calcification (Cunningham & Balon 1986). Temperature also affects the number of vertebrae and fin rays that develop in *F. majalis* (Fahy 1972, 1979, 1980, 1982). As in other vertebrates, retinoic acid concentration is important in patterning

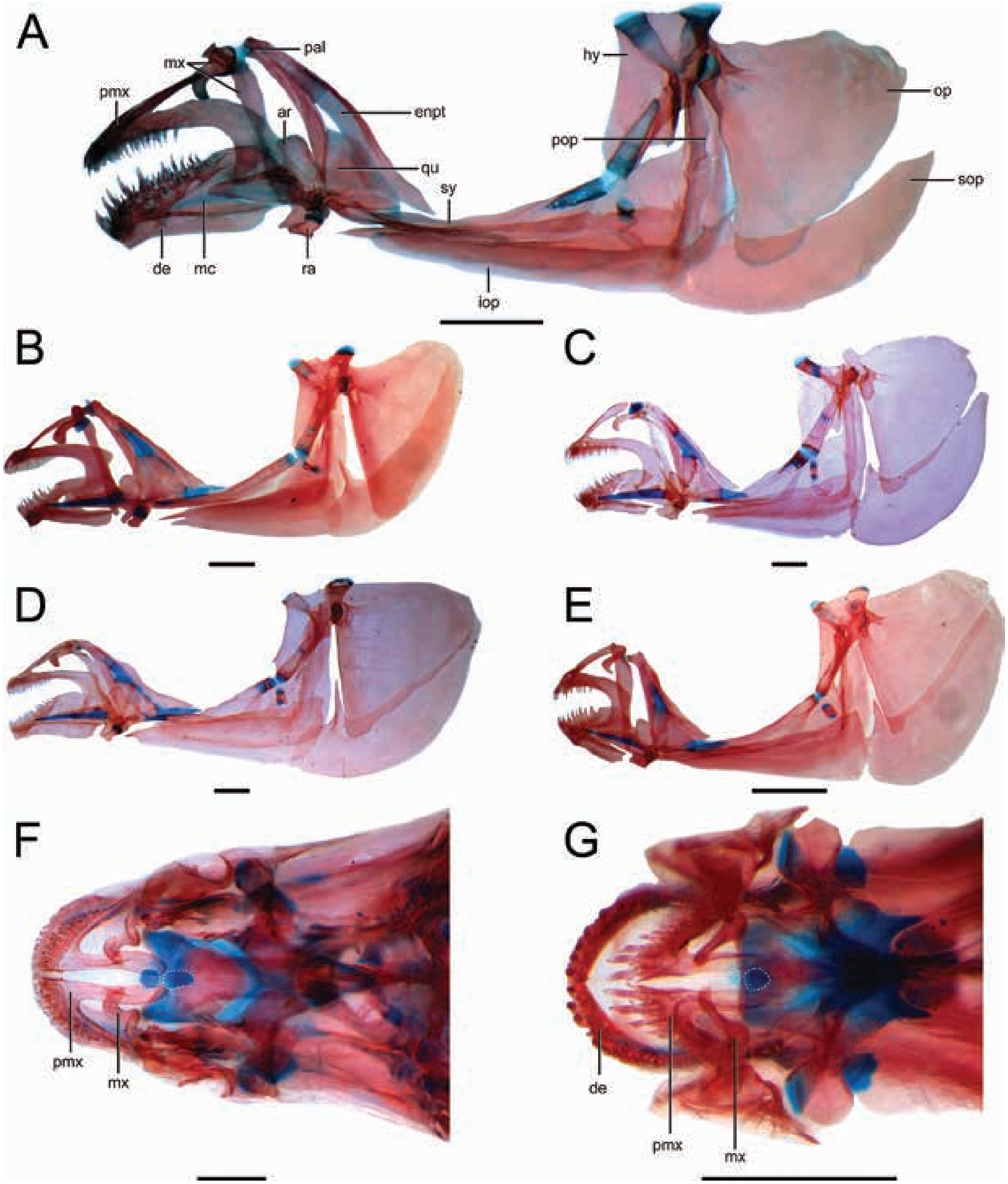

Figure 30.13. Aspects of the jaw morphology are diagnostic of the Topminnows, Fundulidae. Long, ascending premaxillary processes connected by a ligament to the ventral process of the anterior maxilla allows twisting of the maxilla that in conjunction with the lower jaw acts to increase force, an important adaptation in species that feed primarily by biting or picking (note the conical teeth lining both jaws). Medial view of right jaws, suspensoria, and opercular series in cleared and stained (A) Blackstripe Topminnow, *Fundulus notatus* (KU 18021); (B) Northern Plains Killifish, *F. kansae* (KU 14726); (C) Mummichog, *F. heteroclitus* (KU 15351); (D) Longnose Killifish, *F. similis* (KU 1282); and (E) Bluefin Killifish, *Lucania goodei* (KU 17993). The interhyal was left attached to the suspensorium. Dorsal view of snout region in cleared and stained (F) Longnose Killifish (KU 12827) and (G) Bluefin Killifish (KU 17993). Rostral cartilages are outlined with white dotted lines. Anterior is at left. Scale bars = 1 mm (from Ghedotti & Davis, 2013; used with permission of MJG). Key: ar = articular; de = dentary; enpt = endopterygoid; hy = hyomandibula; iop = interopercle; mc = Meckel's cartilage; mx = maxilla; op = opercle; pal = palatine; pmx = premaxilla; pop = preopercle; qu = quadrate; ra = retroarticular; sop = subopercle; sy = symplectic.

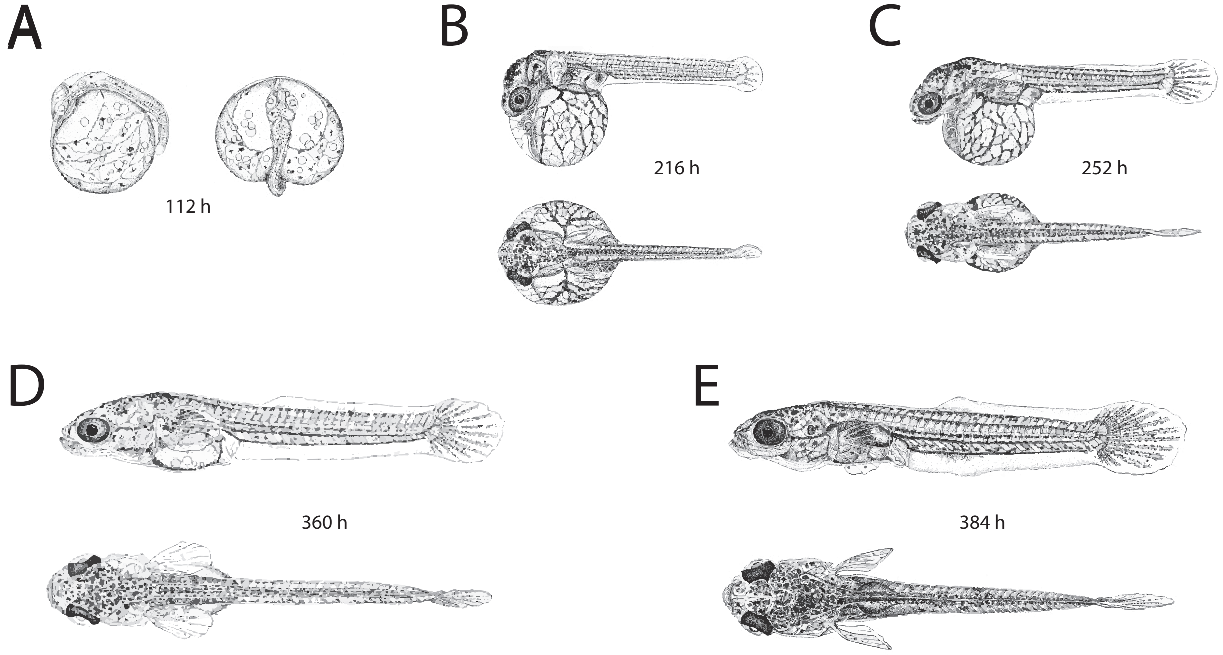

Figure 30.14. Embryos and larvae of the Mummichog, *Fundulus heteroclitus* (reared at 20°C; times are hours post-fertilization; magnification times 22). (A) Embryo at 112 h, lateral view (*left*), dorsal view (*right*), stage 27; (B) Embryo at 216 h just before hatching, stage 33; (C) Free embryo (newly hatched) at 252 h, stage 35; (D) Embryo at 362 h with yolk sac almost absorbed, stage 38, lateral view (*upper*), dorsal view (*lower*); (E) Larvae at 384 h, yolk sac absorbed, stage 39, lateral view (*upper*), dorsal view (*lower*). Stages 1–39 are detailed by Armstrong & Child (1965) (redrawn from Armstrong & Child 1965).

fundulid anatomy during development. In *F. heteroclitus*, however, exposure to excess retinoic acid during gastrulation caused deletion of the anterior cranial skeleton and duplication of pectoral fins (Vandersea et al. 1998ab).

GENETICS

Karyology

Karyotypes are known for 30 Topminnow species: 28 species of *Fundulus* (Ghedotti & Davis 2013) and two species of *Lucania* (Berdan et al. 2014). Most species have the diploid number (2n) of 48 with 24 pairs of acrocentric or nearly acrocentric chromosomes, the typical karyotype for cyprinodontiforms (Chen 1971; Gold et al. 1980). Variation involves reductions in number and the presence of long metacentric chromosomes not present in species with 2n = 48. This suggests evolution by centric fusion of once separate chromosomes (Chen 1971; Howell & Black 1981; Berdan et al. 2014). Other evolutionary factors (e.g., pericentric or paracentric inversions) are indicated by species-specific differences in arm lengths (Chen & Ruddle 1970; Chen 1971). Intraspecific population differences in arm-lengths occurred in *F. parvipinnis* from three different locations in California (Chen 1971; Kornfield 1981).

Two closely related groups of species, the *Fundulus notatus* species group and the *Fundulus chrysotus-F. xenicus-F. luciae* clade, are interesting examples of interspecific variation in chromosome number. The Blackspotted Topminnow, *Fundulus olivaceus*, and *F. euryzonus* have the ancestral 2n = 48, but *F. notatus* has 2n = 40 in most of its range (Setzer 1968, 1970) and 2n = 44 in the Tombigbee River, Alabama and Mississippi (Howell & Black 1981). In the *F. chrysotus* clade, the sister-species *Fundulus xenicus* and *F. luciae* have the lowest number of chromosomes (2n = 32) known for Topminnows and the next lowest (2n = 34) is known only in the Golden Topminnow, *Fundulus chrysotus* (Ghedotti & Davis 2013). Other deviations from the typical 2n = 48 include 2n = 46 in *Fundulus sciadicus*, Plains Topminnow, and 2n = 44 in *Lucania parva*, *Fundulus cingulatus*, Banded Topminnow, and the *F. nottii* species group.

Standard karyotypes and analysis of gene order provided unusually high resolution of chromosome structure in *Lucania parva* and *L. goodei* (Berdan et al. 2014). *Lucania goodei* has 24 pairs of acrocentric chromosomes (2n = 48), and *L. parva* has 22 pairs of acrocentrics and

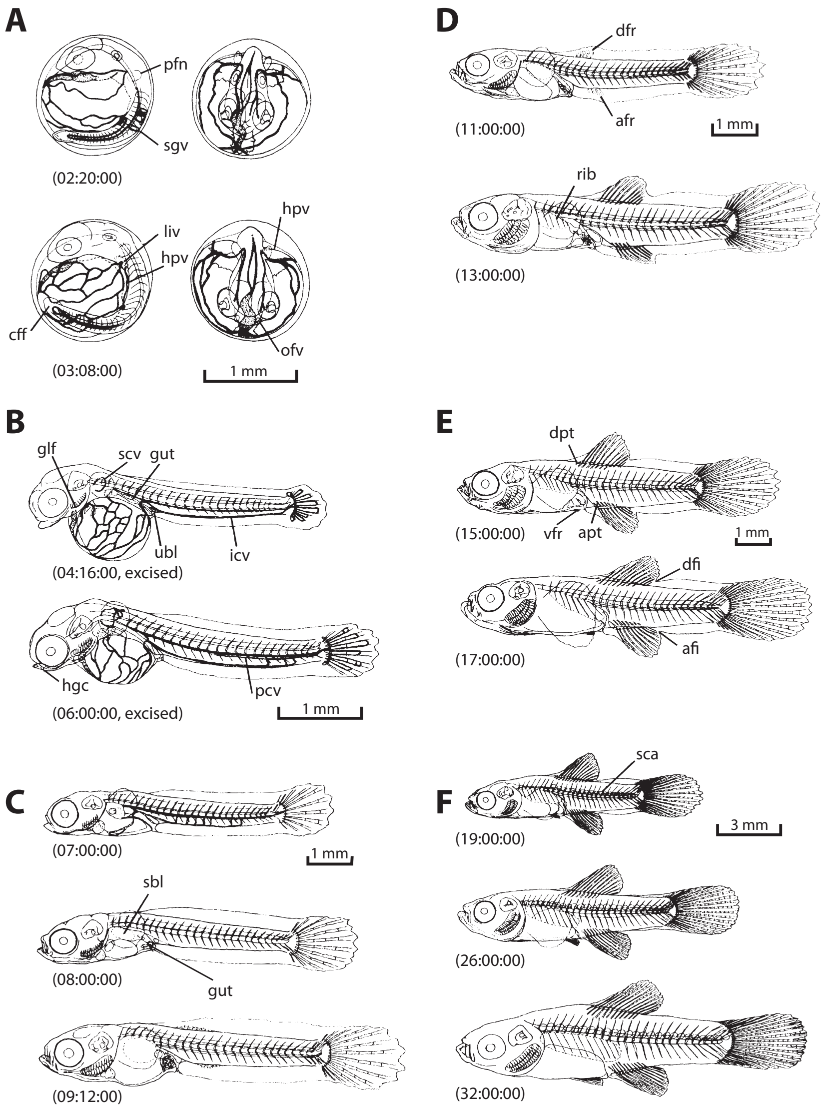

Figure 30.15. Embryos and larvae of the Rainwater Killifish, *Lucania parva* (reared at 25°C; 00:00, days:h): (A) Embryos at days 2–3 post-fertilization (left lateral view, right dorsal view); (B) Lateral view of embryos excised from the egg at day 4; (C) Free embryo (newly hatched) at day 7 and larvae at days 8 and 9; (D) Larvae at days 11 and 13; (E) Larvae at days 15 and 17; (F) Larvae at days 19 and 26 and juvenile at day 32. Scale formation began posterior of midbody and proceeded anteriorly and posteriorly. Only single horizontal rows of scales are indicated, but successive dorsal and ventral rows formed concurrently albeit at slightly slower rates than the midline. Key: afi = anal fin fold indentation; afr = anal-fin ray; apt = anal pterygiophore; cff = caudal fin fold; dfi = dorsal fin fold indentation; dfr = dorsal-fin ray; dpt = dorsal pterygiophore; glf = gill filament; gut = simple gut in B, convoluted gut in C; hgc = hatching gland cell; hpv = hepatic vein; icv = inferior caudal vein; liv = liver; ofv = olfactory vesicle; pcv = profundal caudal vein; pfn = pectoral fin; rib = rib; sbl = swim bladder; sca = scale; scv = subclavian vein; sgv = segmental vessel; ubl = urinary bladder; vfr = pelvic-fin ray (redrawn from Crawford & Balon 1994a).

one large metacentric pair (2n = 46). Linkage maps based on single-nucleotide-polymorphisms and other considerations show that the ancestral condition was 2n = 48 and that the 2n = 46 of *L. parva* is a result of centric fusion between two specific chromosomes seen in *L. goodei*. Interestingly, linkage groups appear highly conserved across widely divergent taxa. The linkage group on each *Lucania* chromosome was syntenic to (represented the same genes as) a specific chromosome in the Medaka (*Oryzias latipes*, order Beloniformes, family Adrianichthyidae, Ricefishes). Obversely, the sequential order of genes in the same linkage group differed markedly between *Lucania parva* and *L. goodei*. Only 50% of the linkage groups showed a significant rank order correlation between the two species.

A similarly constructed linkage map for *F. heteroclitus* (2n = 48) suggests markedly divergent chromosomal structure from *Lucania*. The map shows the expected number of 24 linkage groups (Waits et al. 2016). However, the degree of synteny with *Oryzias* was high, but considerably less than the 1:1 relationship between *Lucania* and *Oryzias*. For example, chromosome 2 in *Oryzias* carries none of the loci used in constructing the linkage map for *F. heteroclitus*, and 12 chromosomes carried loci from ≥2 linkage groups in *F. heteroclitus*. The discordant results for the two Topminnows warrant further investigation.

Sex Determination

Topminnows typically exhibit no evidence of sexually dimorphic chromosomes, but two exceptions exist, both involving male heterogamety (XX-XY systems). One is *Fundulus diaphanus*, in which the X chromosome has secondary constrictions (i.e., other than centromeric) not seen in the Y (Chen & Ruddle 1970). The other exception is *F. parvipinnis*, which shows geographic variation. One population exhibits sex-associated chromosome morphology involving two biarmed X chromosomes in females and one in males (Chen & Ruddle 1970), but another population lacks this distinctive karyotype (Kornfield 1981).

Although the only examples of karyotypic heterogamety in Topminnows are the two XX-XY systems just described, inferences regarding cryptic heterogamety (i.e., not expressed in gross chromosome morphology) can be derived from interspecific crosses indicating the Haldane rule is in effect; that is, if F_1 hybrids of either sex are rare or inferior with reduced viability or sterility then that sex is likely to be heterogametic. Haldane rule indications of male heterogamety occur in crosses between *Lucania parva* and *L. goodei* (Fuller 2008). Hybrid males are 50% sterile in crosses of female *Lucania parva* and male *L. goodei* but fully fertile in the reciprocal cross. As such, cryptic heterogametry occurs in male *L. goodei* but apparently not in male (or female) *L. parva*. Male heterogamety is also indicated in crosses of *Fundulus grandis* × *F. pulvereus* and *Fundulus chrysotus* × *F. kansae*; without giving sample sizes or which reciprocal crosses were attempted, Drewry (1962) commented that male hybrids are sterile. On the other hand, female heterogamety is indicated for the *F. notatus* species group (Vigueira et al. 2008). Female offspring of *Fundulus euryzonus* × *F. notatus* and *Fundulus euryzonus* × *F. olivaceus* crosses are sterile, but the males are not. This effect was not expressed in the F_1 hybrid sex ratio, which was close to 1:1 in all interspecific crosses. The sex difference in fertility was not seen in *Fundulus notatus* × *F. olivaceus* hybrids, possibly because they are more recently diverged species (Vigueira et al. 2008).

The possibility of temperature-dependent sex determination (TSD) in Topminnows is explored only in a New York population of *Fundulus heteroclitus*. Field collections and experimental tests designed to test for TSD found no deviation from a 1:1 sex ratio in a region where TSD was demonstrated for another atherinomorph species, the Atlantic Silverside, *Menidia menidia* (Conover & Demond 1991). The authors emphasized that further work might reveal TSD in other populations of *F. heteroclitus*, and they suggested that the frequency of TSD in fishes is under investigated. Based on *Menidia*, populations most likely to show TSD are those at northern latitudes with short growing seasons and those, like *F. heteroclitus*, in which females are larger than males (Conover & Demond 1991). The advantage of TSD in such situations is multifactorial, but one factor is that female offspring produced early in the year have a relatively long growing season and thereby are larger and more fecund at breeding age than females hatched later in the year. This type of TSD is more likely to occur in species in which male fitness is less affected by body size (Conover & Demond 1991).

Clonality

Clonal all-female forms originating from hybrids between *Fundulus diaphanus* and *F. heteroclitus* occur in two brackish water situations in Nova Scotia (Dawley 1992). This was initially discovered in a location where F_1 hybrids and both parental species were common, but males were absent (Fritz & Garside 1974). Another study discovered all-female hybrids and both parental species at a second locality 125 km away and determined that they exhibited either gynogenesis or true parthenogenesis (Dawley 1992). Gynogenesis was assumed because true parthenogenesis is not reported in fishes. In gynogenesis, the female produces diploid rather than haploid eggs, and embryogenesis is stimulated by contact with sperm from a male of a normal, bisexual (gonochoristic) species, presumably *Fundulus diaphanus* or *F. heteroclitus* in this example. Sperm stimulate development but make no paternal genetic contribution to the offspring, which are replicates of the mother. Most individuals of the unisexual *Fundulus diaphanus-F. heteroclitus* population are diploid, but occasional egg-sperm syngamy (fusion of two nuclei) has produced a few triploids (Dawley et al. 1999).

In unisexual forms originating from hybridization, each hybrid origin results in a clone that perpetuates itself independently of clones originating from other hybrid individuals. For the unisexual *Fundulus diaphanus-F. heteroclitus*, allozyme genotypes and tissue-grafting experiments suggested that most individuals in the two Nova Scotia populations represent a single clone (Dawley 1992; Dawley et al. 2000). This is potentially at variance with results from microsatellite DNA that revealed ≥15 clones with the two populations differing in the predominant clone (Hernandez Chaves & Turgeon 2007). Obversely, all of those clones carried the same mtDNA haplotype from *F. diaphanus* (Hernandez Chaves & Turgeon 2007), a result consistent with a single hybrid origin from a cross between a female *Fundulus diaphanus* and a male *F. heteroclitus*. The variety of microsatellite clones might have arisen by mutations in a single ancestral clone. Microsatellites are rapidly evolving, noncoding DNA segments that are not expected to influence allozyme genotypes or histocompatibility. The predominance of different microsatellite clones in the two locations supporting unisexual *Fundulus* might reflect genetic drift in frequencies of multi-locus genotypes that arose by mutation. Nevertheless, the number of hybrid origins of unisexuality in this system remains an open question (Hernandez Chaves & Turgeon 2007).

Artificial Interspecific Hybridization

Many combinations of Topminnow species produced hybrid offspring in the laboratory (Hubbs & Drewry 1959; Drewry 1962, 1967; Fritz & Garside 1974; Fuller 2008; Vigueira et al. 2008). Successfully hatched offspring can be produced from nearly any attempted intrageneric combination (Hubbs 1967). The major exceptions seem to be crosses involving females of the closely related *Fundulus majalis* and *F. similis*. In crosses between female *Fundulus majalis* and male *F. heteroclitus* or *F. diaphanus*, the embryos reached the eyed stage but failed to hatch; however, the reciprocal crosses resulted in successful hatching (Newman 1914). The same results occurred in the reciprocal crosses of *Fundulus similis* × *F. grandis* (Hubbs & Drewry 1959). Other examples of differences are indicated in developmental success between the reciprocal male-female crosses of some species-pairs, but the results are inconclusive because of small sample sizes. For example, female *Fundulus grandis* × male *F. xenicus* hybrids failed to hatch, but the reciprocal cross resulted in survival of some offspring through the larval stage, but these results were based on sample sizes of six and 13 fertilized eggs (Hubbs & Drewry 1959). In the same study, intergeneric *Fundulus grandis* × *Lucania parva* hybrids from both reciprocal crosses survived through the yolk-sac stage.

With four exceptions, experiments with reproductive compatibility among fundulid species provided no information on hybrid sex ratios or fertility. Exceptions include two studies of species in the *Fundulus notatus* group (Thomerson 1967; Vigueira et al. 2008), a study of *Fundulus grandis* × *F. pulvereus* hybrids (Drewry 1962), and one of hybridization between *Lucania parva* and *L. goodei* (Fuller 2008). All four studies found viable and fertile hybrids, but there is evidence of partial reproductive isolation (see sex determination and reproductive isolation subsections, this section).

Interspecific Hybridization in the Wild

Natural interspecific hybridization is interestingly scarce for a variety of species-pairs of *Fundulus* with broad sympatry, despite the relative ease of obtaining hybrids from such pairs in the laboratory. This is true for coastal and interior species in North America. A coastal exception is ongoing hybridization with evidence of backcrossing between the more freshwater species *Fundulus diaphanus* and the euryhaline *F. heteroclitus* at several localities with intermediate salinity in the Nova Scotia area (Hernandez Chaves & Turgeon 2007). With this exception, no reports are apparently available of hybridization for broadly sympatric Atlantic Ocean and Gulf of Mexico species. These include the following sets: Atlantic Coast, *Fundulus heteroclitus*, *F. luciae*, *F. majalis*, and *F. pulvereus*; coastal Gulf of Mexico, *Fundulus grandis*, *F. xenicus*, *F. similis*, and *F. pulvereus*. Drewry (1962) found no discernible morphological hybrids in a search among several thousand individuals of *Fundulus grandis* and *F. pulvereus* from a site of co-occurrence over multiple years in Texas. These examples of geographically overlapping species involve members of different subgenera or highly divergent clades within subgenera (Ghedotti & Davis 2013). Similarly, and except for the *F. notatus* group, nearly all instances of sympatry among species of *Fundulus* in the North American interior involve members of divergent species groups or different subgenera. The apparent scarcity of hybridization likely reflects long evolutionary histories with ample time for ecological and behavioral divergence.

In contrast, naturally occurring hybrids and local genetic introgression are not uncommon in studies of closely related Topminnows. Two generalities from these situations are that areas of hybridization (hybrid zones) usually lie along clear ecological gradients where species habitat affinity or fitness differences contribute to reproductive isolation and that reproductive isolation (where characterized) is incomplete but the abundance and spatial extent of hybrids are limited. Documented examples include the following coastal situations (see next four subsections for details): hybrid zones between northern and southern subspecies of *F. heteroclitus* on the Atlantic Coast in the New Jersey area, and *F. parvipinnis* along coastal California-Baja California; hybrid zones in Florida between *Lucania goodei* and *L. parva*, *Fundulus grandis* and *F. heteroclitus*, and *F. majalis* and *F. similis*. Hybrid zones in the interior of North America involve western and eastern subspecies of *F. diaphanus* in the Great Lakes-St. Lawrence River system, and multiple examples of contact and hybridization among the three species of the *Fundulus notatus* species group (*Fundulus notatus*, *F. olivaceus*, and *F. euryzonus*).

Ancient genetic interaction among members of the *F. notatus* group is indicated by conflicting results from nuclear and mtDNA analyses of phylogenetic relationships. Nuclear genes indicate that *F. olivaceus* is sister to *F. euryzonus*, but the mtDNA of *F. olivaceus* is sister to that of *F. notatus* (Duvernell et al. 2013). This is an example of deep introgression (Near et al. 2011) in which introgressive hybridization was sufficiently long ago that mtDNA evolution has resulted in reciprocal mtDNA monophyly between *Fundulus notatus* and *F. olivaceus*.

Reproductive Isolation

Tests of interspecific mate preferences among Topminnows are few. One exception is a study of mate choice in multiple-male and multiple-female mixtures of two subspecies of *F. heteroclitus* (McKenzie 2013). A genetic analysis of fertilized eggs collected over 32 days revealed a strong bias toward con-subspecific matings. Other studies of mate preferences involved no-choice experiments measuring the relative propensity for isolated homospecific and heterospecific pairs to undergo courtship or spawning activity. These included studies of two sets of closely related species: *Lucania parva* and *L. goodei* and the *Fundulus notatus* species group, *Fundulus notatus*, *F. olivaceus*, and *F. euryzonus*. In both groups heterospecific pairs mate and produce offspring, but homospecific pairs mate more quickly and produce more offspring (Fuller et al. 2007; Vigueira et al. 2008; Berdan & Fuller 2012).

Mate choice by *Lucania* males is a factor in behavioral isolation as demonstrated in a series of no-choice mating studies. Male *Lucania goodei* and *L. parva* courted females of both species, but they more readily courted conspecific females (Gregorio et al. 2012). Reinforcement of preference was evidenced as greater discrimination in males from areas of sympatry compared with those from areas of allopatry. Degree of preference did not differ between males of the two species. Asymmetry in preference occurred in another study, however, in which a single female was simultaneously presented to a male of each species (Berdan & Fuller 2012). In this situation, *L. goodei* males only courted conspecific females, but male *L. parva*, although preferring conspecific mates, readily courted and mated with *L. goodei* females. This asymmetry made sense in terms of fitness (Berdan & Fuller 2012) because offspring from crosses between male *Lucania goodei* and female *L. parva* had lower fitness than those from the reciprocal cross (Fuller 2008). A further indication of male preference for conspecific females is that male-male aggression was greater in the presence of a conspecific female than in the presence of a heterospecific female (Fig. 30.16). This effect was greater for *Lucania goodei* than *L. parva*, a result that, as mentioned for the mate-choice results, makes sense in light of lower fitness of offspring from male *L. goodeia* by female *L. parva* crosses.

Postzygotic reproductive isolation can be expressed as barriers to gene flow based on endogenous selection (genetic malfunction of hybrids) or exogenous selection enforced by the environment. Endogenous selection occurs when hybrid inferiority is not a function of the external environment. An example is the previously mentioned sterility of female hybrids from crosses of *Fundulus euryzonus* with *F. notatus* or *F. olivaceus* (see sex determination subsection, this section; Vigueira et al. 2008). Exogenous selection occurs when the external environment influences hybrid fitness. The roles of endogenous and exogenous selection were explored in a laboratory study of the euryhaline *Lucania parva* and the freshwater *L. goodei* (Fuller 2008). Fertilized eggs and progeny from various types of crosses (F_1, F_2, and backcrosses) were reared in conditions

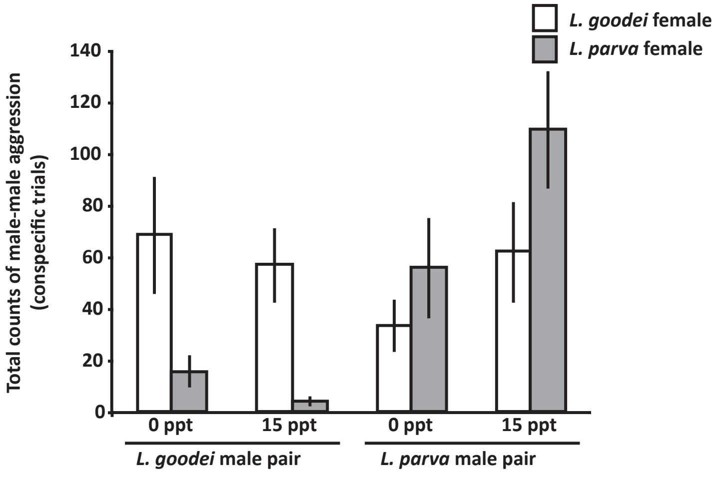

Figure 30.16. Total aggression between pairs of breeding conspecific males of the Rainwater Killifish, *Lucania parva*, and Bluefin Killifish, *L. goodei*, exposed to a conspecific or heterospecific breeding female at two different salinities. Error bars ± SE (sample sizes given in Berdan & Fuller 2012; redrawn from Berdan & Fuller 2012).

of low and high salinity (0–8 versus 15–20 ppt). Only endogenous selection was involved in early egg survival (embryogenesis and survival to day 5 post-fertilization). Male F_1 hybrids from one of the reciprocal crosses (female *Lucania parva* × male *L. goodei*) were 50% sterile and F_2 progeny (from $F_1 \times F_1$ crosses) and backcrosses to *L. goodei* had lower egg survival than F_1 hybrids and offspring from conspecific matings; salinity had no effect. Endogenous and exogenous selection was implicated for hatching success in embryos surviving >5 days. Hatching success was reduced in F_2 progeny and in backcrosses to *L. goodei*, and the effect was more pronounced at high salinity. Hence, natural introgression should be asymmetrical with the freshwater form, *L. goodei*, introgressing more readily into the euryhaline *L. parva* than vice versa (Fuller 2008). Differences in karyotype and in gene order within chromosomes (see karyology subsection, this section) likely have played a role in reproductive isolation and ecological divergence of these two species (Berdan et al. 2014).

A topic of interest in evolutionary biology regards the roles of prezygotic versus postzygotic isolation as the factor precipitating speciation. Two forms in alloparty might, by chance or as a result of local adaptation, show only one form or both forms of isolation. In *Lucania*, saltwater and relatively freshwater populations of *L. parva* exhibit partial postzygotic isolation (reduced hatching success in interpopulational crosses) in an apparent absence of prezygotic isolation (Kozak et al. 2012). This suggests that postzygotic selection likely preceded the evolution of previously described prezygotic isolation (conspecific mate preferences) between the freshwater species, *Lucania goodei*, and a saltwater ancestor like *L. parva*. Adaptation to different salinity regimes might be an important factor in initiating speciation (Kozak et al. 2012).

The strength of reproductive isolation in the *Fundulus notatus* complex varies depending on the situation. Analyses of mtDNA, microsatellite DNA loci, and nuclear introns in contact zones between the wide-ranging species *Fundulus notatus* and *F. olivaceus* revealed assemblages comprising the two parental species, F_1 hybrids, and multigenerational backcross progeny (Duvernell et al. 2007, 2013). The contact zones occur in the transition between small tributaries supporting *F. olivaceus* and mainstem habitats supporting *F. notatus*, although two of 10 contact zones showed the reverse condition. Hybridization is usually rare (Thomerson 1966; Duvernell et al. 2013), but mixed genotypes occur at most sites of contact; frequencies range from zero to near random-mating expectations (Schaefer et al. 2011b; Duvernell et al. 2013). Amount of mixing varies negatively with steepness of the environmental gradient from tributary headwaters to the downstream confluence (Schaefer et al. 2011b). Introgression of mtDNA sometimes extends well beyond the zone of contact between the two species, but nuclear-gene introgression usually is restricted to the contact zone and does not extend beyond expectations for individual dispersal (Duvernell et al. 2007). These results, together with the previously discussed interbreeding experiments with the *F. notatus* group (Vigueira et al. 2008), suggest that mate-choice preferences and postzygotic selection act to reduce levels of hybridization and nuclear genetic introgression in most areas of contact.

The presence of reversed *Fundulus notatus* × *F. olivaceus* hybrid zones (*F. olivaceus* downstream and *F. notatus* upstream) raises the question of whether the two orientations differ in genetic structure and inferred dynamics of introgression (Schaefer et al. 2016). A genomic analysis of single-nucleotide polymorphisms in the Cossatot River (normal orientation), Arkansas, and the Glover River (reversed), Oklahoma, found similar structures (Schaefer et al. 2016). Both hybrid zones were dominated by individuals of mixed ancestry (about 50%), primarily F_1 and F_2 individuals, and they both showed the same pattern of asymmetry in introgression (higher in *F. notatus*). Simulation modelling suggested that the reversed hybrid zones originate primarily as a result of neutral stochastic processes together with strong endogenous factors (genetic) and weak exogenous factors (environment).

Contact with limited hybridization also occurs between *Fundulus olivaceus* and the third species of the *F. notatus* complex, *F. euryzonus*, an endemic in the Lake Ponchartrain drainage, Mississippi and Louisiana. The structure of the contact zone in a tributary of Lake Ponchartrain differed from those between *Fundulus olivaceus* and *F. notatus* in manifesting no clear upstream-downstream pattern (Schaefer et al. 2009). Instead, local habitat features (species diversity, canopy cover, emergent vegetation) seemed more important in structuring contact between the two species. Analysis of mtDNA and four nuclear genes in 244 individuals revealed only nine with hybrid ancestry and only one of those was a putative F_1 hybrid. Other individuals with mixed ancestry included four *Fundulus euryzonus* and one *F. olivaceus* with heterospecific mtDNA and three individuals heterozygous at one to two nuclear loci diagnostic for the two species. In the Lake Ponchartrain drainage, all three species of the *F. notatus* complex occur together at several sites, but hybridization at these sites is unverified (Suttkus & Cashner (1981).

Allopatric Divergence and Secondary Contact

A number of Topminnow species occur over wide geographic areas with regional differences in both historical and present environmental regimes. The associated patterns of genetic variation are explored, especially thoroughly in *Fundulus heteroclitus* on the Atlantic Coast. A northern subspecies, *Fundulus heteroclitus macrolepidotus* (Newfoundland to New Jersey), and a southern subspecies, *F. h. heteroclitus* (New Jersey to northern Florida), intergrade in coastal areas of New Jersey (Fig. 30.17), forming clines documented across a variety of characters. The characters include, among many others (McKenzie 2013), allozymes (Ropson et al. 1990), egg morphology (Morin & Able 1983), adult morphology (Able & Felley 1986), mitochondrial DNA (Gonzalez-Vilasenor & Powers 1990; Bernardi et al. 1993; McKenzie 2013), microsatellite DNA loci (Adams et al. 2006; Duvernell et al. 2008), and a large number of nuclear single-nucleotide polymorphisms (SNPs) (Strand et al. 2012; Fig. 30.18). The cline is replicated in tributaries to Chesapeake Bay, Virginia, and Delaware Bay, Delaware, where intergrade populations separate bay populations of *F. h. heteroclitus* from tributary populations of *F. h. macrolepidotus* (Smith et al. 1998; Whitehead et al. 2011a) that likely are relicts of Pleistocene times when the zone of contact between the two forms was shifted farther southward (Able & Felley 1986; Smith et al. 1998; Whitehead et al. 2011a). Given the amount of evidence for evolutionary divergence in these two forms, under several species concepts they would be treated as two separate species with relatively narrow zones of contact and hybridization (see clines and natural selection, and functional genomics and gene regulation subsections, this section; salinity tolerance subsection, physiology section).

Calculations based on cline width for microsatellite DNA loci and the assumption of selective neutrality in the geographic transition between the northern and southern subspecies of *F. heteroclitus* indicated secondary contact about 15,000 years ago about the time of the last glacial retreat (Adams et al. 2006). The level of mtDNA divergence between the two forms suggested secondary contact occurred after about 1 million years of allopatric divergence (Bernardi et al. 1993). The pattern of mtDNA variation suggested the northern form survived glaciation in multiple northern refugia and expanded outward from these refugia in the post-Pleistocene (Haney et al. 2009).

Four other pairs of fundulid taxa display patterns of genetic variation explained by allopatric divergence and post-Pleistocene secondary contact. Mitochondrial DNA variation in *F. parvipinnis* on the California and Baja California Pacific Coast shows clinal variation with a break on the Baja Peninsula that approximates the geographic limits of a northern subspecies, *Fundulus parvipinnis parvipinnis*, and a southern subspecies, *F. p. brevis* (Bernardi & Talley 2000).

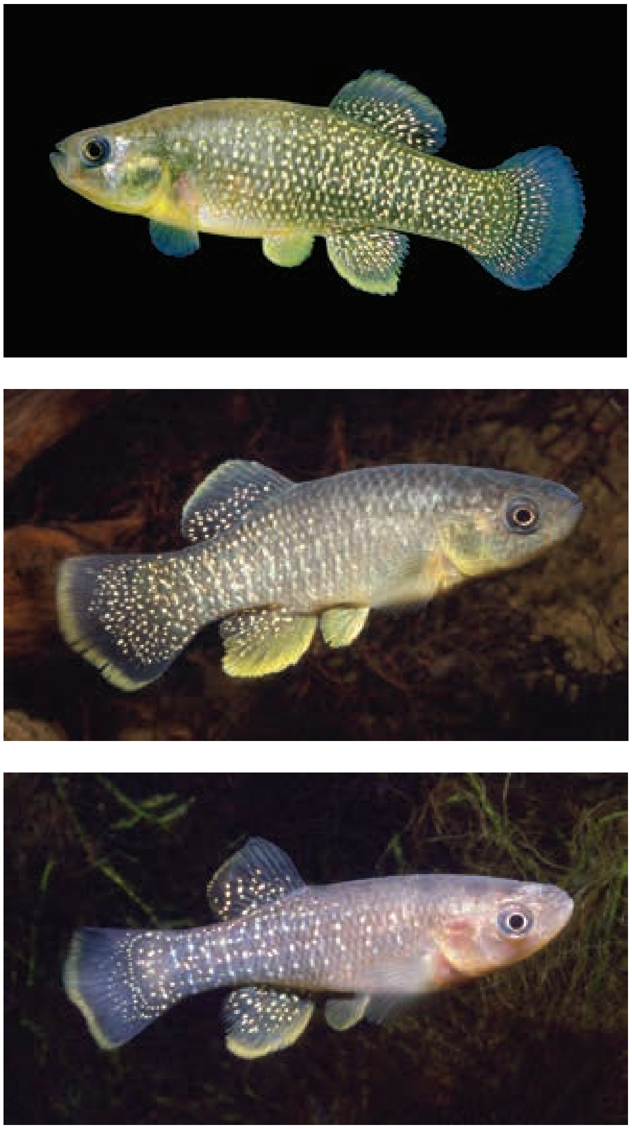

Figure 30.17. Mummichogs, *Fundulus heteroclitus*, often referred to colloquially as Atlantic killifish, are recognized as a northern subspecies, *F. h. macrolepidotus* (*upper*) (from Newfoundland to New Jersey) and a southern subspecies, *F. h. heteroclitus* (*lower*) (from New Jersey to northern Florida); individuals from New Jersey (*middle*) occupy a well-defined contact zone that is at the center of clinal variation in many morphological and genetic characters (Fig. 30.18) (*upper*, Heckscher State Park, East Islip, Suffolk County, New York; *middle*, Barnegat Bay, Ocean County, New Jersey; *lower*, Guana River State Park, St. Johns County, Florida) (courtesy of John Brill).

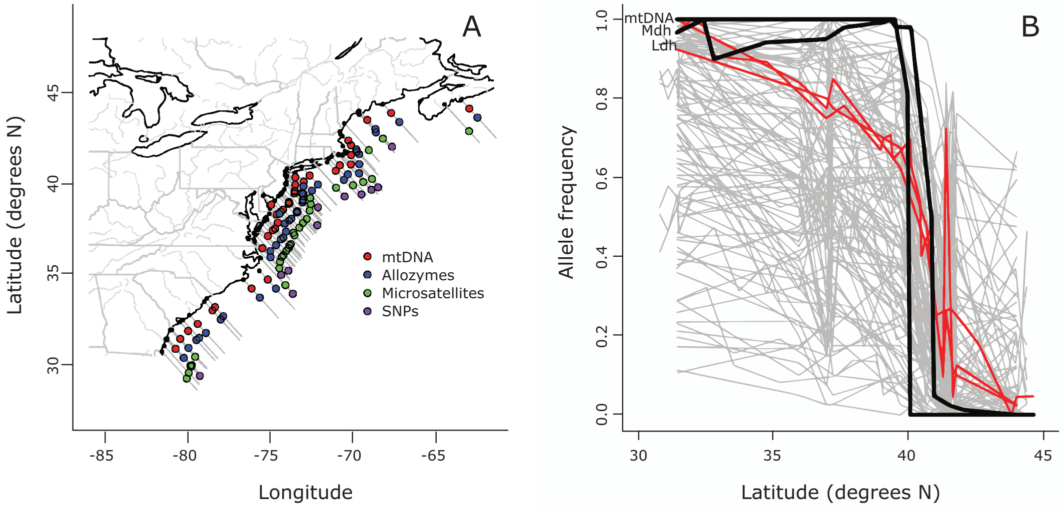

Figure 30.18. Clinal variation is well documented between northern and southern populations of the Mummichog, *Fundulus heteroclitus*. The average midpoint of various clinally distributed loci is centered at about 40 degrees north. Of 301 loci examined, 47% exhibited a clinal pattern of variation. Analysis and simulations indicate clinal patterns can be attributed to demographic history (secondary contact after isolation) without invoking diversifying selection (Strand et al. 2012; see text for details). (A) Location of sampled populations for 301 loci (1 mitochondrial DNA, mtDNA, red; 16 allozymes, blue; 8 microsatellite DNA, green; and 285 single nucleotide polymorphisms, SNPs, violet). (B) Allele frequencies of clinal loci used in a Genomic Co-Co plot analysis to visualize the distribution of the cline location (coincidence) and slope (concordance) for large numbers of loci; mtDNA and Mdh allozyme clines are highlighted in black, and Ldh allozyme and Ldh SNP clines are highlighted in red (redrawn from Strand et al. 2012).

In *F. diaphanus*, analyses of morphology and genetic markers (mtDNA and a nuclear gene, X-src intron) indicated that the ranges of the western subspecies (*Fundulus diaphanus menona*) and eastern subspecies (*F. d. diaphanus*) represent post-glacial dispersal from two Pleistocene refugia, one in the upper Mississippi River region (western form) and one on the Atlantic Coast (eastern form) (April & Turgeon 2006). That study and a subsequent analysis of microsatellite DNA (Rey & Turgeon 2007) resolved a contact zone covering about 150 km of the St. Lawrence River east of Lake Ontario. Nuclear markers indicated that the western form, *F. d. menona*, expanded eastward to Lake Ontario, but populations between Michigan and Lake Ontario carry the mtDNA of *F. d. diaphanus* (April & Turgeon 2006). In two Atlantic Coast sister-species, allozyme variation revealed a narrow contact zone (about 30 km wide) with hybridization in Flagler County, Florida, between *Fundulus majalis* (northern) and *F. similis* (southern) (Fig. 30.19), which at that time was considered a subspecies of *F. majalis* (Duggins et al. 1995). A similarly narrow, coastal zone of contact and hybridization (<40 km) in the Flagler County area exists for another pair of morphologically similar species, *Fundulus heteroclitus* (Fig. 30.17) and *F. grandis* (Fig. 30.19) as evidenced by allozymes (Duggins et al. 1989) and variation in mtDNA and a nuclear locus (Gonzalez et al. 2009).

Other Phylogeographic Patterns

Microsatellite DNA variation in estuarine populations of *Fundulus grandis* in the Gulf of Mexico was consistent with the possibility of separate southern refugia on the Eastern and Western Gulf Coasts (Williams et al. 2008). A negative relationship existed between latitude and genetic diversity on both sides of the Gulf and an apparent phylogeographic break occurred somewhere between Mobile Bay and western Florida although the area was not sampled. A weaker discontinuity occurred between western populations and Mobile Bay on the northern Gulf Coast.

The closely related *Fundulus notatus* and *F. olivaceus* (Fig. 30.1) have had markedly different evolutionary histories, despite broad sympatry over much of the central United States (Duvernell et al. 2013). Analysis of amplified fragment length polymorphism markers, mtDNA, and several nuclear sequences in *F. notatus* revealed deep phylogenetic structure reflecting pre-glacial drainage patterns, but *F. olivaceus* showed shallow structure indicating

Figure 30.19. Some species pairs of Topminnows, Fundulidae, have extremely narrow and apparently stable coastal zones of contact and hybridization. For example, the Longnose Killifish (*upper*), *Fundulus similis*, and Striped Killifish (not shown), *Fundulus majalis*, maintain a 30-km wide contact zone off the coast of Flager County, Florida. Similarly and along the same stretch of coast, the Gulf Killifish (*lower*), *Fundulus grandis*, and the Mummichog (Fig. 30.17), *Fundulus heteroclitus*, maintain a 40-km wide contact zone (*upper*, Key West, Florida, *lower*, Boca Chica Key, Florida) (courtesy of John Brill).

Figure 30.20. The *Fundulus catenatus* species group, all of which are all decidedly upland rocky stream dwellers, comprises three colorful species (see Fig. 30.21B for geographic distributions): Northern Studfish (*upper*), *Fundulus catenatus* (from Indiana); Southern Studfish (*middle*), *Fundulus stellifer* (from Swamp Creek, Elmore County, Alabama); and the Stippled Studfish (*lower*), *Fundulus bifax* (from Hillabee Creek, Tallapoosa River drainage, Tallapoosa County, Alabama). Interestingly, mtDNA haplotypes indicated the Indiana population of the Northern Studfish was more closely related to Ozarkian populations in Missouri than to populations immediately to the south in the Green River drainage, Kentucky (see Fig. 30.21AB and text for details).

recent occupation of most of its geographic range. Historical contingency is indicated for habitat relationships and life-history traits of the two species. As a consequence of allopatric divergence among populations of *F. notatus*, the Ouachita Highlands and Mobile basin populations have converged onto *F. olivaceus* in habitat associations and various features of life history (Duvernell et al. 2013). On a smaller scale, a microsatellite DNA survey of the two species in the Saline River, Illinois, demonstrated greater genetic isolation among populations of *F. olivaceus*, a result predicted from its more headwater distribution compared with *F. notatus* (Earnest et al. 2013).

Geographic variation in allozymes and mtDNA in the Northern Studfish, *Fundulus catenatus*, a highlands species (Figs. 30.4, 30.20, and 30.21), are consistent with dispersal between highland regions rather than an older Pleistocene vicariance hypothesis for the zoogeography of

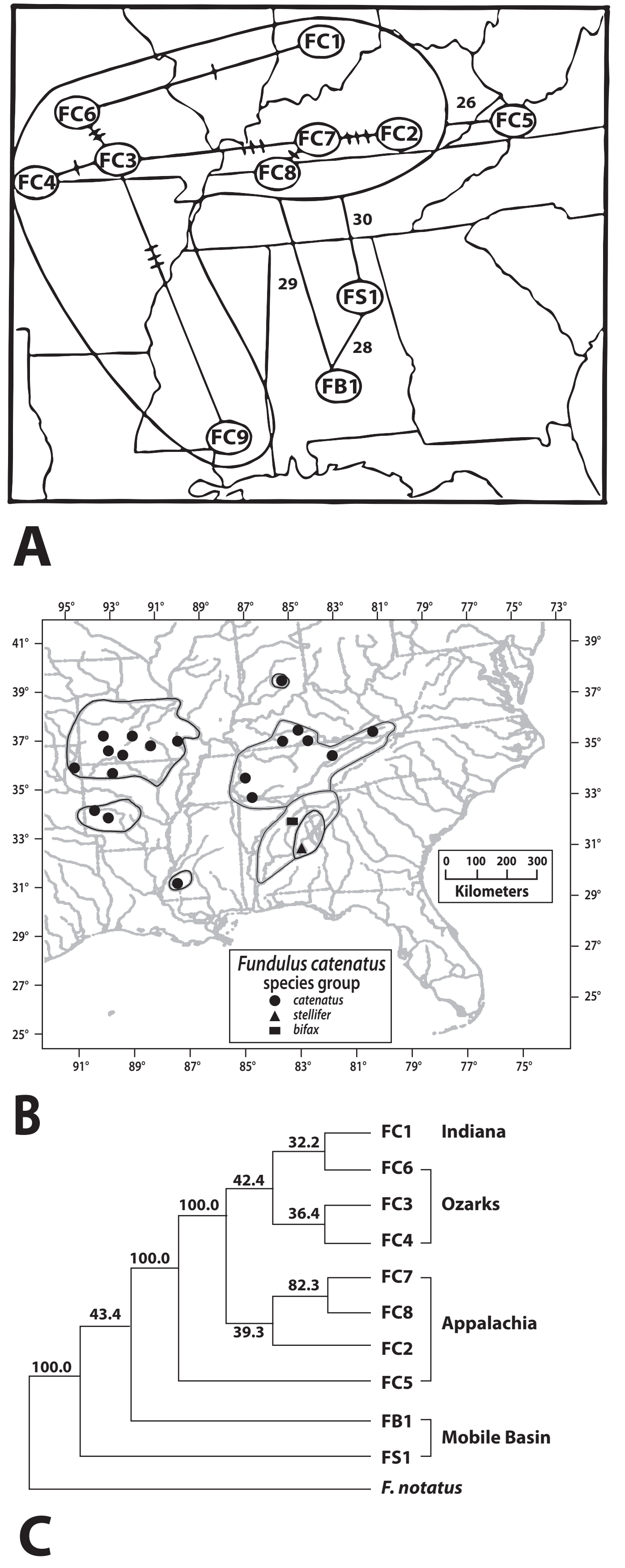

Figure 30.21. (A) Phylogeographic relationships among Ozark-Appalachian mtDNA haplotypes of the *Fundulus catenatus* species group from an analysis using 17 restriction sites (FB, *Fundulus bifax*, Stippled Studfish; FC, *Fundulus catenatus*, Northern Studfish; FS, *Fundulus stellifer*, Southern Studfish). Each unique number after the species code designates a unique haplotype. Numbers or slash marks along the branches indicate the minimum number of restriction site differences between haplotypes. (B) Geographic relationships of species in the group (polygons) and sampled populations (symbols, *inset*). (C) Phylogenetic relationships among Ozark-Appalachian mtDNA haplotypes of the *Fundulus catenatus* species group (species coded as in (A). Numbers above branches indicate the percentage of time a given clade was distinguished in 100 bootstrapped trees (redrawn from Strange & Burr 1997).

the species (Grady et al. 1990; Strange & Burr 1997). Levels of divergence were weak among populations in different highland regions (Ouachita, Ozark, and Appalachian Highlands). For mtDNA and allozymes, a population from Indiana fell within a clade composed of Ozark populations rather than with the geographically closer Appalachian populations. This was confirmed in a geographically more extensive analysis of mtDNA and four nuclear sequences (Hundt et al. 2017). Indiana populations grouped with western populations along with populations from the Cumberland River basin and separately from populations in the Tennessee River.

Phylogeographic studies often provide perspective on taxonomy. For example, based on mtDNA, the coastal California and Baja California subspecies *Fundulus parvipinnis parvipinnis* and *F. p. brevis* are no more closely related to each other than they are to *F. lima* in interior waters of the Baja Peninsula (Bernardi et al. 2007). This needs assessment with nuclear data, but corroboration would dictate taxonomic revision. Another example is from an analysis of variation in mtDNA and allozymes in the *F. zebrinus* group (Fig. 30.22). A sharp phylogeographic break between the Red and Arkansas Rivers, Oklahoma and Texas, resulted in elevation of two previously recognized subspecies to species: *Fundulus zebrinus* from the Red River south and west to the Rio Grande system and *F. kansae* from the Arkansas River system north to the Platte River of Nebraska and Wyoming (Kreiser 2001; Kreiser et al. 2001). Finally, a phylogeographic study of the *F. catenatus* species group identified a wide-ranging western and northern form, *F.* cf. *catenatus*, as a separate species from *F. catenatus* in the Tennessee River (Hundt et al. 2017).

Mitochondrial DNA barcoding with partial cytochrome oxidase subunit I (COI) sequences indicated that further study of variation within named species will uncover additional species of *Fundulus*. A survey aimed at estimating the frequency of cryptic species in North American fishes found that, of the 26 named fundulid species examined, three (11%), *Fundulus diaphanus*, *F. catenatus*, and *Leptolucania ommata*, harbor at least one unconfirmed candidate species each (April et al. 2011). This was based on the working hypothesis that >2% divergence in COI sequences indicates a potential unrecognized species, as subsequently confirmed for *F. catenatus* (Hundt et al. 2017).

Clines and Natural Selection

Widths and positions of molecular genetic clines in *Fundulus* generally are explained by secondary contact and subsequent dispersal without invoking natural selection (Bernardi & Talley 2000; Rey & Turgeon 2007; Duvernell et al. 2008; Strand et al. 2012). This does not question the presence of adaptive genetic differences in populations at the clinal extremes (DiMichele et al. 1986; Burnett et al. 2007). The assumption, however, is that such differences are not needed to explain the clines for most markers. Under natural selection, the shape and positions of clines for individual markers should vary considerably depending on strength of selection. Instead, concordance among clines is the rule. This is best seen in *F. heteroclitus* (Fig. 30.17), where overall concordance is seen among a large number of markers (SNPs for protein-coding genes and noncoding microsatellites) expected to show different levels of susceptibility to natural selection (Duvernell et al. 2008; Strand et al. 2012; Fig. 30.18). Exceptions include especially steep clines for mtDNA and SNPs for various protein-coding nuclear genes (Strand et al. 2012; McKenzie 2013).

Figure 30.22. The Northern Plains Killifish (*upper*), *Fundulus kansae*, and the Plains Killifish (*lower*), *Fundulus zebrinus*, were long considered subspecies, but genetic analyses and morphological comparisons revealed the presence of two distinct, evolutionary lineages with *F. kansae* plus the extinct †*F. detillae* being sister to *F. zebrinus*. The trio composes the subgenus *Plancterus* (*upper*, Salt Creek, Missouri River drainage, Cooper County, Missouri; *lower*, Terlingua Creek, Rio Grande drainage, Brewster County, Texas) (courtesy of John Brill).

A relatively fine-grained geographic study of genetic structure in the coastal contact zone between the two subspecies of *Fundulus heteroclitus* (*F. h. heteroclitus* and *F. h. macrolepidotus*) challenges the prevailing view that populations are randomly mating admixtures free of selection against hybrids (McKenzie 2013). Separate multilocus hybrid indices from two divergent types of markers (microsatellite loci and SNPs in protein-coding genes) indicated trimodal genetic structure near the center of the cline; most individuals represented F_1 hybrids or one or the other parental type. This and the analysis of cline widths for mtDNA and nuclear SNPs suggested that natural selection or assortative mating likely play a role in maintenance of the hybrid zone. The clines for mtDNA and several nuclear genes with mitochondrial functions were especially steep, and within-population cytonuclear disequilibrium (nonrandom positive associations between mtDNA and nuclear alleles) was significant. These results suggest a role for inter-subspecies incompatibility between nuclear and mitochondrial genes as a factor in selection against hybrids and thereby maintenance of the hybrid zone (McKenzie 2013). For example, a nuclear gene coding for mitochondrial malate dehydrogenase tightly covaries with mtDNA, and these form two of the steepest clines among all markers examined to date in the species (Strand et al. 2012; McKenzie 2013; Fig. 30.18).

Other studies suggesting an important role for natural selection in maintaining hybrid zones in coastal populations of *Fundulus* include situations of apparent secondary contact involving two different sister-species pairs in the same small area on the Atlantic Coast (Flagler County, Florida). For both pairs, the zones of transition are extremely steep: about 30 km between *Fundulus majalis* and *F. similis* (Duggins et al. 1995) and about 40 km between *Fundulus heteroclitus* and *F. grandis* (Duggins et al. 1989; Gonzalez et al. 2009). The authors concluded that these clines represent zones of secondary contact maintained by natural selection. The clines are too narrow to be explained by low dispersal rates after the hypothesized post-Pleistocene establishment of secondary contact (Duggins et al. 1989, 1995).

Functional Genomics and Gene Regulation

A large literature demonstrates the importance of change in gene expression on adaptive physiological divergence in *Fundulus* (reviewed by Crawford et al. 1999 and Burnett et al. 2007). Some of this material, which is primarily focused on *F. heteroclitus*, is reviewed elsewhere in this chapter (see physiology section). Advances in technology over the past two decades have fostered the field of functional genomics in which large numbers of loci with known functions can be assayed simultaneously for levels of gene expression. Studies of functional genomics in *Fundulus* took advantage of a database of >40,000 expressed sequence tags from heart and liver of *F. heteroclitus* (Paschall et al. 2004). Expressed sequence tags (ESTs) are short nucleotide sequences derived by reverse transcription from messenger RNA in the tissues. These can be isolated, sequenced, and identified with reference to available databases. The ESTs identified for *F. heteroclitus* enabled a burst of gene expression studies on the species. These include studies of gene expression in embryology (Bozinovic et al. 2011, 2013), cardiac function (Oleksiak et al. 2004), and response to pollution (Fisher & Oleksiak 2007; Bozinovic et al. 2011). We focus here on studies relevant to adaptation to physicochemically different environments and its possible role in speciation.

Studies of functional genomics in *F. heteroclitus* provide considerable support for physicochemical barriers to gene flow as an important factor in maintaining clines for particular sets of genes (Whitehead & Crawford 2006; Whitehead et al. 2011a). Whitehead & Crawford (2006) examined fish from five localities bracketing the coastal cline between *Fundulus h. heteroclitus* and *F. h. macrolepidotus*, where temperature is the most obvious environmental gradient. After two months of acclimation to common conditions, liver transcriptomes (all messenger RNAs in liver samples) from the fish were analyzed for levels of gene expression in 329 genes from central metabolic pathways. A small number (58, 18%) exhibited significant regression with habitat temperature. Of these 58 temperature-related genes, 13 (22%) regressed significantly on temperature after correcting for phylogenetic nonindependence with a microsatellite DNA tree (phylogram) used as a neutral model of divergence. Nine of the 13 genes resolved as adaptive were previously associated with thermal acclimation in the Common Carp, *Cyprinus carpio*. Adaptive divergence in gene regulation between the two subspecies of *F. heteroclitus* is further indicated by comparisons with the southern species *F. grandis* (Oleksiak et al. 2002). Pattern of expression across 907 genes in fish acclimated to 20°C for >6 months showed that the southern subspecies (*F. h. heteroclitus*) is markedly more similar to *F. grandis* than it is to the northern subspecies (*F. h. macrolepidotus*).

Patterns of gene expression in the river-to-estuary transitions between *Fundulus heteroclitus macrolepidotus* and *F. h. heteroclitus* in Chesapeake Bay also revealed evidence of adaptive divergence (Fig. 30.23). In two separate rivers,

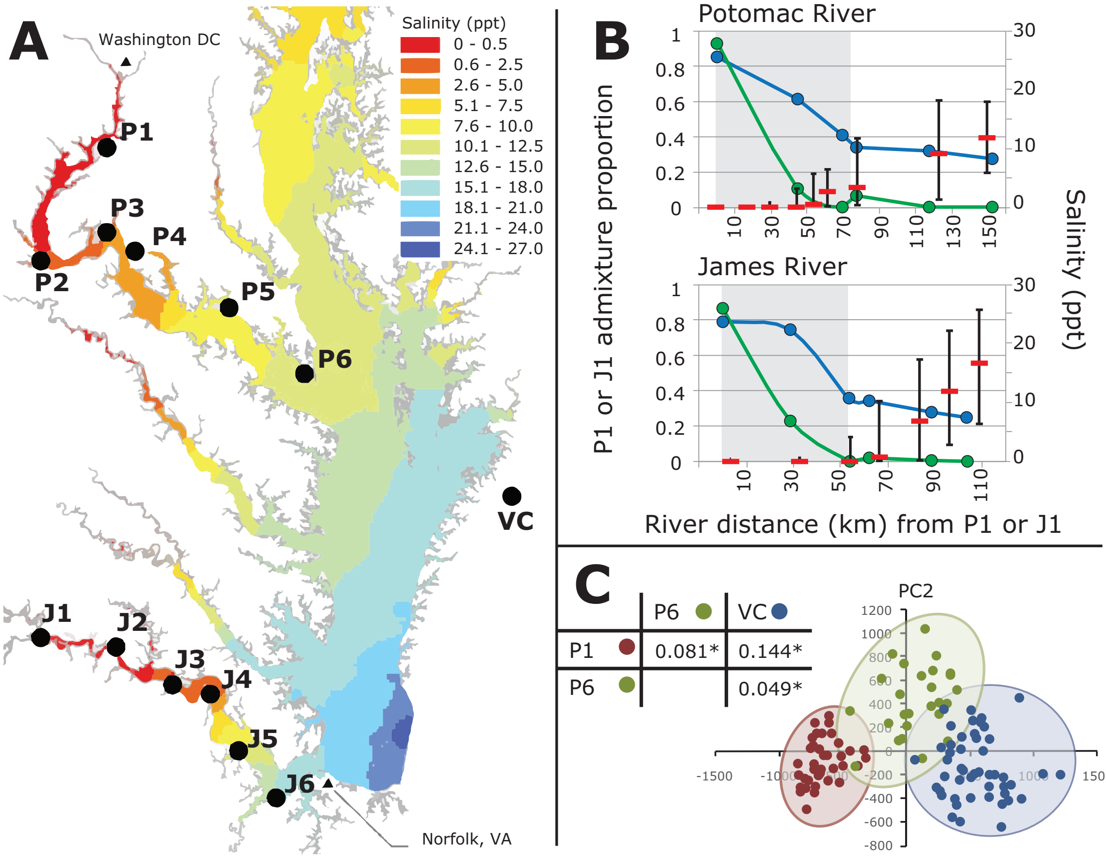

Figure 30.23. Population genetic clines across parallel salinity gradients in the Potomac and James Rivers, Chesapeake Bay, for Mummichogs, *Fundulus heteroclitus*. The steep clines in allele frequency for both mitochondrial and nuclear markers were centered at nearly identical salinities along the parallel gradients (about 0.4–0.5 ppt, indicated by the boundary between shaded and non-shaded areas) (Whitehead et al. 2011a). (A) Map of sampling locations along the Potomac River (P1–P6) and James River (J1–J6). Heat overlay indicates 20 year (1986–2005) average salinity (ppt). (B) Line graphs show clines in admixture proportions for mitochondrial (green line) and microsatellite (blue line) markers plotted against river distance from the most extreme upper-estuary (freshwater) population (P1 or J1). Red squares with black bars indicate 20-year average salinity and range for fixed sampling locations. (C) Cluster diagram illustrates multi-locus microsatellite genotype divergence of individuals along principal components axes for selected populations across the salinity gradient, including P1 (freshwater; red points), P6 (meso-haline; green points), and VC (coastal marine; blue points), and the inset table provides pairwise genetic distances (Cavalli-Sforza and Edwards' chord distance) between populations (star indicates statistical significance, P<0.001, permutation test) (redrawn from Whitehead et al. 2011a).

the transition is marked by a steep cline in mtDNA and microsatellite DNA frequencies and changes in gill structure that are associated with a salinity gradient from the fresh water (<0.5 ppt) of the incoming river to the increasingly saline waters of the estuary (0.5–27 ppt) (Whitehead et al. 2011a). A transcriptome analysis of fish acclimated to 32 ppt and then acutely exposed to hypoosmotic water (0.5 ppt) showed differential patterns of physiological response and gene expression that were consistent with adaptive differences in gene regulation. Such differences might restrict gene flow across a salinity barrier at about 0.5 ppt.

Other studies have compared microevolutionary patterns (among populations within species or subspecies) of gene expression in osmotically divergent environments with macroevolutionary patterns (between taxa). The results are consistent with the hypothesis that differential adaptation to salinity was a factor in speciation. One example is a study of the euryhaline (wide salinity range) *F. heteroclitus* and the more strictly saltwater species, *F. majalis* (Whitehead et al. 2013). Fish of both species were captured from the same or nearby sites in three different areas in Virginia: two nearby locations in Chesapeake

Bay, one brackish (about 12 ppt) and one near-seawater (30 ppt), and a more distant near-seawater (31 ppt) area. After acclimation to 32 ppt, subsets of the fishes were transferred to salinities of 32 (control), 5 (both species), 0.4 (*F. majalis*), and 0.1 (*F. heteroclitus*). Transcriptomic analysis of gill tissues revealed a large set of genes showing the same acclimation-time effect in both species, indicating an evolutionarily conservative response to hypoosmotic challenge. A second set of genes showed species-specific acclimation-time effects, primarily reflecting diminished gene activity in *F. majalis* or no acclimation time-course response in *F. majalis* relative to *F. heteroclitus*. The genes involved in species-specific responses generally followed expectations based on gene expression in *F. heteroclitus* populations adapted to different osmotic conditions. This conforms to the prediction that adaptive microevolutionary responses producing osmotically divergent populations within species can contribute to macroevolutionary divergence producing osmotically divergent species.

A similar conclusion was reached in a study of *Lucania parva*, a euryhaline species, and *L. goodei*, a freshwater species (Kozak et al. 2014). Analysis of gene expression in gill tissue at 4,051 genes during acclimation to a hyperosmotic challenge (acclimated at 0.2 ppt, transferred to 15 ppt) revealed a conserved set of 429 genes showing similar responses in the two species and a set of 310 genes showing species-specific responses, many of which have known or potential osmoregulatory functions. This study also identified SNPs from the transcriptomes of *Lucania parva* and *L. goodei* and used these to analyze sequence divergence in expressed genes. The two species differed in a number of genes involved in ion-transport, as predicted from the salinity tolerance differences between the two species. The sequences of many of these same genes also differed between populations of *L. parva* from different salinity regimes (fresh versus brackish). This was not seen in within-species comparisons of widely separated populations from the same salinity regime (*Lucania parva*, brackish water; *L. goodei*, fresh water), validating the conclusion that adaptation to different osmotic environments explained the sequence differences between freshwater and saltwater populations not differences in geographically separate populations. Thus, factors contributing to macroevolutionary (between species) divergence in adaptation to different salinity regimes are reflected in microevolutionary processes of divergence among populations within species. The two species also showed a high degree of transcriptome sequence divergence for a set of genes with functions in reproduction, a finding consistent with hybrid sterility between the two species (Kozak et al. 2014).

An unexpected result regarding the roles of natural selection and processes of neutrality (e.g., genetic drift) emerged from the previously described study of gene expression in *Fundulus heteroclitus* and *F. majalis* (Whitehead et al. 2013). In laboratory experiments, hundreds of genes in both species appeared neutral to hypoosmotic challenge (expression independent of salinity). As expected from neutrality, the within-species population similarities in gene expression showed a negative relationship with geographic distance. This indicated neutrality and isolation-by-distance as seen in a parallel analysis of supposed neutral markers (microsatellites). However, the interspecific comparison of a shared subset of 384 neutral genes departed from expectations based on neutrality. Genes that up-regulated (up) in hypoosmotically challenged *F. heteroclitus* from Georgia (relative to the northern subspecies *F. h. heteroclitus*) almost invariably down-regulated (down) in *F. majalis* from Georgia and vice versa. In contrast, neutrality predicted four equally common classes of interspecific pattern (up-up, down-down, up-down, and down-up). The authors were unable to choose among various alternative explanations and suggested that gene expression divergence is more complicated than generally appreciated (Whitehead et al. 2013).

The recent completion of high-density linkage maps for *Lucania goodei* and *L. parva* are the first for a fundulid species (Berdan et al. 2014). The markers, 915 for *L. goodei* and 766 for *L. parva*, are SNPs developed from ESTs; thus, the majority represent genes encoding proteins that can be identified. These maps promise to provide the basis for a variety of genomic approaches to the study of the evolutionary biology of Topminnows.

Knowledge of an important class of regulatory genes, the Hox genes, is rather rudimentary in Topminnows. Hox genes are arranged in uninterrupted sets (clusters) that are especially important in specifying anterior-posterior relationships in the developing embryo. A polymerase-chain-reaction survey of Hox genes in *F. heteroclitus* suggested the presence of four Hox clusters that are orthologous (related by vertical descent) with the four clusters present in land vertebrates (Misof & Wagner 1996). Later work revealed, however, that the ancestral teleost underwent a whole-genome duplication that would have increased the number of Hox clusters from four to eight (Hoegg et al. 2004). Thus, some of the genes identified by Misof & Wagner (1996) likely are paralogous representatives (duplicates not necessarily serving the same function) of genes in the four land-vertebrate Hox clusters (i.e., homologous genes that have evolved by duplication and code for protein with similar but not identical functions).

Conservation Genetics

Geographic surveys of mtDNA variation provided perspective on the conservation genetics of two declining species: *Fundulus sciadicus* with two disjunct regions of occurrence (Ozark Highlands and central Great Plains) and *F. lima* in Baja, California. Remarkably low mtDNA diversity but with strong geographic partitioning was detected in a survey of *F. sciadicus* (Li et al. 2009). Only seven variable sites (nine haplotypes) were found among the control region sequences (966 base pairs) of 181 fish from 21 locations. When the data were examined by drainage, 93% of total diversity was attributable to differences among drainages. This structure was only weakly associated with region because the Lamine River population from the Ozark Highlands grouped with populations from Nebraska rather than with other Ozark Highland populations. The range of this declining species is fragmented with four genetically distinct populations defined by river as follows: Niobrara-Platte, Nebraska, and the Lamine, Osage-Gasconade, and Spring systems, Missouri. Four populations, one in each of those units, were identified as of particular interest for conservation management because they appeared to retain greater genetic diversity than others within their systems of occurrence. For *F. lima*, four of the six known populations, all from isolated oases on Baja, California, were examined (Bernardi et al. 2007). Three of the four were resolved as reciprocally monophyletic and recognized as separate evolutionarily significant units for conservation purposes. The fourth was represented by a single individual that, although assumed to be *F. lima* based on locality of capture, was mitochondrially sister to the southern subspecies of the coastal species *F. parvipinnis*.

PHYSIOLOGY

Many species of *Fundulus*, including *F. heteroclitus* and its sister-species *F. grandis*, reside in coastal marshes that are subject to frequent and episodic oscillations in salinity, dissolved oxygen, and temperature (Marshall 2003). Topminnows inhabiting tidally influenced coastal areas can become trapped in small bodies of water that experience high temperature, low dissolved oxygen, or vary dramatically in salinity because of evaporation or precipitation. The resilience of many *Fundulus* to these dynamic habitats makes them a popular model for the study of the physiological and genomic basis of environmental stress tolerance (Burnett et al. 2007). Other factors also contribute to the suitability of Topminnows as experimental models, including the large population sizes of many of the species and the ability of researchers to easily capture and transport these fish and then to successfully culture and breed them in the laboratory.

A large biochemical and physiological experimental toolkit was developed over the last 70 years for several Topminnows (Burnett et al. 2007), and the complete, annotated genome for *F. heteroclitus* is sequenced. Despite their relatively diminutive size, research on Topminnows has provided important insights into marine and freshwater teleostean physiology. Endocrinology in general, and reproductive endocrinology in particular, were the subjects of many studies in *F. heteroclitus* and other Topminnow species (Pickford & Phillips 1959; Potts & Evans 1966). A role of the pituitary hormone, prolactin, in freshwater adaptation in teleost fishes was first described in experiments with *F. heteroclitus*. Euryhaline fish that have had their pituitary gland (hypophysis) surgically removed cannot survive an acute transfer from sea water to fresh water. Hypophysectomized *F. heteroclitus*, however, can survive long term in fresh water if they are given exogenous injections of ovine prolactin before transfer to fresh water (Pickford & Phillips 1959). The current transport model of active sodium and chloride secretion for marine fishes was first described largely based on studies on isolated opercular membranes from *F. heteroclitus*. In addition, Topminnows harbor significant interspecific and intraspecific variation in tolerance to salinity (Scott et al. 2004, 2005; Whitehead et al. 2011b, 2012a; Brennan et al. 2015), hypoxia (Richards et al. 2008), temperature, and anthropogenic pollution (Whitehead et al. 2012b). Many species are somewhat irregular lunar (full moon) or semilunar (new moon and full moon) spawners (Taylor 1999; see Bosker et al. 2013) and produce embryos that are tolerant of desiccation stress during aerial exposure. A laboratory strain of the Mummichog, the Araski strain, however, was isolated and has a constant annual cycle as well as a consistent daily spawning cycle that allows detailed study of reproductive cyclicity in this species (Shimizu 1997). Both the yearly and daily cycles of *F. heteroclitus* are primarily endogenous, based on internal cyclicity, and are broadly independent of day length and temperature cues (Roberts et al. 2003; Shimizu et al. 2003; Shimizu 2003). Further, several populations of *Fundulus heteroclitus* and *F. grandis* have become highly tolerant to extremely elevated concentrations of dioxins, polychlorobiphenyl, and metals at concentrations toxic to most aquatic species, including control populations of *F. heteroclitus* captured from comparably clean field sites (Whitehead et al. 2012a). These examples highlight some of the fascinating attributes of physiology in *Fundulus*.

Here, the focus is on salinity, temperature, and dissolved oxygen tolerances; little of substance is apparently available on pH tolerance of Topminnows. The most in-depth research is available for brackish or estuarine species of *Fundulus*. When possible, summaries of tolerances of some freshwater Topminnows are also included.

Salinity Tolerance

Fundulus spp. occupy habitats ranging in salinity from fresh water to hypersaline tide pools at several times the strength of sea water (Griffith 1974a; Nordlie 1987). Most species of *Fundulus*, even those considered marine stenohaline (narrow range of salinities) (Table 30.1), can survive in the laboratory to salinities ≤20% sea water (about 7 ppt). In contrast, members of *Fundulus* differ significantly in their lethal hyperosmotic limits to exposure during acute, incremental increases in environmental salinity (Griffith 1974a). Data from salinity studies were used to categorize species into three groups based on their hyperosmotic limits (Whitehead 2010; Whitehead et al.2011a; Table 30.1): stenohaline fresh water (species living in fresh water and tolerant of salinities <20–26 ppt); intermediate salinity tolerance (those living in fresh water to sea water but tolerant of salinities ≤60–75 ppt); and salinity tolerant (those described as extremely halotolerant with upper lethal limits of ≤115 ppt). After superimposing those comparative hyperosmotic data on a molecular phylogeny of the genus, a tolerant phenotype was proposed as the likely ancestral state, and the less tolerant, freshwater stenohaline phenotype was considered derived. At least five independent freshwater invasions occurred within the genus, and in each case, freshwater clades experienced a similar contraction in salinity tolerance and had significant reduced osmotic tolerance ranges than species of intermediate and high salinity tolerance (Whitehead 2010).

Of the 40 extant species of *Fundulus*, about 15 species, including *F. olivaceus* and *F. notatus*, live in fresh water and have a limited tolerance to brackish water above the isosmotic point (equal osmotic pressures) (i.e., about 10–12 ppt). The largest number of freshwater *Fundulus* live in rivers draining into the northern Gulf of Mexico and are distributed as far north as the Ohio, Tennessee, Missouri, and Arkansas Rivers (Griffith 1974a). In comparison, the number of species along the Atlantic Slope and Great Lakes region is smaller. The Speckled Killifish, *Fundulus rathbuni*, and Lined Topminnow, *Fundulus lineolatus*, are obligatory hyperosmoregulators (maintain internal osmotic concentration above surrounding water) but have small realized niches in the southeastern region of the United States. *Fundulus diaphanus* resides exclusively in fresh water, spanning a large geographical region along the eastern United States and Great Lakes region, although the species can tolerate relatively high salinities in the laboratory (i.e., categorized as intermediate in salinity tolerance).

Although tending to inhabit brackish to marine environments (Kaneko & Katoh 2004; Wood & Grosell 2008, 2009), euryhaline Topminnows tolerate exposure to salinities approaching fresh water to several times the strength of sea water. *Fundulus heteroclitus* has the largest halotolerance among the euryhaline species within the genus (Griffith 1974a). This species forms shoals (groups) that often move short distances during an incoming tide to feed at lower salinities. Although most fish retreat with outgoing tides to deeper waters approaching marine salinities, some are often stranded in pools during ebb tide where salinities are subject to considerable variation (Marshall 2003). Euryhaline species of *Fundulus* appear to cope with short bouts of fresh water by making rapid physiological adjustments (Marshall 2003; Wood & Grosell 2008, 2009), but also can make more substantive compensatory adjustments to their ion-transporting epithelia during prolonged exposure to fresh water (Wood & Grosell 2008). Interestingly, euryhaline species of *Fundulus* retain a gill surface morphology that is characteristic of marine fishes even when exposed to hypoosmotic conditions (Feldmeth & Waggoner 1972; Griffith 1974a; Whitehead 2010). The *F. heteroclitus* gill may make this physiological transition to a hypoosmotic morphology at a salinity between 2.0 and 0.5 ppt (Copeland 1950; Philpott & Copeland 1963). In fresh water, the surface morphology of mitochondrion-rich (MR) gill cells of *F. heteroclitus* exposed to 0.1 ppt is consistent with the surface characteristics of cuboidal cells (Laurent et al. 2006), which are presumed to be the site of active sodium uptake in freshwater-acclimated fish.

Ion transport in freshwater-acclimated species of *Fundulus* has some unique features. In most freshwater teleosts, the active uptake of chloride is linked with bicarbonate excretion in MR cells (Wood & Goss 1990; Perry 1997) via a presumptive chloride-bicarbonate exchanger. In comparison, freshwater-acclimated *F. heteroclitus* have no appreciable active chloride uptake at the gill (Wood & Marshall 1994; Patrick et al. 1997; Wood & Laurent 2003; Scott et al. 2004), which is one of the reasons the species has high tolerance to water nitrites considering that nitrates are typically accumulated from the water using chloride-transport systems (Tomasso & Grosell 2005). Despite this lack of active chloride uptake, euryhaline *Fundulus* populations (i.e., *Fundulus heteroclitus* and *F. grandis*) that are most tolerant to acute transfer from sea water to fresh water are best able to restore plasma chloride regulation in the face of hypoosmotic stress. These populations must limit plasma chloride loss and even restore homeostasis of plasma chloride despite the absence of active uptake of chloride from the water (Wood & Marshall 1994). Clearly, this puts a greater emphasis for the regulation of paracellular ion loss (passing through intercellular space) in *Fundulus* than perhaps in other teleost species, which can stimulate chloride homeostasis by upregulating their active uptake mechanisms for this ion (Goss et al. 1992). Development of a mechanism to minimize paracellular chloride loss at the gills was proposed as an important evolutionary step allowing certain populations of brackish-tolerant *Fundulus* to survive in fresh water (Scott et al. 2004) and thought to delineate intraspecific and interspecific variation in hypoosmotic tolerance. Later studies also suggested that gastrointestinal accumulation of ions from the diet is also an important mechanism of maintenance of the ion homeostasis in *F. heteroclitus* during hypoosmotic exposure although this is still an active area of research (Bucking et al. 2013).

Populations of *Fundulus heteroclitus* also harbor significant intraspecific variations in osmotic compensatory abilities (Scott et al. 2004). For instance, *F. heteroclitus* along the U.S. Atlantic Coast north of a phylogenetic break at about 40.5 degrees north latitude (*F. h. heteroclitus*) are more tolerant of freshwater transfer than are fish south of this latitude (*F. h. macrolepidotus*). Specifically, both populations could maintain osmotic homeostasis when transferred from 32 ppt to 0.4 ppt, but diverged in their compensatory abilities when challenged down to 0.1 ppt in parallel with divergent transformation of gill morphology (Whitehead et al. 2011a). *Fundulus heteroclitus* acclimated to full-strength sea water (i.e., 32 ppt) can tolerate abrupt transfer from sea water to 0.4 ppt with no significant effects to plasma sodium and chloride concentrations or plasma osmolality over 14 days post-transfer (Whitehead 2010; Whitehead et al. 2011a). Genes involved in cell volume regulation, nucleosome maintenance, ion transport, energetics, mitochondrion function, transcriptional regulation, and apoptosis (cell death) exhibited population-dependent and salinity-dependent patterns of expression during acclimation; northern populations showed greater plastic responses to hypoosmotic challenge than their southern counterparts. Cytokine and kinase signaling pathways coordinate the genome regulatory response to osmotic challenge, and the signaling is coordinated through a transcription factor (HNF-4α). These genome responses support a hypothesis of regulatory mechanisms particularly relevant for enabling extreme physiological flexibility. The northern subspecies appears to be more tolerant of low temperature in addition to low salinity extremes than the southern species (Scott et al. 2005; Fangue et al. 2006; Healy & Schulte 2012). Together, these data suggest that northern populations were derived from populations with wider salinity or temperature tolerance ranges and that this could have contributed to their successful invasion of newly-emergent estuarine habitats following the retreat of Pleistocene glaciers north of the Hudson River (habitats that would initially have had relatively low salinity and low temperature).

Fundulus heteroclitus with northern mitochondrial haplotypes (*F. h. heteroclitus*) do not occur south of New Jersey, except in extreme upper-estuary (Chesapeake Bay and Delaware Bay) habitats characterized by low salinities (Whitehead et al. 2012b). Population genetics of *F. heteroclitus* populations along the tidal rivers of the Chesapeake Bay region demonstrated a sharp genetic discontinuity of nuclear and mitochondrial allelic frequencies at water salinities of 0.1–0.5 ppt. No other discernable barrier to gene flow was apparent along these parallel salinity clines (Fig. 30.23). Further, in the laboratory, *F. heteroclitus* from the upper-estuary habitat, upstream of the genetic discontinuity, exhibited tolerance to extreme hypoosmotic challenge, but populations that occur in brackish or marine habitats downstream of the discontinuity lost osmotic homeostasis more severely and took longer to recover (Whitehead et al. 2011). Comparative transcriptomics revealed a core transcriptional response associated with acute and acclimatory responses to hypoosmotic shock and suggested novel mechanisms were enabling extreme osmotic tolerance (Whitehead et al. 2011a, 2012b). It was not the well-known effectors (cell responses) of osmotic acclimation, but rather the lesser known immediate-early responses that appeared important in contributing to differences. Further, of the genes that varied in expression among species, those that are putatively involved in physiological acclimation were more likely to exhibit non-neutral patterns of divergence between fresh and brackish water populations.

Temperature Tolerance

Estimates of optimal temperature ranges are available for multiple species of *Fundulus* based on the water temperatures of field sites at the time of fish capture or from measures of the optimal temperature preference for growth, feeding, or fertilization in laboratory studies (reviewed by Nordlie 2006). Optimal temperature data for many species of *Fundulus* are also published in non-peer reviewed forums, such as webpages (EOL 2015; Fishbase 2015), or in anecdotal reports targeting the killifish hobbyist community. Although these data are useful as benchmarks for optimal fish holding in the laboratory, they often show little correlation to the precise thermal limits derived in the laboratory using standard experimental methodologies (e.g., critical thermal maxima, CT_{max}; critical thermal minima, CT_{min}) (Beitinger et al. 2000). Thermal limits for *Fundulus* are typically derived using the critical thermal approach using loss of equilibrium or opercular spasms (CT_{max}) as the endpoint following incremental changes in water temperature over time. In general, the range of temperatures at which species were captured in the field is narrower than the ranges between the critical lower and upper thermal tolerance limits of *Fundulus* in the laboratory (Nordlie 2006). Even though estimates differ from thermal tolerance experiments and field observations, both support *Fundulus* spp. as extremely eurythermic.

As a general rule, species of *Fundulus* have CT_{max} higher than those of most other fishes (reviewed by Beitinger et al. 2000; Nordlie 2006). CT_{max} in *F. heteroclitus* varied between 38.1°C following acclimation to 10°C and 41.7°C when acclimated to 30°C (Burnside 1977). In comparison, CT_{max} for this species was estimated as 32.2°C following acclimation at 7°C and 44.1°C following acclimation to 36°C (Bulger & Tremaine 1985). CT_{max} estimates for *F. heteroclitus* were influenced 1–2°C depending on the time of day of testing (Bulger 1984) and also by the salinity and dissolved oxygen level of water during testing. A population of *F. heteroclitus* derived from the most northern range of the species (i.e., Nova Scotia) and acclimated to 15°C had a CT_{max} of 30.5°C at a salinity of 32 ppt and 34°C at 14 ppt (Garside & Jordan 1968). Acclimation temperature also affects the acute upper thermal limit of *F. majalis*, a marine *Fundulus* distributed along the Atlantic Coast from New Hampshire to Florida. CT_{max} of *F. majalis* was 37.2°C following 10°C acclimation and 40.9°C following 30°C acclimation (Burnside 1977).

Similarly, most species of freshwater *Fundulus* have high CT_{max} and are considerably eurythermic. *Fundulus olivaceus* had a CT_{max} estimate of 38.8°C and *F. sciadicus* had one of 37.0°C when acclimated to 26°C (Smale & Rabeni 1995). *Fundulus notatus* has a CT_{max} of 38.3°C at an acclimation temperature of 26°C and a CT_{max} of 41.6°C when acclimated to 30°C. Thermal preference and tolerance of *Fundulus notatus* and *F. olivaceus* populations was assessed from southern (30–31 degrees N), middle (34 degrees N), and northern (37–41 degrees N) portions of the range (all acclimated to 25°C). Across latitudes, *F. olivaceus* showed no difference in thermal preference (about 26.4°C), but *F. notatus* populations from middle latitudes preferred warmer temperatures (27.6°C) than either northern (25°C) or southern (26.3°C) populations. Temperature tolerance (CT_{max}) was generally similar for the two species and increased with latitude; presumably, the more northerly populations experience greater thermal variation overall, which selects for more eurythermic traits. The peak CT_{max} was 41.8°C for *F. olivaceus* from the northern extent of its range. These data also suggest that freshwater species of *Fundulus* have CT_{max} values as high as those reported for many marine species when acclimated to warm water before testing (Stewart 2015). In a study of a population in Nova Scotia, *Fundulus diaphanus*, which is a species that lives almost exclusively in fresh water despite its moderate salinity tolerance, had a CT_{max} of only 33.5°C at 14 ppt and 27.5°C in fresh water (acclimated to 15°C) (Garside & Jordan 1968). *Fundulus diaphanus* living at its southernmost geographical range of South Carolina may possibly have much higher CT_{max} values, but no published values are available.

Estimates of CT_{min} are available for only a few species; however, the measurements that do exist support that *Fundulus* spp. have moderate to extreme tolerances to cold-water temperatures. *Fundulus grandis* captured in Florida have a CT_{min} of about 4°C, and *F. diaphanus* from freshwater habitats in Nova Scotia have a CT_{min} of 6°C. In comparison, *F. heteroclitus* captured in Nova Scotia tolerate water temperatures down to about −1.5°C. These limited data support large interspecific variation in CT_{min} between euryhaline versus freshwater species of *Fundulus*. In general for fishes, CT_{min} is more strongly influenced by acclimation temperature than is CT_{max}, although this relationship is not thoroughly demonstrated in *Fundulus* (Nordlie 2006). The impressive cold tolerance of these species is consistent with the cold-water temperatures at which these animals are captured alive. *Fundulus heteroclitus* was captured in waters ≥−1.5°C and *F. diaphanus* ≥−0.5°C. In the wild, *Fundulus* spp. will often burrow in sediments to avoid extreme cold temperatures. Despite this, southern species of *Fundulus*, including *F. grandis* and other species distributed along the Gulf of Mexico and Florida Peninsula, are subject to winter fish kills linked to precipitous drops in water temperature (reviewed by Nordlie 2006).

The realized thermal niche (i.e., conditions observed in the wild) of *F. heteroclitus* from the Gulf of St. Lawrence in Canada to Florida represents a dramatic thermal gradient (about −0.91°C/latitude change between 26–60 degrees N) (Baumann & Doherty 2013). Between 33–39 degrees N along the Atlantic Coast of North America, sea surface temperatures decrease by as much as 2.55°C/degree latitude change, representing by far the steepest thermal cline in the world. Populations of *F. heteroclitus* distributed from cold northern to warm southern Atlantic Coast habitats are a classic evolutionary model for the study of adaptive variation along a thermal gradient (Schulte 2001; Healy et al. 2010; Dhillon & Schulte 2011; Healy & Schulte 2012). *Fundulus heteroclitus* is eurythermic with the ability to maintain aerobic scope (difference between minimum and maximum oxygen consumption rate) over a wide temperature range (Healy & Schulte 2012), but CT_{max} values are higher in the southern subspecies, *F. h. macrolepidotus* (Fangue et al. 2006). *Fundulus heteroclitus* showed a clinal variation in cardiac lactate dehydrogenase-B (LDH-B) enzyme concentration because of differences in the rate of transcription. In general, LDH-B concentration and the rate of transcription are higher in the northern subspecies, *F. h. heteroclitus*, such that the kinetics at lower temperatures are similar to that found in southern subspecies, *F. h. macrolepidotus*, at higher temperatures (Crawford & Powers 1992). Despite a contemporary increase in water temperature along the U.S. Atlantic Ocean over the last 40 years due to global climate change, no significant change in the allelic frequency of the LDH-B isozyme in *F. heteroclitus* populations has occurred (Bell et al. 2014). These data suggest that despite the influence of temperature on LDH-B isozyme frequency, no microevolution of LDH-mediated thermotolerance has occurred. The enzymatic concentrations of at least three glycolytic enzymes, including LDH-B, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and pyruvate kinase (PYK) decreased as a function of increasing temperature among six other species of *Fundulus* (Pierce & Crawford 1994, 1997). The enzymatic activities of LDH-B, GAPDH, and PYK in the heart ventricles of northern *F. h. heteroclitus* is significantly greater than those measured in *F. h. macrolepidotus*, helping to explain the enhanced metabolism of cardiac tissue in more northerly populations of *F. heteroclitus* (Podrabsky et al. 2000).

Thermal tolerance in *F. heteroclitus* is cyclic with increased ability to tolerate higher temperatures at times of day when temperatures are likely to be higher even when maintained at a constant temperature in the laboratory (Bulger 1984). *Fundulus heteroclitus* likely physiologically anticipate daily variation and change enzyme activity in preparation for predictable thermal changes. Alteration of enzyme activity generally is associated with mechanisms of increased thermal tolerance.

Dissolved Oxygen Tolerance

Dissolved oxygen is another major physicochemical variable influencing the distribution of species of *Fundulus* in the environment. Fishes can elicit a diverse suite of compensatory responses to aquatic hypoxia (low dissolved oxygen), which include physiological and biochemical responses or behavioral changes aimed at enhancing survival in low oxygen environments or providing additional flexibility in coping with temporal and spatial fluctuations in oxygen. One common measure of assessing the magnitude of hypoxia tolerance is measuring the frequency and threshold of aquatic surface respiration (ASR) during gradual reductions in dissolved oxygen and the survival time to severe hypoxia. A second more refined measure involves quantifying the critical partial pressure of oxygen (P_{crit}). The P_{crit} is the dissolved oxygen tension in which the resting metabolic rate becomes dependent upon the external partial pressure of oxygen (Richards 2011). The dissolved oxygen level required for the onset of ASR behavior is slightly below or closely associated with the P_{crit}. However, in some species, estimates of P_{crit} do not always predict the lower lethal dissolved oxygen limits. Regardless, these measures provide important insights into the capacity and compensatory responses to hypoxia in fishes.

Comparatively, more anecdotal information is available on the ranges of optimal dissolved oxygen concentrations of *Fundulus* based on measurements of dissolved oxygen at fish capture sites than on the tolerance limits for these species from laboratory studies (reviewed by Nordlie 2006). Topminnows, like many other fishes, exploit the higher oxygen content water in the surface film to offset hypoxic conditions (Stierhoff et al. 2003). The data that do exist suggest that most species of *Fundulus* are tolerant of extremely low levels of dissolved oxygen for extended periods of time. Although hypoxia tolerance is partly explained by the capacity to take advantage of ASR and aerial respiration (Stierhoff et al. 2003), *F. heteroclitus*, which has a limited capacity to perform aerial respiration, do not seem to avoid hypoxia until dissolved oxygen levels drop to about 1 ppm (Smith & Able 2004). Regardless, *F. heteroclitus* are capable of extended survival in low dissolved oxygen even when unable to perform these behaviors. The species also has a limited capacity to perform aerial respiration. In a study monitoring multiple fish species within a marsh pool with no access to the air-water interface, *F. heteroclitus* had the longest survival time to exposure to low oxygen conditions; 50% of the population tolerated ≤4 h exposure to 0.23 ppm dissolved oxygen levels (Smith & Able 2004). Richards et al. (2008) calculated a P_{crit} of 63.9 torr for *F. heteroclitus*. Despite being extremely hypoxia tolerant (i.e., tolerant to lethal levels of dissolved oxygen, about 10% of the measured P_{crit} after 15 h exposure), *F. heteroclitus* likely has many unique features in its oxygen cascade to function as an oxygen conformer over a wide range of dissolved oxygen tensions (Richards et al. 2008). These data help explain why *F. heteroclitus* is the dominant species in many salt-marsh pools experiencing frequent tidal and diel hypoxic events.

At least three other marsh species of Topminnows are highly tolerant of hypoxia. *Lucania parva*, a mesohaline (brackish water) Topminnow, tolerated dissolved oxygen of 0.91 ppm before initiating ASR and a median dissolved oxygen lethal level of 0.23 ppm for ≤60 minutes (Smith & Able 2004). *Fundulus grandis* can oxygen regulate to a dissolved oxygen level (P_{crit}) of 34 torr (1 torr = 133.3 Pa) below which animals begin to accumulate blood lactate and metabolically conform (oxygen consumption declines) to further reductions in dissolved oxygen (Virani & Rees 2000). *Fundulus grandis* from Mobile Bay, Alabama, which encounters episodic bouts of hypoxia in nature, have a significantly reduced P_{crit}. Hypoxia tolerance in *Fundulus grandis* and *F. parvipinnis* exhibits a consistent yearly cycle with increased ability to tolerate hypoxia in the summer when hypoxic conditions are more likely to occur (Wells 1935; Virani & Rees 2000; Love & Rees 2002). In comparison, *F. grandis* from hypoxic regions tend to have higher metabolic rates in hypoxic waters than fish captured in historically normoxic waters, however, P_{crit} did not significantly vary between populations (Everett & Crawford 2010).

Measures of hypoxia tolerance in freshwater Topminnows are rare, but data indicate a high tolerance for hypoxic waters. For example, *F. pulvereus* has lower lethal limits of 0.88 ppm at 26°C in fresh water (Smale & Rabeni 1995), and *F. notatus* tolerated dissolved oxygen levels of 1.2 ppm and used ASR (Rutledge & Beitinger 1989). In this latter study, hypoxia impaired upper lethal temperature tolerance, but this was offset if animals had access to ASR during hypoxia. In fact, *F. notatus* can live in the absence of dissolved oxygen if they have access to subsurface oxygen (Lewis 1970). *Fundulus* spp. often can survive brief hypoxic conditions by performing ASR. Overall, the study supported the ability of *F. notatus* to acclimatize to low dissolved oxygen levels at times of the year when hypoxia was particularly prevalent and noted morphological adaptations facilitating ASR (e.g., dorsally oriented mouth, flattened head) (Lewis 1970).

Topminnows elicit a suite of biochemical and molecular mechanisms (Martínez et al. 2006) to tolerate hypoxia. In *F. heteroclitus*, the roles of different levels of expression of various glycolytic genes, including LDH-B, vary as a result of exposure to hypoxia and vary among populations (Schulte 2001). Although the activities of glycolytic enzymes are influenced by hypoxia, tissue responses are heterogeneous, suggesting a complex interplay between the change in metabolism and supply of oxygen to tissues during reduced access to environmental oxygen (Richards et al. 2008). Metabolic depression appears to be a mechanism for reducing the metabolic requirements of tissues, but *Fundulus* spp. can also stimulate oxygen independent ATP production when environmental oxygen is limiting (Richards et al. 2008). This type of acclimation to hypoxia, however, is compromised in fishes exposed to the toxin polychlorinated biphenyl (PCB) (Kraemer & Schulte 2004). Further, temperature will likely influence oxygen tolerance given that high temperature and low dissolved oxygen frequently co-occur as a function of the negative relationship between dissolved oxygen and water temperature.

Vision

Topminnows are diurnal and occupy well-lit surface environments that provide a backdrop for the evolution of vision systems. Cyprinodontiforms, in general, tend to have receptors covering a greater portion of the spectrum compared with deep water groups that live in a more light-restricted environment (Munz 1958). Topminnows have rod and cone cells that may be paired (double cones) and of varying sizes. Individual cones typically produce a single opsin that responds to specific wavelengths. Opsin gene duplication occurred early in cyprinodontiform evolution (Ward et al. 2008b) and provided a framework for greater color discrimination and well-studied sexual selection linked to color phenotypes (Houde & Endler 1990). Some species have ellipsosomes, which appear to be analogous to spectral filtering or light funneling oil droplets found associated with other vertebrate photoreceptors (Nag & Bhattacharjee 1995). *Fundulus heteroclitus* has four cone types (red $\lambda = 563$, blue $\lambda = 463$, violet $\lambda = 400$, and ultraviolet, UV $\lambda = 363$), and cones are most dense in the dorsal, central, and nasal regions of the retina (Flamarique & Harosi 2000). *Lucania goodei* has five cone types (red $\lambda = 570$, yellow $\lambda = 540$, blue $\lambda = 455$, violet $\lambda = 405$, and UV $\lambda = 360$), and both *L. goodei* and *F. heteroclitus* are somewhat unique among fishes in expressing both violet and UV opsins. UV reception improves prey contrast and detection in visually oriented fish predators in surface waters (Browman et al. 1994). Prey reaction distances were increased (i.e., fish recognized prey farther away), and pursuit of prey was more precise under UV spectra than full spectra (Browman et al. 1994). UV vison may be especially effective when viewing prey from below in UV-rich environments due to scatter at the surface. Both male nuptial coloration and the proportions of various cone types of *L. goodei* differ among habitats (Fuller 2002). In swampy habitats with lower UV and blue transmission, individuals have fewer UV cones and more blue color morphs compared with clearer water spring habitats (Fuller et al. 2003). Population differences are driven by plasticity in the abundance of types of cones as opposed to structural changes in photopigments (Fuller et al. 2003). How much of this difference may be ontogenetic is unclear because some fishes express UV sensitive opsins early in life (Beaudet & Hawryshyn 1999).

BEHAVIOR

Movement and Migratory Behaviors

Movement and residence of dominant taxa among multiple habitats has important implications for population dynamics and trophic linkages among disparate environments (Kneib 1986; Hunter et al. 2009). As surface feeders that are often numerically dominant in shallow, structured environments, Topminnows play an important role in mediating energy exchange between terrestrial and aquatic systems (Levin et al. 2001). During summer months (July–September), *Fundulus heteroclitus* shows limited movement in salt-marsh creeks, establishing home ranges of about 36 m, which persist through tidal and osmotic fluctuations. Mark-recapture estimates of population size and movement rates concluded that little emigration from or immigration to creeks occurred during this period (Lotrich 1975). In autumn and winter, net directional movement upstream into lower salinity habitat apparently occurs (Fritz et al. 1975). In marsh ponds, *F. heteroclitus* are less likely to emigrate with frequent flooding possibly because flooding introduces prey and improves water quality (Halpin 2000; Hunter et al. 2009). In contrast, three Topminnows (*Fundulus chrysotus*, *F. confluentus*, and *Lucania goodei*) all showed directional movement in response to rising water in Florida Everglades wetlands (Hoch et al. 2015). In a mark-recapture study of fish passage through various types of road crossings in small Ouachita Mountain streams, Topminnows (primarily *F. catenatus*) showed an order of magnitude lower proportional movement through open-box (3%) and culvert (7%) crossings, the two extremes in observed water velocity, relative to fords (33%) or natural reaches (14%). Their relative inability to pass through culvert crossings might be related to high water velocity, but other aspects of crossing configuration (e.g., shading, predator avoidance) apparently influenced passage success (Warren & Pardew 1998). In fresh water, *F. notatus* movement rates in a small Mississippi River tributary in Illinois were substantially greater (mean = 23 m/day) than that of *F. olivaceus* (<1 m/day) in a small Pascagoula River tributary in Mississippi. The differences were hypothesized to be related to local population density or the amount of available habitat structure; *F. notatus* populations were smaller, and the surrounding habitat contained less woody structure (Alldredge et al. 2011).

Predator Avoidance

Most Topminnows spend the majority of their time near the surface (Figs. 30.4 and 30.24), making them susceptible to aquatic and terrestrial predators. A number of escape behaviors can be seen, including jumping out of the water, fleeing, diving to deeper water, or burying in the substrate. My own (JS) observations from years of sampling Topminnows by dip net is that some species (e.g., Western Starhead Topminnow, *Fundulus blairae*; *F. notatus*; *F. olivaceus*; and *F. euryzonus*) remain at the surface while being pursued and will rarely dive. Other species (e.g., *Fundulus catenatus* and *F. chrysotus*) will dive to deeper waters as soon as they are approached. Among species, clear differences also exist in the responsiveness to predators (flight reaction distance). *Fundulus notatus* will begin evasive movements at a greater distance than *F. olivaceus*, possibly an evolved response to greater levels of predation experienced by *F. notatus* in larger river habitat. In *F. diaphanus*, flight reaction distance was not correlated with school size. This response suggests that in larger groups when predators are detected earlier,

Figure 30.24. A male Russetfin Topminnow, *Fundulus escambiae*, in typical Topminnow position just under the surface of the water in Pitt Spring, Econfina Creek, Bay County, Florida, in October. The reverse image is the reflection of the male on the underside of the water's surface. The Russetfin Topminnow is relatively narrowly restricted in distribution to the Gulf Coastal Plain in the Suwannee and Aucilla Rivers east to the Perdido River, Florida and Alabama, where it inhabits vegetated sloughs, swamps, and quiet backwaters and pools of streams (Page & Burr 2011) (courtesy of © Isaac Szabo / Engbretson Underwater Photography).

fish may delay flight responses while assessing the threat (Godin & Morgan 1985).

Some Topminnows jump out of the water to avoid predation, a fairly common observation among fishes. Topminnows will evidently target temporary terrestrial habitat to avoid piscivorous fishes. When pursued by predators, *F. dispar* can leap onto the bank and remain there momentarily until the threat is gone (Boschung & Mayden 2004). A close relative, *F. lineolatus*, will also jump onto the shore when threatened. This species orients itself, via sun compass, for directional escape responses that will ensure it leaves the water and can reliably return with a subsequent jump (Goodyear 1970). Evidence for this phenomenon comes from the fact that orientation of jumping behavior is less accurate on overcast days. *Lucania parva*, which is prey of the Bluegill (*Lepomis macrochirus*) and other piscivores, can avoid predators by jumping out of the water onto lily pads and remaining still for ≤10 s. Bluegills apparently counter this strategy by biting through the lily pad from below (Baylis 1982).

Not all Topminnows are primarily oriented toward the water's surface or escape by jumping. *Fundulus seminolis* is most commonly found in the mid to lower portions of the water column (Wildekamp 1996; Fig. 30.25), and *F. zebrinus* will burrow into the substrate with only the eyes and mouth remaining exposed to avoid predators or thermal stress in unshaded, open, shallow plains streams (Minckley & Klaassen 1969). Burrowing behavior also occurs in *Fundulus luciae* and *F. heteroclitus* (the likely origin of the colloquial name mud minnow) (Chidester 1920).

The ability of Topminnows to avoid predation may be significantly affected by exposure to environmental contaminants or parasites. Exposure of developing *F. heteroclitus* to lead and methylmercury reduced the ability of juveniles to avoid predation by decapod crustaceans (Weis & Weis 1998; Zhou & Weis 1998). *Fundulus diaphanus* is an intermediate host for a trematode parasite (*Crassiphiala bulboglossa*), which modifies host shoaling behavior to facilitate completion of the life cycle in the final host, the Belted Kingfisher (*Megaceryle alcyon*). Infected *F. diaphanus* spend less time in shoals and when in shoals, are more likely to assume peripheral positions (Krause & Godin 1994).

Most Topminnow species form shoals of loosely affiliated individuals. For most species, these are groups of <20 individuals that remain near the surface and forage without apparent directional movement (Arndt 1971). When startled in aquaria settings, Topminnows may form tighter clusters that hover near the bottom. *Fundulus diaphanus* shoals are loose aggregations in which individuals do not exhibit shoal or site fidelity and assort based on body length. This species also assorts based on prevalence of visible external metacercarial parasites, likely as a result of less parasitized individuals avoiding more obviously parasitized fish (Hoare et al. 2000). Shoal size in *F. diaphanus* is related to hunger, perceived food, and threat of predation. Shoals are smaller when individuals are hungry, identify a potential food source, and are not in the presence of a predation threat (Hensor et al. 2003; Hoare et al. 2004). Larger shoals did not respond to predators any sooner but did show less variation in individual responses (Godin & Morgan 1985). The closely related *F. waccamensis* swims in the more open water away from shore where it forms notably larger shoals (Wildekamp 1996), perhaps an adaptation to decrease risk from open-water predators.

Figure 30.25. A Seminole Killifish, *Fundulus seminolis*, swims through dense aquatic macrophyte beds in October in the Rainbow River, Withlacoochee River drainage, which heads from a first magnitude spring in Marion County, Florida. The Seminole Killifish, which is one of the largest freshwater Topminnows (≤16 cm TL), is endemic to Florida where it can be common in lakes and quiet stream pools (courtesy of © Isaac Szabo / Engbretson Underwater Photography).

Feeding Behavior

Most Topminnows are opportunistic surface feeders. Starhead Topminnows, *Fundulus dispar*, form pairs or small groups to cruise at the surface where they feed heavily on terrestrial insects trapped by the surface tension (Forbes & Richardson 1920; Gunning & Lewis 1955; Boschung & Mayden 2004). Even more active feeding can occur, including *F. chrysotus* leaping out of the water to capture flying insects (Boschung & Mayden 2004).

Cleaning Behavior

Several Topminnows engage in intra- or interspecific cleaning behavior. Conspecific or interspecific cleaners are stimulated by vertical posturing or tail-standing by the individual seeking to have ectoparasites picked from its body. *Fundulus xenicus* is the recipient of cleaning by conspecifics and other species of Topminnows (Hastings & Yerger 1971). *Fundulus majalis* along the Atlantic Coast and *F. similis* along the Gulf Coast are cleaned of leeches and flukes by the Sheepshead Minnow, *Cyprinodon variegatus* (Fig. 30.26). The cleaning behavior is elicited by *Fundulus similis* or *F. majalis* adopting a head down, vertical position (Able 1976; Ross 2001). *Lucania parva* removed external parasites from conspecific individuals as well as those of *C. variegatus* (Able 1976).

Alarm Substances

Alarm pheromones in fishes were originally thought to be restricted to ostariophysans, which possess large epidermal club cells that produce the substances (Brönmark & Hansson 2012). When exposed to alarm substances in aquaria, the stereotypical response includes excited swimming activity, typically with the body angled downward. This may be an adaptation to dive into cover, bury in the substrate, or possibly stir up sediment to reduce visibility. Since their first description, alarm pheromones and the neural pathways responsible for the behavioral responses are documented broadly across most teleost groups (Døving & Lastein 2009). *Fundulus olivaceus* secretes an alarm pheromone, which results in fright reactions of nearby individuals. Interestingly, the response seems to be to remain motionless at the water surface and not increase activity or dive (Reed 1969; Ross 2001; Boschung & Mayden 2004). This is generally consistent with some of the other observed antipredator behaviors in this species (e.g., remaining at the surface when pursued). In a laboratory experiment, *F. catenatus* was exposed to chondroitin sulfate, fragments of which elicit an antipredator response in Zebrafish, *Danio rerio* (Mathuru et al. 2012). When Northern Studfish were exposed to chondroitin individuals significantly reduced their movement and were more likely to move to the bottom of the aquarium relative to control fish. These responses suggest chondroitin serves as an alarm-cue component in this species (Farnsley et al. 2016), although expansion and replication of this study is warranted.

REPRODUCTION

Reproduction in Topminnows involves deposition of a relatively small number of large, clear, and adhesive eggs

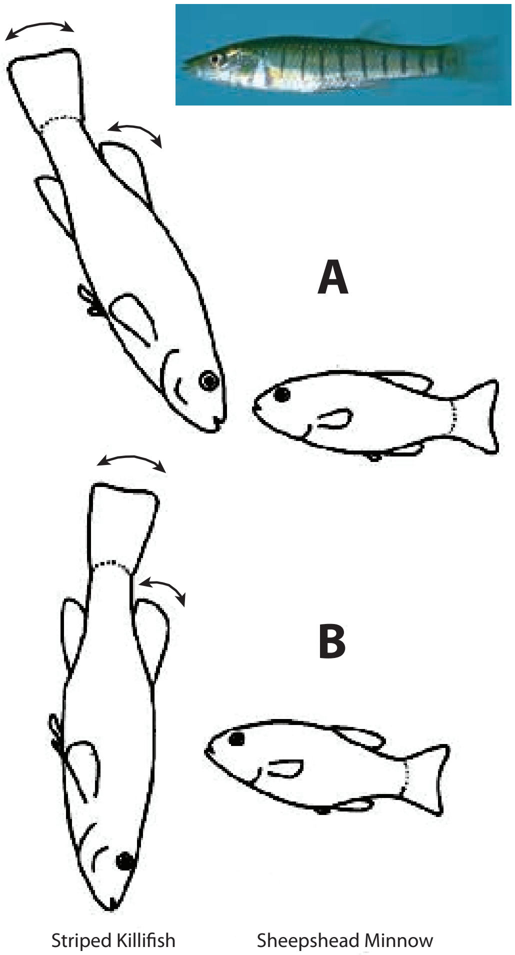

Figure 30.26. Head-down pose of the Striped Killifish, *Fundulus majalis*, soliciting cleaning of external parasites (e.g., leeches, trematodes) by the Sheepshead Minnow (*Cyprinodon variegatus*). (A) The Striped Killifish maintains the pose by rapid beating of the pectoral fins and balancing movements of the partially expanded dorsal and anal fins. (B) The angle of the pose varies (45–90 degrees) with more acute angles apparently being associated with an increase in motivation to be cleaned. Cleaning periods are brief (10–15 s) (redrawn from Able 1976).

onto some kind of substrate, typically aquatic vegetation (Table 30.2). Topminnows are sexually dimorphic (often seasonally, though some fixed morphological differences exist) with females being larger and males more colorful. Most reports of reproductive behavior involve males maintaining some kind of loose territory or home range where females are courted and potential competing males are chased away.

Spawning periods are long, extending from early spring through late summer with southern populations of some species reproducing year round. Female reproductive allotment quantified as gonadosomatic index (GSI) is typically 6–15% (Kneib & Stiven 1978; Fisher 1981; Greeley & MacGregor 1983; Cheek 2011; Lang et al. 2012; Fig. 30.27) with occasional reports of values as high as 25–30% for species with shorter reproductive periods (Edberg & Powers 2010). Ovaries of preserved females usually have a small number of mature eggs and others in multiple stages of development, indicating that spawning occurs repeatedly in small batches (e.g., Lang et al. 2012; Figs. 30.28 and 30.29). This is consistent with observations of prolonged daily spawning in controlled environments (Vigueira et al. 2008; Cheek 2011). A number of the coastal species spawn in cycles related to spring tides (Fig. 30.30). Spawning involves a brief courtship resulting in fertilization of a small number of relatively large eggs with adhesive chorionic coverings or filaments. As in most fishes, development time is largely dependent on temperature and egg size (Gillooly et al. 2002; Schaefer 2012). However, some Topminnows that spawn in tidal or ephemeral habitats can suspend development when embryos are out of the water, similar to the developmental diapause in some of the annual killifishes, such as *Austrofundulus limnaeus* (Järvikilli) (Machado & Podrabsky 2007). Fundulid eggs in suspended development may hatch within minutes after being resubmerged (Foster 1967b).

The North American Topminnows generally reach sexual maturity in <1 year, often within months of hatching, but there is variation. For example, in North Carolina, young-of-the-year of the small-bodied *Fundulus luciae* (40 mm maximum SL) breed at 20–25 mm SL within 2–3 months of hatching, but the larger *F. heteroclitus* (about 130 mm maximum SL) does not breed until the following year (Kneib 1978; Table 30.2). Sexual maturity at age 1 is also true of *F. stellifer* (100 mm maximum SL) in Georgia (Edburg & Powers 2010) and various species of *Fundulus* at northern latitudes: Starhead Topminnow, *F. dispar*, in Illinois (Taylor & Burr 1997) and coastal species in northern latitudes with short growing seasons (Able & Fahay 1998).

Sexual Dimorphism

Typically, male Topminnows are more brightly colored and have longer dorsal and anal fins than females. Some species, such as the studfishes in the *Fundulus catenatus* species group (Figs. 30.4 and 30.20) and other species (Figs. 30.2, 30.3, 30.24, and 30.31) are brilliantly colored over their entire body or parts of their body, and still others are more subdued in coloration but show differences in pigment patterns (e.g., intensity of spotting, dorsal fin spot, vertical bars in males versus lateral stripes in females) (Figs. 30.1, 30.3, 30.32, and 30.33). Juvenile col-

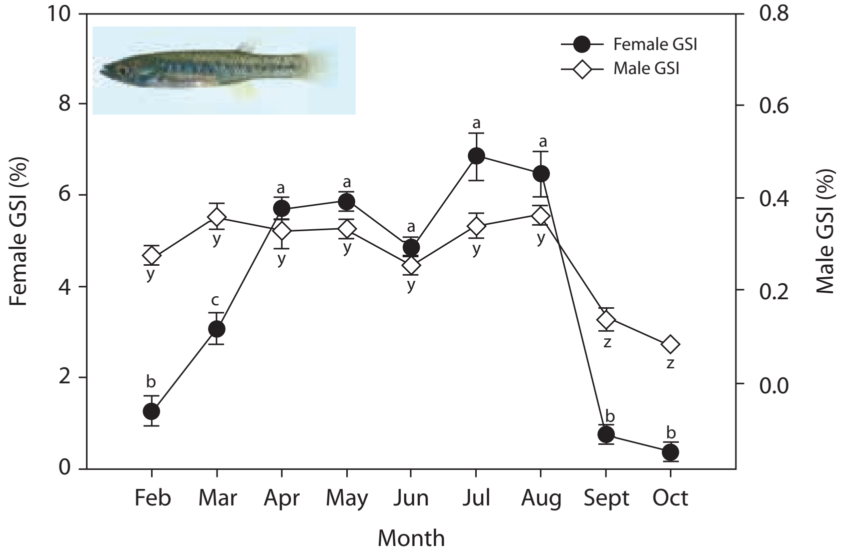

Figure 30.27. Plot of mean (± SE) female and male gonad somatic index (GSI) by month for the Saltmarsh Topminnow, *Fundulus jenkinsi*, captured in flooded estuarine marshes of the Gulf of Mexico, Mississippi. In October, SE bars are hidden by the data point symbol. Data points with the same letters within gender are not significantly different ($P < 0.05$) (redrawn from Lang et al. 2012; *inset* photograph of the Saltmarsh Topminnow, 27 mm SL, Tchoutacabouffa River system, Mississippi, May, by and used with permission of Stephen T. Ross).

oration is similar to adult coloration, except that lateral barring is often more prominent and sexually dimorphic color patterns are absent, only appearing at sexual maturity. In most Topminnow species, females attain slightly larger sizes than males. Size dimorphism in Topminnows is much less pronounced than it is in Livebearers (Poeciliidae), and a few species, such as *F. xenicus*, have males that typically attain larger sizes than females (Boschung & Mayden 2004; Fig. 30.31).

Spawning Season

The spawning period for most Topminnows is long, generally extending from March to September (Figs. 30.27 and 30.28) with peak effort toward the beginning of this period. Substantial variability occurs among species. For example, gravid females of the Southern Studfish, *Fundulus stellifer*, were captured only from March to May with GSI values reduced by June (Edberg & Powers 2010). In a small Louisiana stream, syntopic populations of *F. olivaceus* and *F. euryzonus* females showed peaks in number and size of mature eggs in April and May with these indicators decreasing by August (Fig. 30.29). Topminnows frequenting more saline (and also more southern and less seasonal) environs, such as *Fundulus confluentus*, *F. grandis*, and *Lucania parva*, have nearly year-round spawning in at least some parts of their range. The length of the breeding season for *F. grandis* varies geographically along the northern Gulf of Mexico. The season is January to November in Louisiana and March to October in Texas and may be year-round in parts of Florida (Greeley & MacGregor 1983; Nordlie 2000a).

Daily, Semilunar, and Lunar Spawning Periodicity

During the reproductive period, most Topminnows spawn repeatedly, often daily (Lang et al. 2012). Some of the best evidence for this is that ovaries tend to contain a relatively small proportion of mature eggs even during the peak reproductive period. During the spawning season, female *Fundulus catenatus* carry ≤250 eggs, most of which are not mature (Fisher 1981). *Fundulus heteroclitus* females have ≤512 eggs from March–August, but during the peak spring reproduction, the average female has just 17 ripe eggs (Kneib 1978; Kneib & Stiven 1978). *Fundulus luciae* produced just 9–10 mature eggs / female (Kneib 1978), *F. olivaceus* females carried on average 5–7 mature eggs / female, and *F. euryzonus* females carried 5–9 mature eggs / female during peak reproductive months from April to June in northeastern Louisiana (Blanchard 1996; Fig. 30.29). In captive breeding settings, all of the Topminnows studied produced small (1–20) clutches of eggs daily for weeks at a time (Foster 1967b; Vigueira et al. 2008; Cheek 2011; Berdan & Fuller 2012).

For many estuarine Topminnows, the reproductive period may extend for many months, but spawning activity is primarily semilunar with most activity occurring during spring (biweekly) tides (Fig. 30.30). When this reproductive pattern was first described in *Fundulus heteroclitus*, it was the first such pattern seen in a temperate cyprinodontiform (Greeley & MacGregor 1983; Greeley 1984). In such species, female GSI values are greatest during spring tides at which time eggs are deposited in areas that are subsequently out of the water and immersed again for hatching in the next tidal cycle. In addition to *F.*

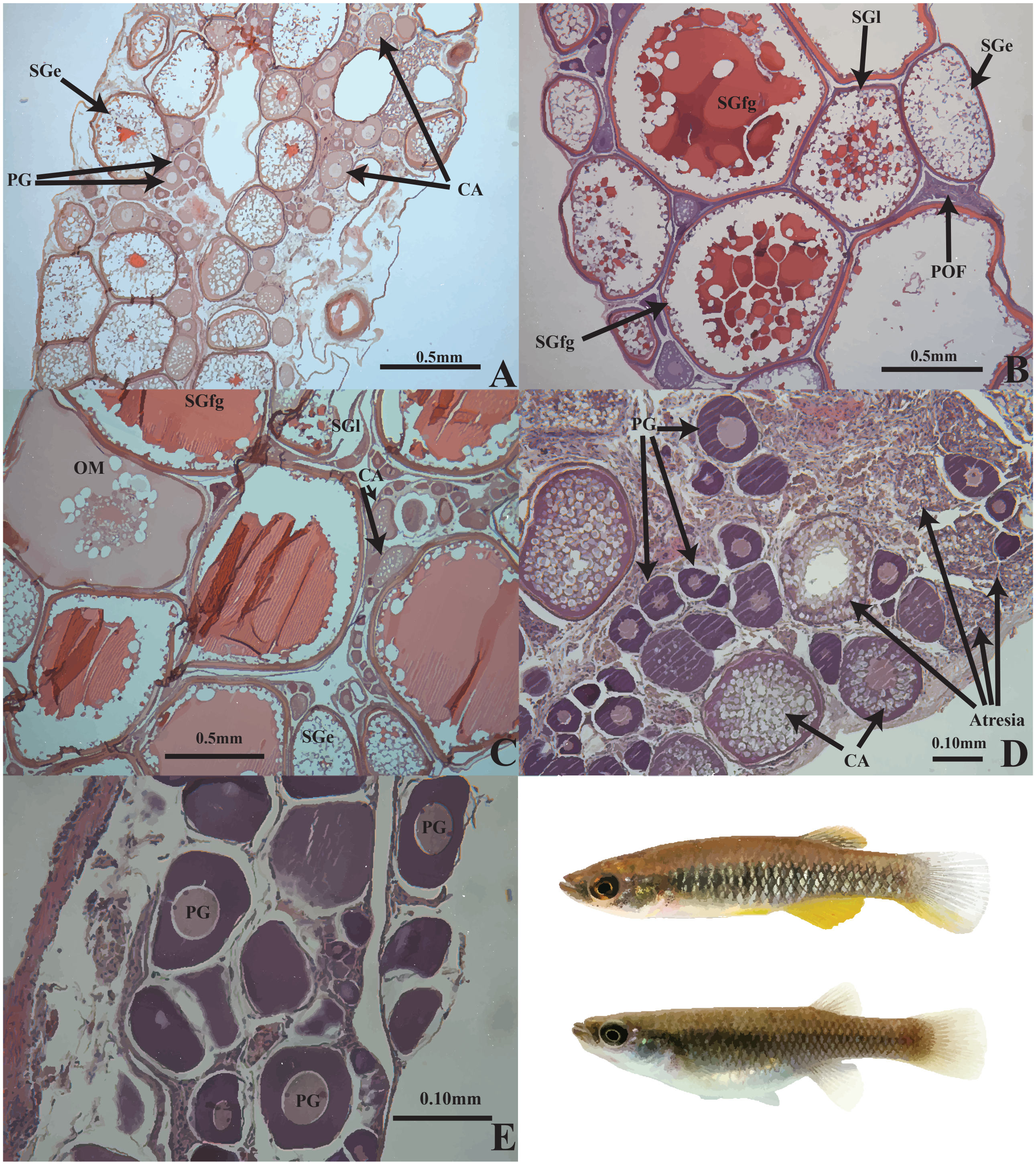

Figure 30.28. Spawning phases of female Saltmarsh Topminnows, *Fundulus jenkinsi*, captured in flooded estuarine marshes of the Gulf of Mexico, Mississippi, as illustrated by photographs of ovarian histological sections. The inset depicts a 50 mm TL male (*upper*) and 53.5 mm TL female (*lower*) Saltmarsh Topminnow. (A) Oocyte developing in March; (B) spawning capable in June; (C) actively spawning in May; (D) oocyte regressing in September; and (E) oocyte regenerating in October. Oocyte stages are labeled as primary growth (PG), cortical alveoli (CA), early secondary growth (SGe), late secondary growth (SGl), full secondary growth (SGfg), oocyte maturation (OM), postovulatory follicles (POF), and atresia (modified from Lang et al. 2012) (courtesy of Erik Lang and Nancy Brown-Peterson).

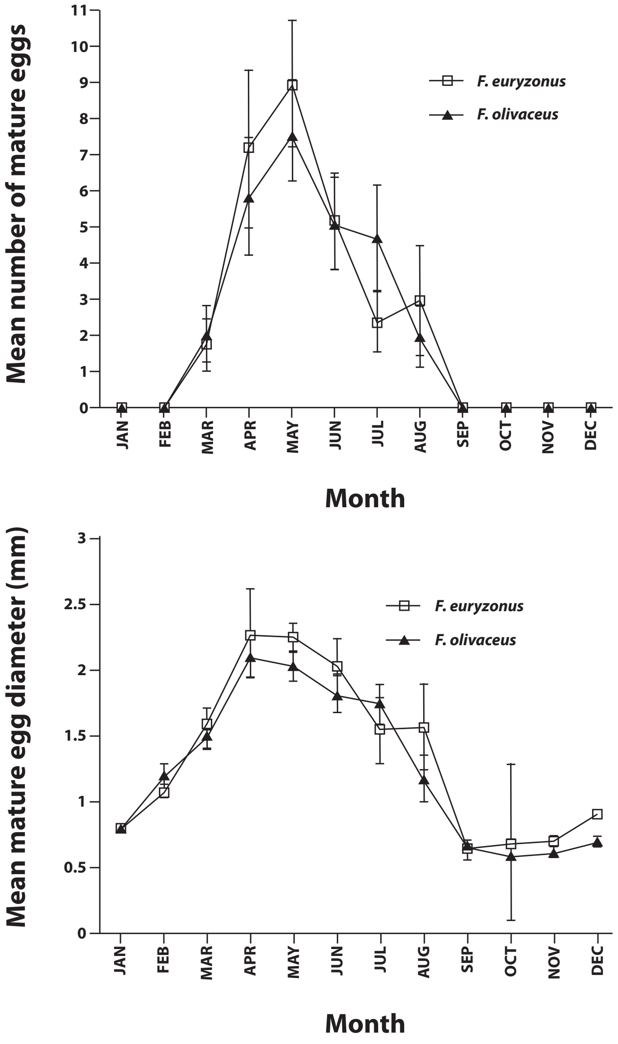

Figure 30.29. (*upper*) Mean number of mature eggs recovered from ovaries of females of the Blackspotted Topminnow, *Fundulus olivaceus* (n = 95 adults, mean = 48.5 mm SL) and Broadstripe Topminnow, *Fundulus euryzonus* (n = 70, mean = 49.1 mm SL) over a 12 month period in Chappepeela Creek, Tangipahoa River, Tangipahoa Parish, Louisiana. (*lower*) Mean egg diameter from measurements of the 20 largest eggs found in ovaries of each individual over time. Error bars are presumably 95% confidence intervals (redrawn from Blanchard 1996).

heteroclitus, this semilunar pattern occurs in *Fundulus confluentus*, *F. grandis*, *F. pulvereus*, *F. xenicus*, Saltmarsh Topminnow, *Fundulus jenkinsi* (Fig. 30.30), *F. majalis*, *F. similis*, and most likely *F. parvipinnis* (Foster 1967b; Lopez et al. 2010; Lang et al. 2012). In *F. grandis*, female GSI values are ≤10% in March–July during the first tide stage. Similar male tidal cycles in GSI are present but less pronounced (Greeley & MacGregor 1983). The tidally synchronized reproductive activity strands embryos out of water for much of the development cycle, likely an adaptation to either protect embryos from predation or to synchronize hatching with favorable conditions to maximize larval growth and survival. The obvious tradeoff involved with this strategy is that adaptations are necessary to protect developing embryos from potentially severe dehydration, temperature, and anoxic stressors (Podrabsky et al. 2010; Warkentin 2011).

Field studies show that tidally synchronized spawning activity in *Fundulus* occurs mainly during a few days around peak spring tides, but geographic variation exists along latitudinal gradients. For example, spawning periodicity in *F. heteroclitus* at southern latitudes on the Atlantic Coast is primarily semilunar, but in Maine it occurs daily during the short (June–early August) breeding season (Petersen et al. 2010). Regardless of whether spawning is semilunar or daily, there is a bias toward greater activity during the higher of the two daily tides on the Atlantic Coast, which means that spawning occurs primarily at night (Hsiao et al. 1994; Petersen et al. 2010). Tidal periodicity on the northern coast of the Gulf of Mexico is more affected by the declination cycle of the moon relative to the equator than by the lunar-phase cycle (Greeley & MacGregor 1983). As a result, only one high tide occurs each day, and the time between spring tides is 13.6 days rather than the 14.8 days on the Atlantic Coast. Consequently, spawning in *Fundulus grandis*, *F. similis*, and *F. jenkinsi* on the northern Gulf Coast shows a periodicity of about 13.6 days (Greeley et al. 1986; Lang et al. 2012).

A detailed study of *F. heteroclitus* in northeastern Florida demonstrated a complex interplay of lunar-semilunar and tidal reproductive cycling in addition to the annual cycle (Hsiao et al. 1994). From February to April, the marsh population showed a lunar cycle with reproductive peaks occurring only during full-moon spring tides. This transitioned to a semilunar cycle from April to September when peaks occurred during both full-moon and new-moon spring tides. Fish brought into the laboratory showed a semilunar pattern of reproductive activity, regardless of whether they were taken from the wild early or late in the season. Remarkably, during days of peak activity, spawning occurred specifically during the higher of the two daily tides. This was reflected in the daily pattern of ovarian activity with follicular maturation and ovulation timed to prepare for spawning during the highest tide of the day. In the laboratory, periodic spawning in *F. heteroclitus* and the closely related *F. grandis* continued even in the absence of daily, tidal, or lunar cues (Hsiao & Meier 1989; Taylor 1999). Even so, synchronization with the spring tides disappeared, suggesting a role for entrainment by external

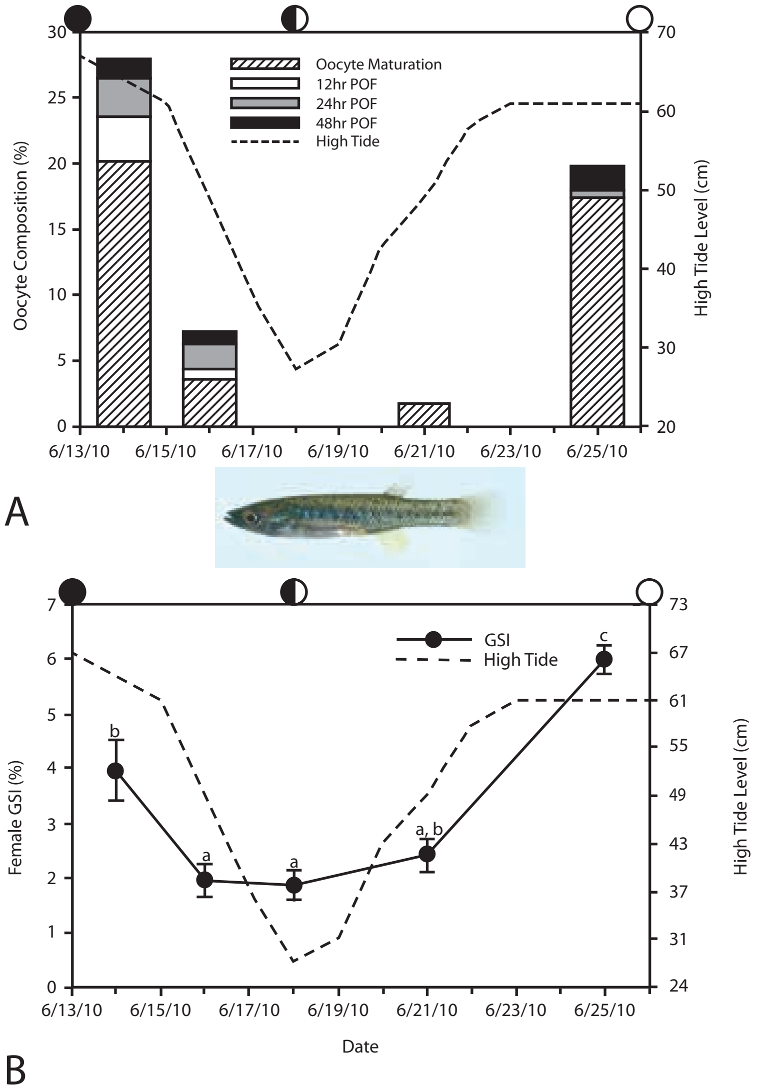

Figure 30.30. As in several coastal Topminnows, females of the Saltmarsh Topminnow, *Fundulus jenkinsi*, spawn primarily during spring tides (tide after new or full moon with greatest difference between high and low tide) but not neap tides (tide after 1st and 3rd quarter moon with least difference between high and low tide) in northern Gulf of Mexico estuarine marshes in Mississippi. Breeding males and females invade high marsh during high tides where fertilized eggs are stranded to incubate in air. Females can spawn over successive days (≤4 days) in the wild with ovarian histology indicating 88% of actively spawning females do so over the April to August spawning season. (A) Percentage of the ovaries of female Saltmarsh Topminnows, captured between two spring tides in June, containing oocytes undergoing oocyte maturation and various stages of postovulatory follicles (POF). (B) Gonad somatic index (GSI) of females relative to high tide level. Moon phases are shown above the graphs on corresponding dates (redrawn from Lang et al. 2012; *inset* photograph, see Fig. 30.27 for details, by and used with permission of Stephen T. Ross).

cues that are not understood (Taylor 1999; see also Bosker et al. 2013).

Eggs

Fundulid eggs are clear, spherical, and covered with adhesive chorionic filaments for attachment to surfaces. Eggs contain conspicuous oil droplets, which are clumped at fertilization but disperse and may number from <10 to >200 (Able 1984). The exact lipid composition and caloric content of eggs is generally unknown. The outer covering consists of a thick (≤13 μm), multilayered chorion, the structure of which varies by species (Kuchnow & Scott 1977). Fertilized eggs range in size from 0.7 (*Lepotlucania*

Figure 30.31. A beautifully colored, breeding male Golden Topminnow, *Fundulus chrysotus*, in June from the St. Marks River, near St. Marks, Wakulla County, Florida. Golden Topminnows inhabit low-flow wetlands, springs, marshes, or lakes where they are associated with often dense beds of aquatic vegetation (Ruud Wildekamp captured the specimen; courtesy of John Brill).

omata) to 3.7 mm in diameter (*Fundulus catenatus*) (Table 30.2). For most species, mature eggs removed from the ovaries are in the range of 1.5–2.0 mm in diameter, and this size usually correlates with female body size. Considerable variation occurs in egg size intraspecifically among populations (JS pers. obs.) and between closely related taxa (e.g., *Fundulus catenatus* 3.5 mm, Fisher 1981; *F. stellifer* 2.75 mm Edberg & Powers 2010; *F. notatus* 1.6 mm, *F. olivaceus* 2.1 mm, *F. euryzonus* 2.1–2.5 mm, Blanchard 1996; Vigueira et al. 2008). Because of their large size and durability, *F. heteroclitus* embryos were a model for a variety of developmental studies (Atz 1986). The chorion of the fertilized egg is thick and durable, allowing in part, for the remarkable desiccation tolerance in some species.

Development

Embryonic dormancy is widespread among fishes and can take the form of delayed hatching, embryonic diapause (suspended development), or anoxia-induced quiescence (inactivity) (Podrabsky et al. 2010). Within cyprinodontiforms, some annual killifishes (e.g., *Austrofundulus limnaeus*) go through two distinct diapause stages, which are required for development of embryos. The North American Topminnows are not annual, and eggs will undergo continuous development from fertilization to hatching under favorable conditions. Under some conditions, a few species (i.e., *Fundulus pulvereus*, *F. heteroclitus*, *F. xenicus*, and *F. confluentus*) delay hatching for extended periods (1–5 weeks) as an adaptation for spawning near the high-tide line during spring tides (Harrington 1959; Taylor et al. 1977; DiMichele & Taylor 1980, 1981; Cunningham & Balon 1985; Martin 1999). The embryos, which are left stranded after tidal recession, complete development and delay hatching until immersed, normally during the next springtide. Laboratory tests revealed successfully delayed hatching (past normal hatching time) of ≥2 weeks in *F. heteroclitus* (Taylor 1999) and 3 months in *F. confluentus* (Harrington 1959). In those instances, eggs were collected from the field and kept in a humid microenvironment but out of water.

Delayed hatching is best studied in *F. heteroclitus* where embryo development proceeds most rapidly (both in time to hatching and in size and developmental stage of the larvae) in eggs that are incubated out of the water for ≥6 days. Air-incubated eggs hatch four days earlier and are 2 mm larger and roughly twice the wet mass of those incubated under water (Tingaud-Sequeira et al. 2009). When embryos were experimentally placed in empty mussel shells at different positions relative to the high tide line, those continually immersed or immersed by daily flood tides failed to hatch and died, but embryos near the high tide mark hatched in 9–14 days when reached by the tides (Taylor 1999).

The accelerated development in air-exposed embryos of *F. heteroclitus* may be, in part, a function of increased oxygen availability to support accelerated metabolic processes (DiMichele & Taylor 1980; Tingaud-Sequeira et al. 2009). Hatching after completion of embryonic development is stimulated by reduced oxygen availability in going from air to water (DiMichele & Powers 1984). Delayed development and terrestrial survival of embryos is not limited to the estuarine Topminnows. Many freshwater Topminnows deposit eggs on vegetation near the surface in what are clearly ephemeral habitats prone to drying. *Fundulus cingulatus* embryos develop fully in damp moss and hatch quickly when immersed in water (Lee et al. 1980). The most rapid and successful development of *Fundulus chrysotus*, *F. sciadicus*, and *F. cingulatus* embryos occurred when they were partially exposed to air (only surface tension keeping embryos moist) (Foster 1967b). Embryos contained within damp peat moss could be stored for longer periods and hatched within a few hours of being placed back in water. Embryos of those same species develop more slowly (≤4–8 days delayed) and less successfully (reported fungal infections) when submerged throughout development.

Development rates and hatching success are also closely tied to temperature and shaped by the evolutionary history of individual species. *Fundulus olivaceus* develops

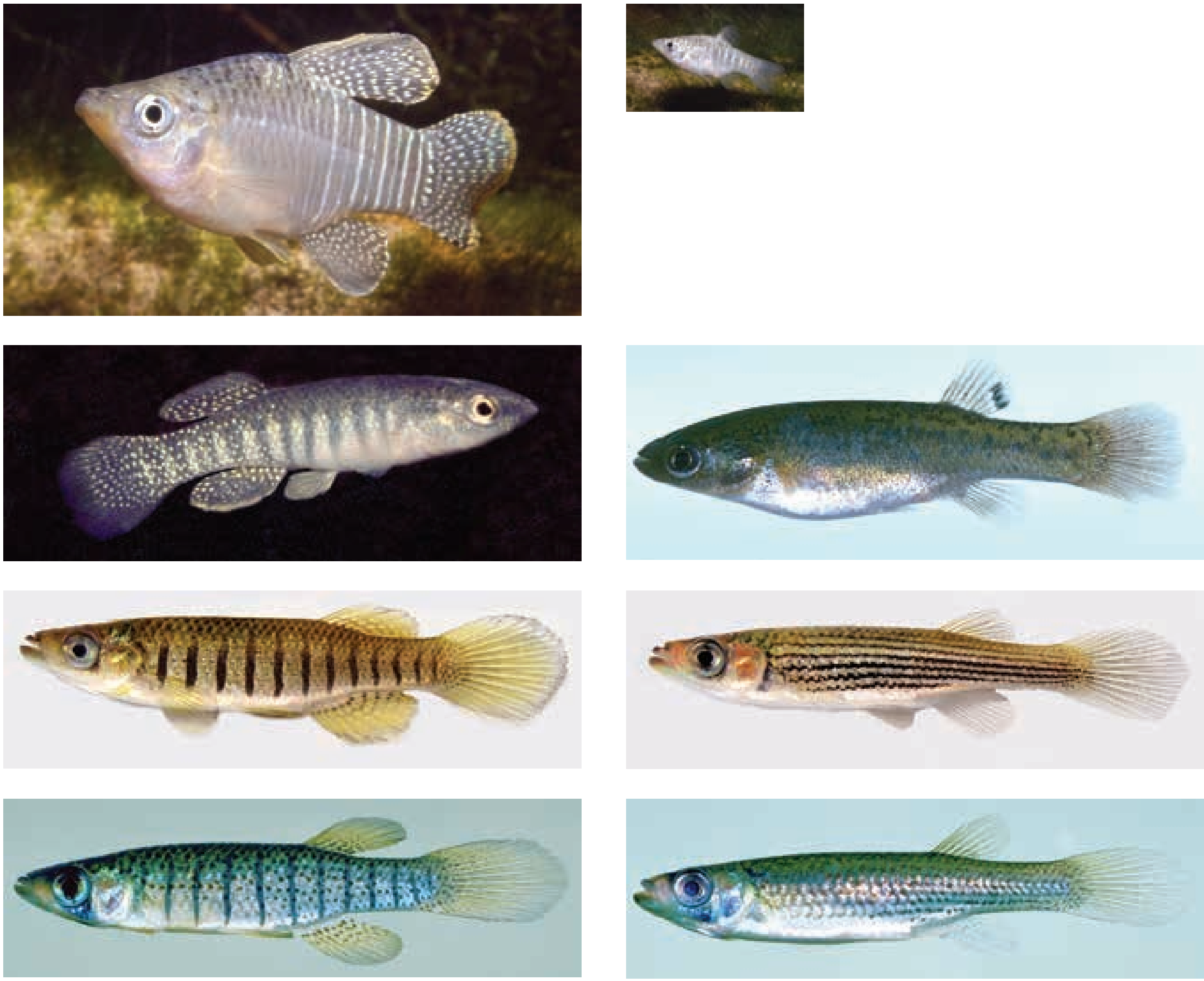

Figure 30.32. Topminnows, Fundulidae, can show sexual dimorphism in size and fin shape but also in pigmentation patterns and coloration, which is often most pronounced during the breeding season (males on *left*, females on *right*): (*upper*) Diamond Killifish, *Fundulus xenicus* (aquarium specimens, original locality unknown); (*upper middle*) Bayou Killifish, *Fundulus pulvereus* (male locality unknown; female 38 mm SL, USM 20733, June, coastal river, Mississippi); (*lower middle*) Lined Topminnow, *Fundulus lineolatus* (October, Hopkins Prairie Lake, Marion County, Florida); (*lower*) Bayou Topminnow, *Fundulus nottii* (male, 46 mm SL, USM 9609, female 47 mm SL, USM 9634, August, coastal river, Mississippi) (courtesy of John Brill, male and female Diamond Killifish, male Bayou Killifish; Nate Tessler, Lined Killifish; and Stephen T. Ross, female Bayou Killifish, male and female Bayou Topminnow).

Figure 30.33. A breeding pair of Pygmy Killifish, *Leptolucania ommata*, in May in Lake Hiawatha, Hillsborough County, Florida. The female (*left*) has two distinct spots connected by a distinctive lateral stripe that is lacking in the male; the male (*right*) has larger fins and an overall iridescence, being especially prominent on the fins, sides of the body, and as narrow bars on the caudal peduncle (courtesy of © 2013 Anthony Terceira).

faster and hatches at a larger size (lower mass-corrected development time) but is less eurythermic than *F. notatus* (Schaefer 2012). Optimal hatching success temperature for *F. notatus* was estimated as 26.5°C, and hatching success remained >25% at high (34°C) and low (16°C) temperatures tested. *Fundulus olivaceus* hatching success optima was estimated as 27.9°C and decreased to <10% at 34°C and 16°C. The faster development in *F. olivaceus* compared with *F. notatus* is consistent with the higher basal metabolic rate in *F. olivaceus* (Schaefer & Walters 2010), and the lower optima in *F. notatus* may be related to the more northern distribution and onset of reproduction earlier in the spring.

Reproductive Behavior

For many Topminnows, accounts of courtship and reproductive behavior are incomplete and assumed to be similar to closely related species. In other cases, detailed descriptions are based on observations in aquaria. Here, we summarize some of the available detailed accounts of reproduction in some well-studied Topminnows. These studies of captive fish may accurately portray courtship and breeding behaviors but may not provide the most valid information on fecundity, sexual maturity, egg size, and duration of development in nature.

Breeding behavior in *Fundulus xenicus* occurs during 3–6 day spans of semilunar spring tides. Males pursue females and male-male aggressive interactions occur frequently. During courtship, a male and female will pair off and swim together with the male above and slightly behind the female (Fig. 30.34). Males will interrupt their courtship to fend off rival males, which sometimes results in a larger challenger replacing a smaller defender. On a selected spawning site the female brings her urogenital papilla into contact with the substrate to deposit a lone egg, while the male clasps the female's side with his dorsal, caudal, and anal fins. Fecundity is low with 20 mm SL females carrying 70–105 eggs, only 15–30% of which are mature. The fertilized eggs are relatively large, 2.0 mm in diameter, and hatch in about 14 days in water temperatures at 25.5°C (Koenig & Livingston 1976). The adult body form is reached in about 30 days, and sexual maturity for both sexes is reached at 19–20 mm SL (Hastings & Yerger 1971). The extent of the larval stage is about 45 days after hatching, when TL is about 15 mm. With 10 days as the earliest age at hatching, the end of the larval period for *F. xenicus* is ≥55 days from activation (Cunningham & Balon 1985). The adult period begins within 49–52 days of hatching (Cunningham & Balon 1986). Sexual maturity is reached at 19–20 mm SL (Hastings & Yerger 1971).

Fundulus catenatus and *F. julisia* males establish and defend small territories in shallow, non-flowing waters near the bank. Patches of regenerated scales commonly seen in mature males may be indicative of male-to-male combat (Boschung & Mayden 2004). Peak spawning for *F. julisia* occurs from late May through early June at water temperatures of 15–20°C. Spawning may extend into August and September when water temperatures are ≤30°C. Courtship involves males approaching females with fins depressed; receptivity to males is signaled by females nipping at spawning substrate, which in turn stimulates a

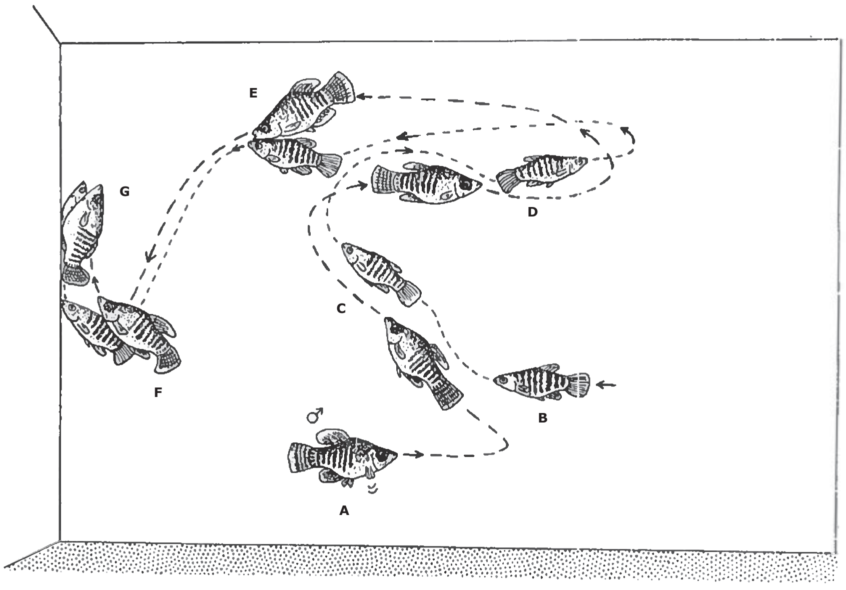

Figure 30.34. Diagram of the courtship and spawning behavior of the Diamond Killifish, *Fundulus xenicus*, from observations in aquaria. (A) Male posts on territory; (B) female enters his territory; (C, D) male pursues female (and male often circles the female, not shown); (E) male jaw-nudges the female; (F) female nips at the potential spawning site; (G) pair clasp in a vertical position, tremble strongly and jerk, and release eggs and sperm (modified and redrawn from Arndt 1971).

spawning embrace from the male and egg deposition in vegetation (Rakes 1989). *Fundulus catenatus* may use Longear Sunfish, *Lepomis megalotis*, nests for spawning in Missouri (Pflieger 1997), but more typically the eggs are deposited in clean, open gravel (Rakes 1989). In Kentucky, 1–4-year-old females have between 28–245 eggs in the ovaries at various developmental stages with mature ova ranging from 0.5–3.5 mm in diameter, suggesting only a small portion of ova are available during a spawning event (Fisher 1981).

Spawning in several other *Fundulus* is similar to the previous descriptions. *Fundulus notatus* courtship includes male lateral displays and opercular flaring followed by the male trailing below and behind the female. When ready, females will swim along vegetation and the pair will assume an S-shape, stop and vibrate for 1–2 s that will terminate with a flip of the male caudal fin (Fig. 30.35). The flip of the caudal fin might serve to release the egg and attach it (via adhesive chorionic filaments) to adjacent vegetation (Carranza & Winn 1954). Spawning behavior is similar in *F. olivaceus* and *F. chrysotus* (Fig. 30.36). Mature males chase conspecific males from a selected spawning area. Courtship involves head bobbing by males (Foster 1967b; Baugh 1981) followed by male-female contact and brief vibrations before egg deposition.

Male *F. olivaceus* have a larger number of dorsolateral spots than females, and females prefer spawning with larger and more heavily spotted males. The density of spots also varies by drainage; populations in more turbid areas have fewer spots indicating spots are a visual signal of mate quality to females (male spot density correlates with male GSI). In predation trials, males with more spots were also more vulnerable to predation, indicating spot phenotypes are under local natural and sexual selection forces (Schaefer et al. 2012). Interestingly, female *F. olivaceus* also have discrete, dorsolateral spots albeit fewer than the males, but female spot density and GSI show no relationship, and females with more spots are not more prone to predation. Among drainages, male and female spot density is correlated indicating selective pressures impacting male spot phenotypes are also being expressed in females (Schaefer et al. 2012).

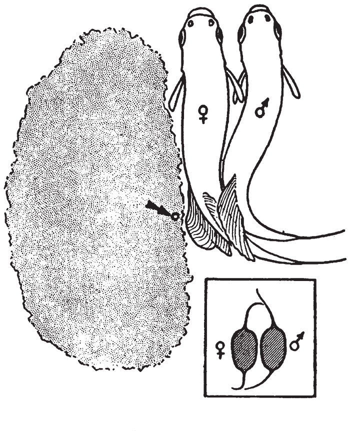

Figure 30.35. Spawning act of the Blackstripe Topminnow, *Fundulus notatus*, against a mass of algae. In this position, the male and female vibrate for 1–2 s with their throats expanded. The vibration is ended with a flip of the caudal fin by the male, which might force the single egg into the algae. Frequently, the egg is released from the female by the vibrations, but it adheres to her by a mucous thread until the flip of the caudal fin pushes it into the vegetation (Carranza & Winn 1954). Arrow indicates an egg. *Inset* is a cross section showing the position of the dorsal and anal fins (redrawn from Carranza & Winn 1954).

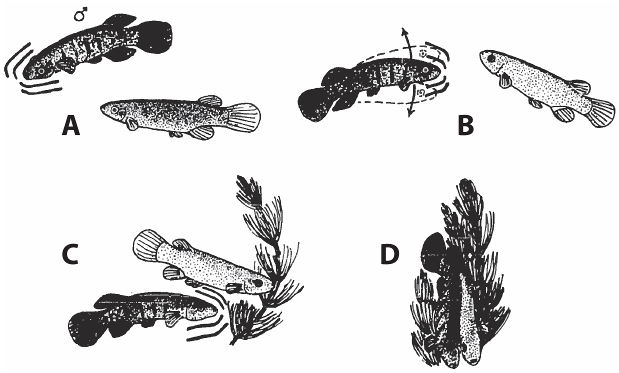

Figure 30.36. Spawning behavior in the Golden Topminnow, *Fundulus chrysotus*, as determined from field observations in Florida and in aquaria: (A) the male displays and head-flicks at female; (B) the male head bobs at female; (C) the female nips at suitable spawning substrate; and (D) the pair clasps against substrate and spawns (redrawn from Foster 1967b).

Courtship behaviors are similar between *F. heteroclitus* (multiple populations) and *F. grandis*. Males slowly approach females with medial fins folded; receptive females are courted through a series of looping (figure-8 pattern) or circular movements while occasionally vibrating the posterior portion of the body in front of the female. Aggressive interactions with nonreceptive females include lateral displays, flared opercula, and tail wags, which typically result in the female fleeing. Receptive females begin looking and nipping at suitable egg deposition sites. Males begin nudging females, and the pair forms an S-shape briefly at which point the pair vibrates and releases eggs and sperm. As many as 70–108 eggs may be released over 2–9 clasps from a large (90 mm TL) female (Able & Hata 1984). Eggs are left above the high-tide mark until the next tidal cycle. The preferred egg deposition site seems to be variable by population and can include vegetation, sandy substrate, or mussel shells (Able & Castagna 1975; Able & Hata 1984).

Breeding behavior in *Leptolucania ommata* is more complex than in some other members of the family. Males defend loose territories or home ranges about 1 m in diameter in dense vegetation at the uppermost portion of the water column (Foster 1967b; Arndt 1971). Males behave aggressively toward other male *L. ommata* by exhibiting sustained fin erection. Male courtship displays include lateral displays, fin spreading, and head flicking (Fig. 30.37). Females respond with a wriggling behavior, which may indicate submission and stimulate the males. Receptive females enter the dense vegetation with a male quickly taking a place along her side. The pair forms an S-shape as the male clasps his mate with dorsal and anal fins. The release of eggs and sperm follow a rapid series of tremblings. Eggs adhere to leaves and stems of the vegetation. Reproduction occurs during their first spring or in the autumn if hatched early in the spring months. The autumn-hatched fish are small (12–14 mm SL) compared with typical adults (15–20 mm SL), which makes *L. ommata* a candidate for the title of smallest freshwater fish in North America. Peak spawning occurs in April and May, but some populations may spawn all year. Mean egg diameters range from 0.7 to 1.1 mm, which varies by female size and is the smallest recorded for any Topminnow. Clutches are small with 1–4 ripe eggs / spawn. Fecundity and breeding season are unknown (Foster 1967b). Life span is likely about 13 months with a 2-year maximum (Freeman & Freeman 1985).

Courtship behaviors and displays are similar between *Lucania goodei* (Figs. 30.3 and 30.38) and *L. parva*, except for head flicking (present in *L. goodei*, absent in *L. parva*). Males guard territories containing fertilized eggs, or other females, from conspecific males and cyprinid predators (Arndt 1971; Fuller & McEachran 2001). Males flare fins in lateral displays (Fig. 30.38), followed by swimming around the female while performing head flicks beneath the female's head (Foster 1967b; Fig. 30.39). The pair come together and vibrate while the posterior portions of the body are pressed together, ending in the deposition of eggs on vegetation. Male *L. parva* defend a territory of vegetation by lateral displays and intense exaggerated back-and-forth swimming over the territory. Head flicking was not observed during courtship. A receptive female extrudes only a single egg in response to fin clasping by the male. Repeated spawning occurs at about 30 s intervals with the same or a different male (Foster 1967b). Hatching occurs in 6–7 days at 24°C (Fig. 30.15), sexes are distinguishable at 29 days, and reproductive behaviors appear by 52 days. Females produce multiple clutches during a prolonged spawning season. A single female may produce

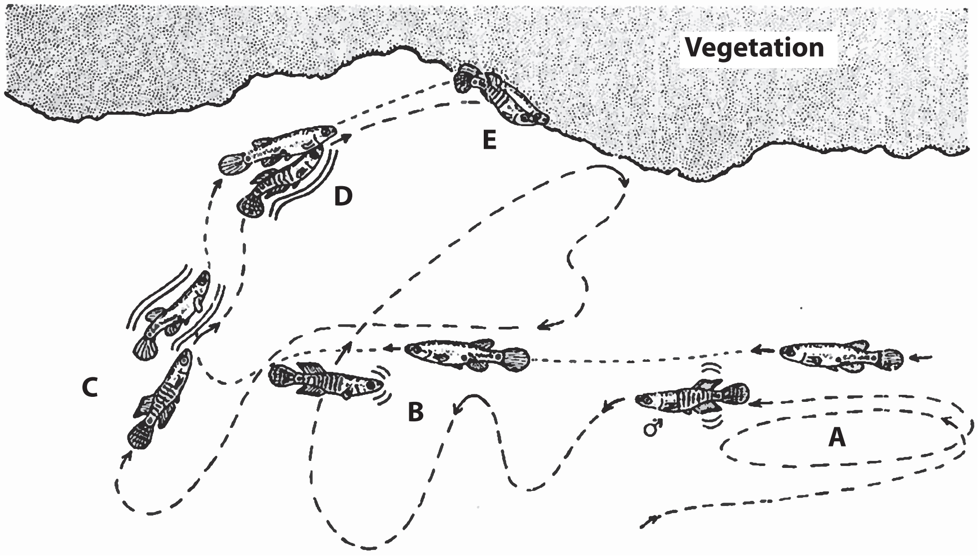

Figure 30.37. Diagram of the courtship and spawning behavior of the Pygmy Killifish, *Leptolucania ommata*, from field observations in Harris Creek and Levy County Lake, Florida: (A) male circles and fin-flicks around a female that has just entered his territory; (B) male continues to circle, often leading up to the vegetation of the potential spawning location, and often facing and head-flicking her; (C) female wriggles, often while swimming toward the spawning location; (D) male swims up below female and contacts and rubs her isthmus; (E) pair swims into and clasps vegetation and with trembling release eggs and sperm (modified and redrawn from Arndt 1971).

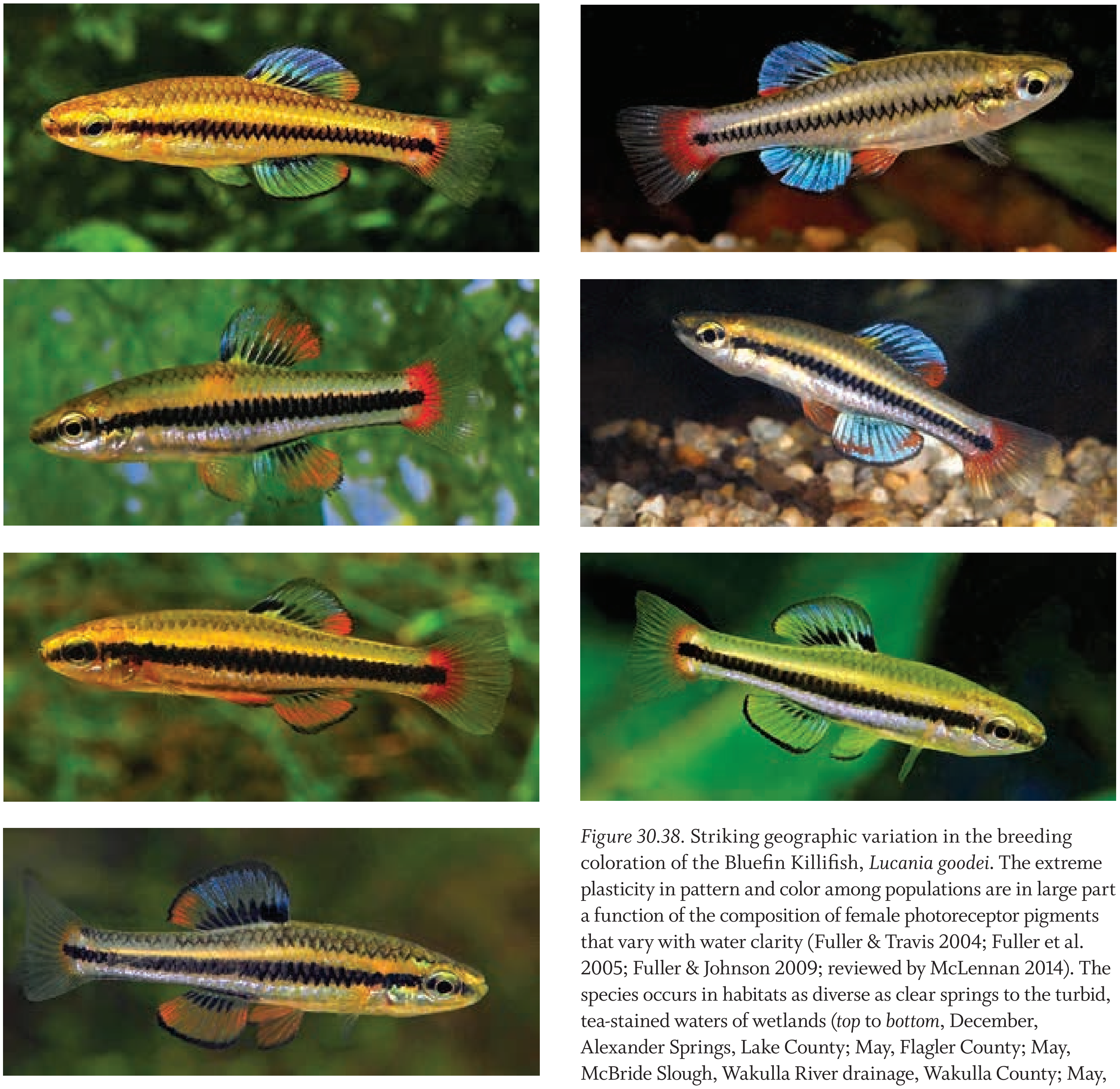

Figure 30.38. Striking geographic variation in the breeding coloration of the Bluefin Killifish, *Lucania goodei*. The extreme plasticity in pattern and color among populations are in large part a function of the composition of female photoreceptor pigments that vary with water clarity (Fuller & Travis 2004; Fuller et al. 2005; Fuller & Johnson 2009; reviewed by McLennan 2014). The species occurs in habitats as diverse as clear springs to the turbid, tea-stained waters of wetlands (*top* to *bottom*, December, Alexander Springs, Lake County; May, Flagler County; May, McBride Slough, Wakulla River drainage, Wakulla County; May, McBride Slough; May, Morris Bridge, Hillsborough River drainage, Hillsborough County; March, Myakka River State Park, Sarasota County; July, Wacissa Spring, Wacissa River drainage, Jefferson County, all Florida) (courtesy of © 2014 Anthony Terceira).

≤104 eggs (Hildebrand & Schroeder 1928; Crawford & Balon 1994b), but clutch size in a mating bout was 15.6 eggs/female, high given the small body size of *L. parva*. Inactivated eggs are clear, with a chorionic filament and a mean diameter of about 1.2 mm (Crawford & Balon 1994a).

ECOLOGY

Habitat

Topminnows are generally adapted to the upper portions of shallow water columns, often near structure or edge habitat. Topminnows occupy this fairly specific niche across a broad geographic range (Table 30.2; Figs. 30.5–30.7). Notable exceptions are *Fundulus zebrinus* and *F. kansae*, which are more bottom-oriented. Some broadly distributed species (e.g., *Fundulus notatus*, *F. heteroclitus*, and *F. grandis*) occur in a variety of ecological conditions, but other species with more restricted ranges (*Fundulus waccamensis*, *F. euryzonus*, *F. bifax*, and *F. jenkinsi*) have more

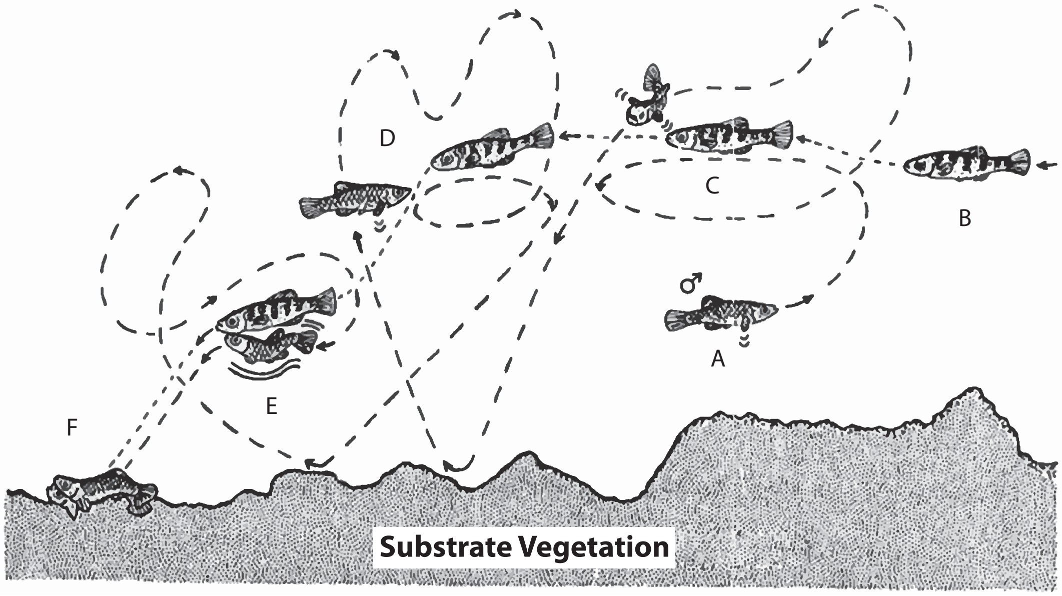

Figure 30.39. Diagram of the courtship and spawning behavior of the Rainwater Killifish, *Lucania parva*, from field observations in Alexander Springs, Florida. (A) Male posts near the bottom of his territory (about 40 cm^2, depending on male size and densities of other males), which is usually over algal beds at depths of 1.2–1.8 m (range 0.3–3.7 m); (B) female enters his territory; (C) male circles around female, occasionally leading her to the bottom; (D) as female slows down, male continues to display and lead, and often stops while facing the female; (E) female moves closer to spawning substrate, the male swims behind her and rubs her isthmus; (F) pair clasps in vegetation, releases eggs and sperm (modified and redrawn from Arndt 1971).

specific habitat requirements. In fresh water, Topminnows can occupy high-gradient streams with cobble or gravel substrate (*Fundulus catenatus*, *F. dispar*, and *F. olivaceus*), springs (*F. julisae*), backwaters and margins of larger rivers (*Fundulus notatus* and *F. euryzonus*), low-flow wetlands, marshes, or lakes associated with aquatic vegetation (Bayou Topminnow, *Fundulus nottii*, *F. blairae*, *F. chrysotus*, *F. cingulatus*, and *Leptolucania ommata*), and shallow plains streams (*F. zebrinus* and *F. kansae*). In brackish or marine habitats, species occur from high marsh habitat, which is often isolated (*Fundulus luciae* and *F. jenkinsi*), down through estuaries and tidally influenced streams (*Fundulus heteroclitus* and *F. grandis*) to more open high-salinity water over sandy substrate (*Fundulus similis* and *F. majalis*); still others occur primarily in the subtidal zone being most common in low salinity or fresh water (*F. diaphanus*) (Fig. 30.40). Because the shallow habitats tend to be low in volume, they often show high seasonal or diurnal variability in physicochemical properties (e.g., temperature, dissolved oxygen, and salinity) that often play an important role in structuring fish assemblages (Matthews 1987, 1998; Peterson & Ross 1991; Smale & Rabeni 1995). Many Topminnow embryos develop most successfully in moist and air-exposed situations, linking these species to more ephemeral habitats. The high tolerances documented for many Topminnows (see physiology section) often make them dominant in these variable environments (Moyle 2002). Three of the eight most abundant species in throw trap surveys of Florida Everglades marsh habitat were Topminnows (*Fundulus chrysotus*, *F. confluentus*, and *Lucania goodei*). Densities of each Topminnow species ranged from 0.1 to >1.0 individuals/m^2 and were generally higher at sites inundated >300 days/year (Hoch et al. 2015).

Freshwater species tend to inhabit slower or lentic freshwater habitats. *Fundulus nottii*, *F. dispar*, and the Southern Starhead Topminnow, *Fundulus blairae*, are not as well studied as other Topminnows, but generally prefer clear waters in vegetated areas of ponds, shallow backwaters, or slow-flowing streams (Ross 2001; Boschung & Mayden 2004). *Leptolucania ommata* occurs in blackwater habitats along the eastern Gulf and Atlantic Coasts, which are typically heavily vegetated sloughs, swamps, and backwaters with reduced flow and low pH (3.8–6.2) (Ross 2001; Boschung & Mayden 2004).

Lucania interioris (Fig. 30.41) is restricted to isolated areas in the partially arid Cuatro Ciénegas basin in the State of Coahila, Mexico. In this setting, *L. interioris* occurs in clear, shallow, marshy pools with outfalls from cool to

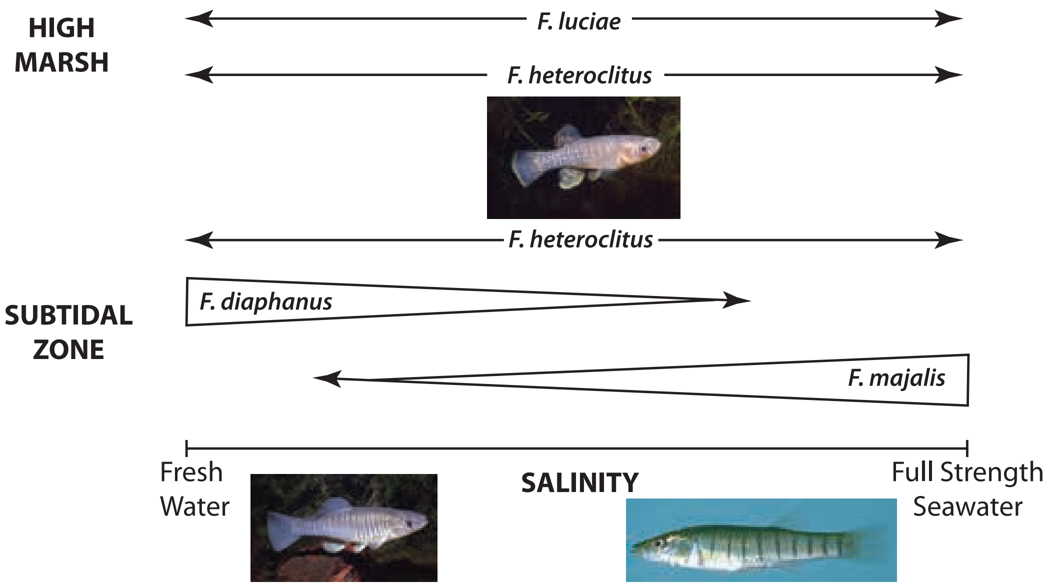

Figure 30.40. The distribution of four Atlantic estuarine *Fundulus* relative to salinity and tidal height zonation. Striped Killifish (*lower left inset*), *Fundulus majalis*, and Banded Killifish (*lower right inset*), *F. diaphanus*, occur primarily in subtidal areas. Spotfin Killifish, *Fundulus luciae*, occur almost exclusively in pools in the upper intertidal zone of marshes. Mummichogs (*upper inset*), *Fundulus heteroclitus*, occur in both subtidal and intertidal regions. Juvenile Mummichogs inhabit intertidal pools almost exclusively, but adults tend to access intertidal areas only at high tide (reviewed by Weisberg 1986) (redrawn from Weisberg 1986; *insets* by and used with permission of John Brill, *upper* and *lower left*, and Stephen T. Ross, *lower right*).

warm freshwater springs. The species also inhabits nearshore regions of small terminal lakes with substrates ranging from deep mud to fine marl detritus, firm clay, and gravel. Such waters are highly alkaline to highly saline with depths of 1.5–45.0 cm and moderate to no current. Vegetation associated with the fish includes abundant *Chara* and flooded terrestrial grasses (Miller et al. 2005).

Of the two species of *Fundulus* that are native west of the Rocky Mountains, *Fundulus lima* has a somewhat unusual habitat preference compared with the eastern members of the genus. *Fundulus lima* frequents clear, low saline tule (bulrush) springs in the interior Pacific Slope waters of Baja California Sur, Mexico. The other species, *F. parvipinnis* (Fig. 30.42), inhabits coastal lagoons, bays, estuaries, and river mouths in salt to fresh water, a range of habitat types exhibited by several wide-ranging Atlantic and Gulf Coast species.

Several other species are predominantly salt to brackish water species, but some show high variability. In Mexico, *F. grandis* (Fig. 30.19) occurs in coastal lagoons, tidal marshes, and rivers at high salinities (76 ppt) but also in fresh water (Miller et al. 2005). In the United States, the species enters tributary streams in the Gulf of Mexico, which are essentially fresh for brief periods but also occurs in large numbers in the Brazos River, Texas, >400 km from the Gulf. The closely related Giant Killifish, *F. grandissimus*, is also a salt to brackish water inhabitant and restricted to the Atlantic Slope of Yucatan Peninsula. *Fundulus persimilis*, the Yucatan Killifish, another Yucatan Peninsula endemic, occurs in brackish to highly saline (51.7 ppt.) estuaries with little or no aquatic vegetation beyond the mangrove borders (Miller et al. 2005).

Segregation along Ecological Gradients

In areas with high fundulid diversity, groups of similar species are often found segregated along distinct ecological gradients. Within freshwater streams, *Fundulus olivaceus* (and in some areas, *F. catenatus*) occur in headwaters upstream of *F. notatus* throughout a broad range of sympatry (Schaefer et al. 2009; Duvernell & Schaefer 2013). In the Lake Pontchartrain drainage, the endemic *F. euryzonus* is found in middle reaches between *Fundulus olivaceus* and *F. notatus* with coexistence of all three species in a few sites (Suttkus & Cashner 1981; Schaefer et al. 2009). Within coastal streams, *Lucania goodei* frequents ponds and pool sections of streams with abundant vegetation and low salinity. It is replaced downstream in higher salinity habitat by *L. parva*, which occupies salt marshes, estuaries, lagoons, springs, and rivers often with salinity >10 ppt (Crawford & Balon 1994b; Jordan 2002; Miller

Figure 30.41. The Cuatro Ciénegas Killifish, *Lucania interioris* (*upper*, mature male; *lower*, young male), is a small (adults usually 20–30 mm SL), narrowly restricted endemic occurring only in saline, often highly alkaline, lakes and pools, marshes, cool to warm springs, and their outflows in isolated parts of the Cuatro Ciénegas basin, Coahuila, Mexico. The species is considered endangered by the American Fisheries Society because of habitat destruction and its narrowly restricted range (photographs in Cuatro Ciénegas basin, Coahuila, Mexico, by and used with permission of John Brill).

Figure 30.42. The California Killifish, *Fundulus parvipinnis*, occurs in coastal habits across a range of salinities from sea water to fresh water (photograph from Marina Del Rey, Los Angeles, California, by and used with permission of John Brill).

et al. 2005; Fuller 2008; Lopez et al. 2011; Stevens et al. 2013). The presence of *L. parva* in a Florida estuary was best predicted by habitat complexity and salinity. In this habitat, abundance was positively correlated with salinity (habitats ranged from 0.1 to 1.9 ppt), and fish occurred almost exclusively in and around beds of *Vallisneria* (Jordan 2002; see also Stevens et al. 2013). Farther downstream in estuaries and subtidal habitat, *L. parva* is replaced by some of the more high-salinity tolerant species.

Competitive exclusion is sometimes invoked as one mechanism generating complementary distributional patterns along well-defined gradients. Indeed, Topminnows sometimes use the full length of such gradients in allopatry. For example, *Fundulus notatus* and *F. olivaceus* occur from headwaters to large rivers in drainages when the other species is absent. *Lucania parva* extends farther into fresh waters in the northern Gulf Coast outside the distribution of *L. goodei* (Burgess et al. 1977; Fuller & Noa 2008). Obversely, evidence clearly exists of local adaptation to conditions along these gradients that shift competitive balances. In *L. goodei*, a variety of fitness metrics are negatively correlated with local salinity, but *L. parva* retains higher fitness measures in higher salinity (Kozak et al. 2014).

Within coastal marsh systems of North America, two sets of geminate (paired) species occur along gradients in salt-marsh habitat (Lopez et al. 2010, 2011). *Fundulus luciae*, *F. pulvereus*, *F. heteroclitus*, and *F. majalis* occur primarily along the Atlantic Coast, and *Fundulus jenkinsi*, *F. xenicus*, *F. grandis*, and *F. similis* occur along the northern and western Gulf of Mexico. *Fundulus luciae* is largely restricted to one of the most extreme environments of any fundulid species. This diminutive fish (26–30 mm SL) prospers in the shallow (2–3 cm) waters of salt marshes along the high tide zone. At low tides, *F. luciae* occurs in shallow ditches with thick muddy substrates. In comparison with other saltwater congeners (e.g., *F. heteroclitus*), *F. luciae* rarely, if ever, leaves its narrow zone of habitat between dry land and water, but *F. heteroclitus* inhabits tributary creeks and bays (Kneib 1978; Halpin 1997). *Fundulus confluentus* is another high marsh species that is quite euryhaline with some permanent freshwater habitat populations in parts of Florida (Boschung & Mayden 2004). *Fundulus heteroclitus* densities in marsh ponds are ≤60 fish/m^2 in the spring with mortality and movement among habitats linked to the frequency of flooding, which enhances water quality and introduces prey items (Hunter et al. 2009). In the subtidal areas, high marsh species are replaced by *Fundulus diaphanus* (primarily in freshwater coastal streams) and *F. majalis* at salinities near full seawater (Fig. 30.40). In the northern Gulf of

Mexico habitats, a similar transition occurs from *F. jenkinsi* in low salinity marsh habitat associated with *Spartina* (Peterson et al. 2003, 2016; Lopez et al. 2011); it is replaced by *F. pulvereus* and *F. xenicus* at intermediate salinities. The more euryhaline *F. grandis* uses a variety of habitats, but *F. similis* occupies more open, high salinity habitat.

Trophic Ecology

For most Topminnows, the mouth is small and upturned or in the superior position, as opposed to terminal as seen in many predatory fishes and inferior as in many bottom feeders. Feeding for many Topminnows includes but is not restricted to the air-water interface. Typical prey items are small and include a wide variety of taxa and different life cycle stages (Table 30.2). Topminnows capture terrestrial insects trapped by the surface tension, snatch flying insects on the wing, feed delicately on periphyton associated with submerged vegetation, exploit varying depths in the water column, and even feed on benthic organisms. Across the family, the most common diet items are larval and adult aquatic invertebrates; terrestrial insects; and the eggs, embryos, and occasionally adults of other fishes (Harrington & Harrington 1961; Arndt 1971; Hastings & Yerger 1971; Atmar & Stewart 1972; Kneib 1978; DuRant et al. 1979; Boschung & Mayden 2004; Champagne 2011).

Many Topminnows ingest considerable amounts of algae and plant detritus, but no evidence exists that this material is digested. On diets of either a filamentous green alga (*Enteromorpha* sp.) or detritus of a salt-marsh, vascular plant (*Spartina alterniflora*), *F. heteroclitus* lost weight and showed higher mortality than another cyprinodontiform (Sheepshead Minnow, *Cyprinodon variegatus*), which gained weight on the alga or maintained on *Spartina* and showed little or no mortality (D'Avanzo & Valiela 1990). Stable isotope studies indicated that *F. heteroclitus* does not assimilate carbon or nitrogen "to any significant degree" and is "functionally at least two trophic levels above primary producers throughout their life history" (Currin et al. 2003:508). The stable isotope signatures for *F. grandis* in the Pecos River, New Mexico, varied considerably depending on location with the lowest values in a salinized reach with a simple food-web structure (East et al. 2017). It consumed primarily fishes in all areas, but in the salinized reach, fishes eaten were primarily an algivore-detritivore, hybrid *C. variegatus* × *C. pecosensis* (Pecos Pupfish), resulting in a relatively low trophic position.

Topminnows are opportunistic feeders with diets varying temporally (Kneib 1978; Weisberg 1986; Lopez et al. 2010) and geographically (Champagne 2011). Diet studies aimed at demonstrating resource partitioning among ecologically similar sympatric populations all concluded that extensive diet overlap occurs and prey availability is likely not important in any competitive interactions (Thomerson & Woolridge 1970; Baker-Dittus 1978; Weisberg 1986; Champagne 2011). Opportunism was clearly demonstrated in a salt-marsh assemblage of Topminnows in Florida during a boom-and-bust cycle in abundance of mosquitos, *Aedes* sp. (Harrington & Harrington 1961). During a 12-day boom, mosquito larvae represented 73% of the gut contents of *Leptolucania parva*, 86% in *F. confluentus*, and 95% in *F. grandis*. During a 20-day period of mosquito scarcity, the diets of all three species shifted toward other animals, such as glass shrimp (*Palaemonetes* sp.), insects, both terrestrial and aquatic, and larval fishes (primarily the Sailfin Molly, *Poecilia latipinna*, and *Gambusia holbrooki*).

Adults of some of the large species (*F. undulus heteroclitus* and *F. grandis*) prey more heavily on fishes and small crabs, and small species (*Fundulus xenicus*, *F. luciae*, and *F. jenkinsi*) feed opportunistically on copepods and amphipods (Hastings & Yerger 1971; Able et al. 2007; Lopez et al. 2010). In a study of the role of piscovory in *F. heteroclitus*, 13% of wild-caught individuals contained fish remains (range across dates of collection, 4.3–24.7%; Able et al. 2007). This involved scavenging and predation, primarily on conspecifics. Although low levels of piscivory, including cannibalism, in *F. heteroclitus* are reported in a variety of other studies, its importance in the trophic ecology of the species is under-appreciated (Able et al. 2007). Predation by *F. heteroclitus* plays a major role in structuring size distributions of dominant snails (Vince et al. 1976) and overall invertebrate assemblage structure (Kneib & Stiven 1982; Kneib 1986). The consumption of prey with some terrestrial link and the subsequent movement of Topminnows from high marsh habitat through subtidal zones means many Topminnows play important roles in ecosystem energy transfer (Kneib 1997; Lopez et al. 2010). Along the Atlantic and Gulf Coasts, *Fundulus confluentus*, *F. heteroclitus*, *F. pulvereus*, and *Lucania parva* may play a significant role in mosquito control in marsh habitats (Chidester 1920; Boschung & Mayden 2004).

In fresh water, most Topminnows prey on various aquatic insects and terrestrial invertebrates at the surface. *Fundulus notatus* and *F. olivaceus* feed on terrestrial invertebrates, aquatic emergent insects, physid snails, microcrustaceans, and filamentous algae (Thomerson & Woolridge 1970; Atmar & Stewart 1972; Fig. 30.43). Floating

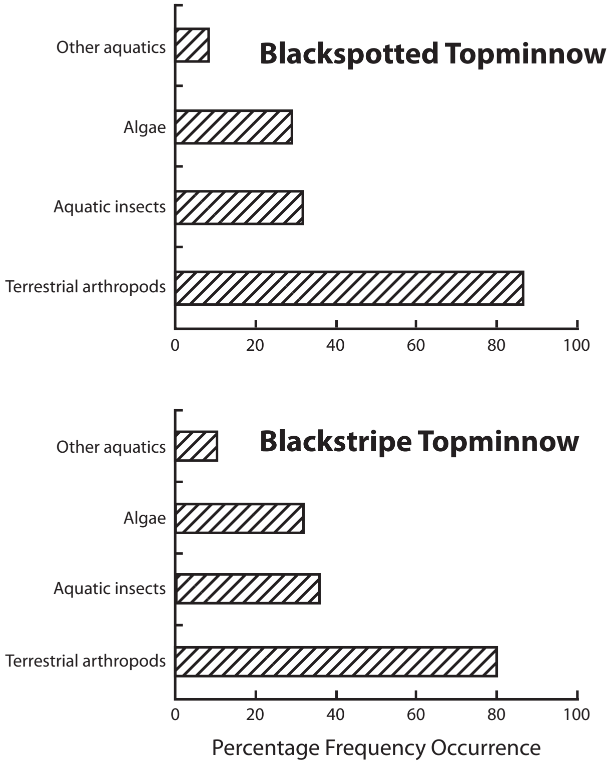

Figure 30.43. Percentage frequency occurrence of food items in guts of adult (>35 mm SL) Blackspotted Topminnows, *Fundulus olivaceus*, and Blackstripe Topminnows, *Fundulus notatus*, from spring, summer, and early autumn in southern Illinois and southeastern Missouri streams (n = 474) (data from Thomerson & Wooldridge 1970). The diets of these two closely related and often syntopic species were highly similar. Terrestrial arthropods (mostly insects, but also spiders, isopods, and centipedes) were most frequent in both species. Aquatic insects included benthic larvae and mid-water forms (corixid beetles). Other aquatic food items included amphipods, cladocerans, and other microcrustaceans. Although algae occurred somewhat frequently, it is uncertain if it was taken incidentally as the Topminnows fed on other prey or intentionally as a food source (Thomerson & Wooldridge 1970).

terrestrial invertebrates are strongly selected in all habitats except stagnant pools where snails are commonly eaten. In laboratory trials, snails were avoided in favor of chironomid larvae and floating terrestrial insects (Atmar & Stewart 1972). Among three coastal drainages, diet varied widely, and the invasive fire ant (*Solenopsis invicta*) was the most abundant prey item occurring in >50% of the stomachs (Champagne 2011). *Fundulus olivaceus* likely feed on the same items based on observations in aquaria. Headwater streams, however, are expected to have more abundant terrestrial input (Kawaguchi et al. 2003) and less primary productivity, possibly selecting for a more specialized diet.

Other freshwater Topminnows feed from a variety of habitats. *Fundulus seminolis* eat mostly benthic food organisms on the bottom or on vegetation often feeding in aggregations over sandy areas (McLane 1955; DuRant et al. 1979; Fig. 30.44). *Fundulus catenatus* select prey from the water column and benthic substrates (DuRant et al. 1979; Ross 2001; Boschung & Mayden 2004). Adults often have sand grains in the gut, indicative of feeding on caddisfly cases and other benthic prey (McCaskill et al. 1972; Fisher 1981; Fig. 30.45). Feeding activity in *F. kansae*, in its shallow, sandy-bottomed, plains stream habitat is especially bottom-oriented (Echelle et al. 1972). The gut contents often include large quantities of sand and filamentous algae along with a wide variety of invertebrates, and a female (50 mm TL) had cannibalized a juvenile (25 mm TL). Summertime counts of feeding acts (nipping and digging in the bottom substratum) and an index of gut fullness increased with daytime temperature; little or no feeding occurred at night. *Fundulus stellifer* has large molariform teeth, which may aid in crushing of large prey (Fig. 30.46). In northern Georgia, the species feeds primarily on chironomids and branchiopods (Edberg & Powers 2010), but anecdotal reports suggest snails form an important part of the diet (Thomerson 1969). *Lucania ommata* rely on vision to pick out larval and adult invertebrates inhabiting the leaves and stems of submersed aquatic vegetation, feeding only during the brightest daylight hours (Arndt 1971).

Predators and Parasites

As small-bodied fishes, Topminnows are preyed on by a wide variety of aquatic and terrestrial predators. Eggs and larvae are likely prey to other small fishes, including Topminnows, which eat their own eggs (Thomerson & Wooldridge 1970; Arndt 1971; Welsh & Fuller 2011). Fundulid affinity for structure in habitats could be adaptive to avoid predation. Because they are easily visible on the surface, birds, such as kingfishers, gulls, terns, and herons likely prey on Topminnows frequently. As part of their predator avoidance behavior, many Topminnows remain at the surface, briefly jump out of the water, or remain motionless (see behavior section). *Lucania parva* leave the water to intentionally land on aquatic vegetation to avoid predators (Baylis 1982). The minimum threshold predator distance for a flight response (synchronized start-start movement by a group of individuals) by schools of *F. diaphanus* was 21.5 cm (Godin & Morgan 1985). I (RCC) have found lifeless but still identifiable *Fundulus grandis* and *F. similis* dropped by Least Terns

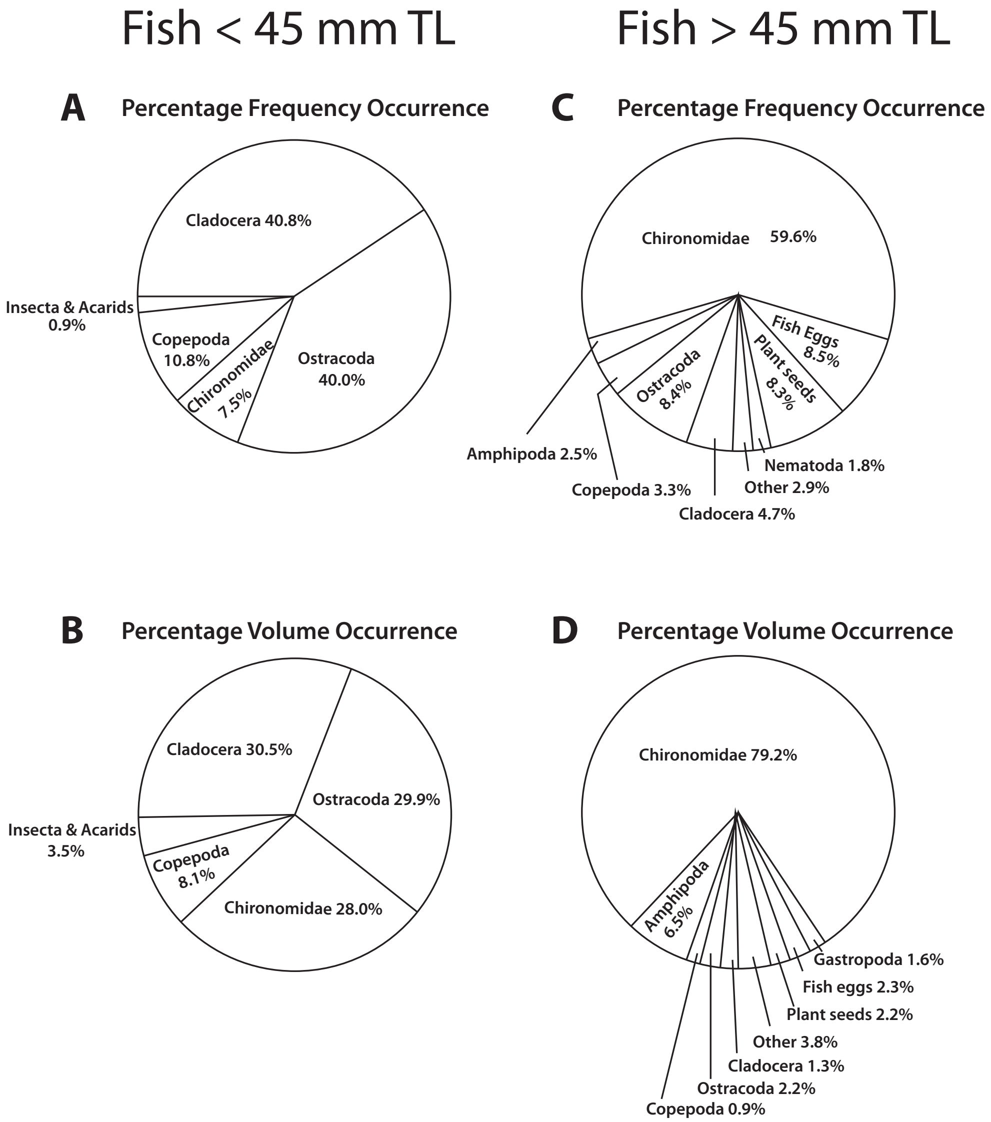

Figure 30.44. Food habits as percentage frequency occurrence and volume for small (*left*) and large (*right*) Seminole Killifish, *Fundulus seminolis*, in Lake Wales and Star Lake, Polk County, Florida (redrawn from DuRant et al. 1979). Most cladocerans, ostracods, and chironomids in the lakes were associated with benthic substrates or vegetation.

on the campus of the University of New Orleans, which is bordered by a canal and the south shore of Lake Pontchartrain. Aquatic and semi-aquatic snakes, such as members of the genera *Nerodia* and *Agkistrodon* (and other reptiles), are frequent predators of Topminnows (Kofron 1978; Ernst & Ernst 2003). Miller et al. (2005) listed garter snakes, genus *Thamnophis*, as predators of *F. lima*, and humans as predators of *F. grandissimus* in Mexico.

The importance of *F. heteroclitus* in marsh trophic ecology and ecosystem function is particularly well characterized. The major predators of adult *F. heteroclitus* are crabs (*Callinectes* sp.) and various wading birds. Patterns of *F. heteroclitus* habitat selection are consistent with tradeoffs between predation risk and optimizing growth rates. Fish use suboptimal habitat for growth in order to avoid heavy predation pressure (Kneib 1982, 1997; Halpin 2000).

Relatively few studies document parasite loads of Topminnows. Harris & Vogelbein (2006) found 22 parasite species in 150 *F. heteroclitus* collected from six localities in the York River, Virginia. Five of the reported parasites were new records for *F. heteroclitus*, and some of the most common parasites found were rarely reported in the literature, indicating a general lack of comprehensive data for fundulid parasites. In some cases, reports indicated less

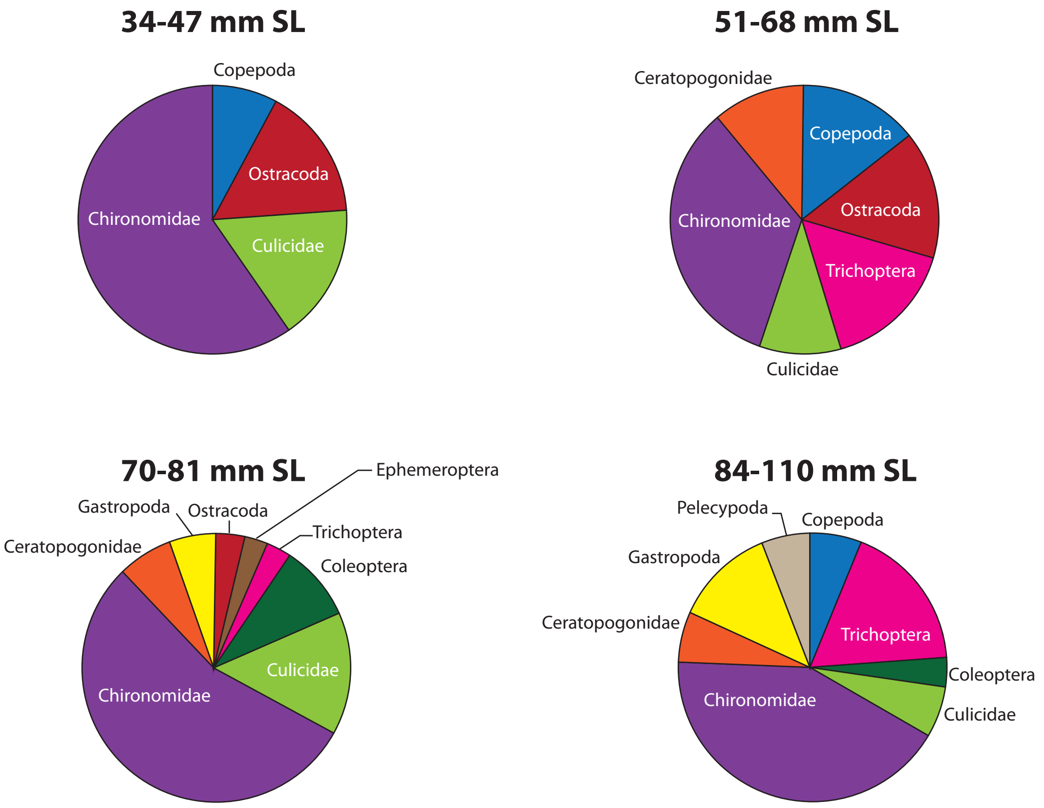

Figure 30.45. Food habits as estimated volume for several size-classes of Northern Studfishes, *Fundulus catenatus*, from multiple localities and seasons in the Missouri Ozarks (n = 100). The data suggests most food items were taken from the bottom, chironomids (midge larvae) were a major diet item for all size groups, ostracods and copepods were used more by small rather than large fish, and large fish had a wider variety of major food items than small fish with increased use of ephemeropterans (mayflies), trichopterans (caddisflies), gastropods (snails), and pelecypoda (bivalves) (data from McCaskill et al. 1972).

host specificity than previously thought for parasite species (Harris & Vogelbein 2006). All individuals examined had external ciliates (*Trichodina* sp. and *Apiosoma* sp.) averaging 3,000/host. Most individuals (97%) were hosts for unidentified metacercaria in gill filaments; the intestinal nematode *Dichelyne bullock* (67%); the gill copepod *Ergasilus fundulid* (79%); and the annelid fin parasite *Myzobdella lugubris* (61%). Combined with other reports, *F. heteroclitus* hosts 77 species of parasites. Records for many other Topminnows are clearly lacking (e.g. three reported parasites of *F. pulvereus*, five of *F. jenkinsi*). The two Topminnows with what appears to be the most robust records are the widespread and fairly common *Fundulus diaphanus* (54 parasite species) and *F. grandis* (31 parasite species) (Harris & Vogelbein 2006). In *F. diaphanus*, greater parasite loads (external trematode cysts of *Crassiphiala bulboglossa*) were positively correlated with time spent in a refuge following attack from model predators (Dowling & Godin 2002). When in groups, parasitized fish were also less likely to shoal and took more peripheral shoal positions, behavior modifications that may be parasite-mediated to facilitate transfer to the final avian host (Krause & Godin 1994).

Information on Topminnows acting as hosts for unionid mussel larvae (glochidia) is generally lacking, but Topminnows apparently do not serve as primary hosts for any host-specialist freshwater mussels. Glochidia of Paper Pondshells, *Utterbackia imbecillis*; Giant Floaters, *Pyganodon grandis*; and Eastern Elliptio, *Elliptio complanata*, all host-generalist species, transformed on laboratory infested *Fundulus diaphanus* (Trdan & Hoeh 1982; Lellis et al. 2013). Glochidia of the Giant Floater likewise reportedly transformed on *Fundulus chrysotus* (Penn 1939), and those of the host-generalist Fluted Shell, *Lasmigona costata*, transformed on *F. catenatus* (Luo 1993). *Fundulus olivaceus* serves as a marginal host (i.e., low rate of transformation) for the Southern Creek Mussel, *Strophitus subvexus*, another host-generalist species, and the Warrior Pigtoe,

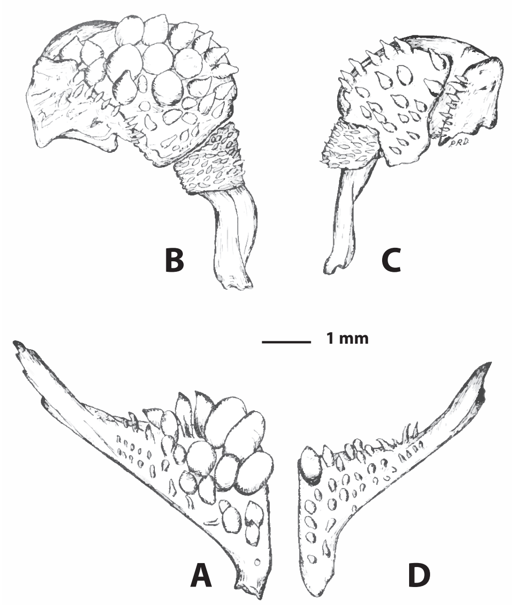

Figure 30.46. Pharyngeal arches of the Southern Studfish (A and B), *Fundulus stellifer* (71.4 mm SL), and Northern Studfish (C and D), *Fundulus catenatus* (70.0 mm SL). Note the heavy, crushing, molariform teeth of the Southern Studfish relative to the thinner, conical teeth of the Northern Studfish. (A) Right lower arch, dorsal view, anterior down. (B) Right upper arch, ventral view, anterior to upper left. (C) Left upper arch, ventral view, anterior to upper right. (D) Left lower arch, dorsal view, anterior down (redrawn from Thomerson 1969).

Pleurobema rubellum (as *furvum*), a cyprinid specialist (Haag & Warren 1997). Other Topminnows likely serve as hosts to other freshwater mussel species (e.g., Wiles 1975ab; Kneeland 2006; Kneeland & Rhymer 2008; Williams et al. 2008 citing M. L. Buntin; M. Hove unpubl.; C. Barnhart unpubl.), but peer-reviewed laboratory trials are needed to assess whether transformation occurs and the strength of the host fish-mussel relationship.

CONSERVATION

The conservation status of a particular species reflects the perceived conditions of population stability or sustainability at local, regional, or global levels. Many wide-ranging species reach the extremes of their distribution in a limited area in one or more states or provinces in North America. State compilations may note that a particular species is of Special Concern or Vulnerable because of the limited distribution or habitat disturbances just within that state even though the species is considered stable throughout the larger extent of its geographic range. The American Fisheries Society included nine Topminnows in the latest list of North American imperiled freshwater fishes (Jelks et al. 2008). One is Extinct, three are Endangered, one is Threatened, and four are Vulnerable (Table 30.2). All nine of the species have or had limited distributions either in the southern United States (†*Fundulus albolineatus*, *F. bifax*, *F. euryzonus*, *F. julisia*, *F. waccamensis*) or Mexico (*Fundulus grandissimus*, *F. lima*, *F. persimilis*, and *L. interioris*). Despite the assessments of the American Fisheries Society, Southeastern Fishes Council (Warren et al. 2000), and other authorities, which clearly identify imperiled species in the family, no Topminnow species is currently protected under the United States Endangered Species Act (USFWS 2015b), but the Barrens Topminnow is proposed for protection (USFWS 2018).

Of the 40 species of Topminnows in North America, 30 (including one extinct species) are recorded from the southern United States, which is clearly the center of diversity for this group. This region supports more native fishes than any comparable size area on the continent north of Mexico (Warren et al. 2000). In the southern United States, >660 species are documented, and about 28% are considered to be Extinct, Endangered, Threatened, or Vulnerable (Warren et al. 2000). Etnier (1997) attributed the imperilment of several southern United States Topminnows to pollution, habitat loss, and small geographic ranges. Others identified invasive species, particularly the Western Mosquitofish, *Gambusia affinis*, as a threat to native Topminnows (Pasbrig et al. 2012). Fundulid species of conservation concern in the southern United States include one Extinct (†*Fundulus albolineatus*; Fig. 30.47); one Endangered (*F. julisia*, proposed for federal protection, USFWS 2018); one Threatened (*F. waccamensis*; Fig. 30.48); two Vulnerable (*F. bifax* and *F. euryzonus*); and one candidate for listing (*F. jenkinsi*) classified as a species of Special Concern by the National Marine Fisheries Service and the states of Louisiana, Mississippi, Alabama, and Florida (Peterson et al. 2003; Lopez et al. 2010, 2011).

A number of Topminnows specializing in springs, headwaters, or wetlands or with limited distributions are of conservation concern. As noted, †*Fundulus albolineatus* is assumed to be Extinct (Fig. 30.47). The last known specimens were captured at the type locality, Big Spring and its outflow Spring Creek in Huntsville, Alabama, in 1889. The only known habitat (Big Spring) was paved in concrete, repeatedly drained, and stocked with Common Carp (*Cyprinus carpio*) and Goldfish (*Carassius auratus*) (Williams & Etnier 1982). Another spring dweller, *F. julisia*, occurs in springs and vegetated pools of sluggish streams. The species only occurs on the Barrens Plateau

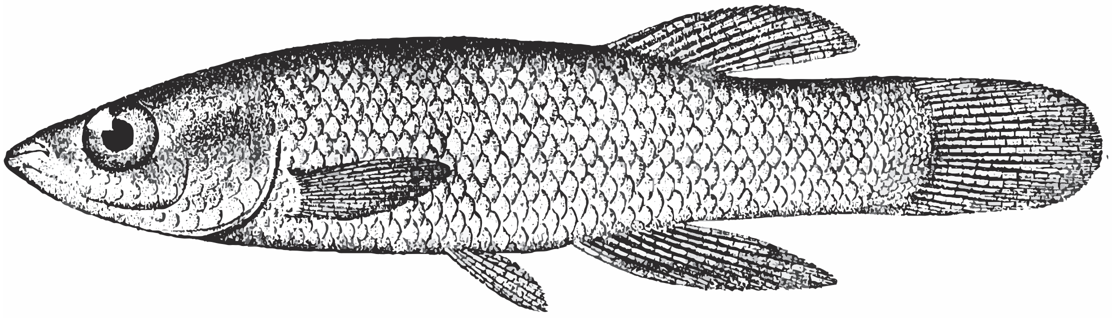

Figure 30.47. The Whiteline Topminnow, *Fundulus albolineatus*, the only recently extinct North American *Fundulus*, is known solely from 25 specimens taken in Big Spring and its outflow, Spring Creek, in Huntsville, Madison County, Alabama, where it was last captured in the 1890s. Spring Creek has been altered drastically since the turn of the century (e.g., over pumped, concrete-lined banks, impounded, invaded by non-native fishes) (Williams & Etnier 1982) (scale about twice natural size; redrawn from an original illustration by S. F. Denton as plate XLIII, fig. 1 in Gilbert 1891).

Figure 30.48. The Waccamaw Killifish, *Fundulus waccamensis* (both adult males) is endemic to Lake Waccamaw, North Carolina. The species is a close relative of the widespread Banded Killifish, *Fundulus diaphanus*. The Waccamaw Killifish is considered Threatened by the Southeastern Fishes Council and American Fisheries Society (Warren et al. 2000; Jelks et al. 2008) because of threatened habitat destruction and a narrowly restricted range (specimens captured in fall 2013; courtesy of Joe Scanlan).

in the headwaters of the Duck and Elk Rivers, Tennessee River drainage, and in the Caney Fork River, Cumberland River drainage (Etnier & Starnes 1993; Bettoli 2015). Only three natural populations remain, but an additional 23 populations were established through extensive reintroduction efforts. Monitoring of 20 reintroduction sites yielded evidence of natural recruitment at roughly half of them (from 8–12 sites/year). Most sites exhibiting successful recruitment of reintroduced populations lack *Gambusia affinis* (Zuber & Mattingly 2012). *Fundulus bifax* is found only in the Tallapoosa River system, western Georgia and eastern Alabama, and in one tributary to the Coosa River, Alabama. Because of its limited range and relative rarity, Warren et al. (2000) and Jelks et al. (2008) recommended Vulnerable status and equivalently Boschung & Mayden (2004) recommended Special Concern status for the species. *Fundulus euryzonus* exhibits an even more limited distribution; the species is endemic to the Lake Pontchartrain drainage in the Amite and Tangipahoa Rivers, southwestern Mississippi and northeastern Louisiana (Suttkus & Cashner 1981). Ross (2001) considered *F. euryzonus* to be of Special Concern in Mississippi because of the small geographic range and ongoing habitat alteration (Vulnerable in Warren et al. 2000; Jelks et al. 2008). Similar disturbances occur in the limited range in Louisiana. Lake Waccamaw in Coastal North Carolina is the home to three unique fish species from different families, including *F. waccamensis*, which likely evolved from isolated populations of *F. diaphanus* (Hubbs & Raney 1946; Krabbenhoft et al. 2009) and is considered Threatened (Warren et al. 2000; Jelks et al. 2008). At the time of description in 1946 and for the next 20 years, *F. waccamensis* was considered endemic to Lake Waccamaw, but a population was discovered in Phelps Lake in northeastern North Carolina in the mid-1970s (Lee et al. 1980). The range extension of about 100 km northeast may be the result of a baitbucket introduction.

Two notable examples of broadly distributed Topminnows exist that are declining throughout their range and may warrant future conservation attention. In 2000–2010 surveys, *Fundulus sciadicus* occurred at only 28% (189 out of 667) of historical occurrence sites. In Nebraska, the center of the distribution, *F. sciadicus* were only found at

34% of historical sites, and marked declines were noted in some drainages that had supported healthy populations. Habitat loss, fragmentation, and assemblage shifts to generalist-type invasives were hypothesized as the largest threats to remaining populations (Pasbrig et al. 2012). *Fundulus blairae* occurs in Gulf Slope drainages from the Brazos River, Texas, eastward to the Escambia River, Alabama and Florida, and northward to the Red River drainage, Louisiana, Oklahoma, and Arkansas. It is afforded Special Concern status in Alabama because of habitat loss (wetlands), pollution, isolated populations, and rarity of specimens at most localities. Similar isolation of *F. blairae* populations also is characteristic in Florida, Mississippi, and Texas. Throughout its extensive range, population isolation, small population size, and ongoing habitat alteration (loss of wetlands) may merit broader Special Concern status for *F. blairae* (Boschung & Mayden 2004).

Coastal areas in the southern United States contain some of the most rapidly developing human communities, which are putting increasing anthropogenic pressure on wetland and estuarine habitats. *Fundulus jenkinsi* has a fairly broad distribution from Escambia Bay, Florida, to Galveston Bay, Texas, but is patchily distributed and more of a habitat specialist than previously thought. Originally thought to be a salt-marsh generalist associated with *Spartina* (Gilbert & Relyea 1992; Peterson et al. 2003), later work narrowed the species habitat preference to lower ends of salinity gradients in dendritic intertidal creek networks within salt marshes (Lopez et al. 2011). This fairly narrow niche within coastal ecosystems is likely to put the species continually at odds with human development of these areas (shoreline hardening, dredging, increased turbidity, and coastal development). Various populations from Florida to Texas are at risk. Local abundances tend to be higher in less developed areas (Weeks Bay, Alabama; Barataria-Terrebonne, Louisiana) compared with areas with greater development pressure (Pensacola Bay, Florida).

In Mexico, four of the eight species of Fundulidae are of conservation concern: *Lucania interioris* is Endangered (Fig. 30.41), *Fundulus lima* is Endangered, and *F. grandissimus* and *F. persimilis* are Vulnerable (Miller et al. 2005; Jelks et al. 2008). *Fundulus lima* inhabits isolated inland ponds, springs, and creeks and is one of only three freshwater endemics to Baja California Sur. It was generally considered abundant before the introduction of exotics (e.g., *Cyprinus carpio*, *Xiphophorus* spp., and *Tilapia* spp.) to promote rural aquaculture, after which monitoring demonstrated low relative abundance compared with exotics (Ruiz-Campos 2000). Introducing the species to some pools within the native range that are not stocked with exotics might help the species persist (Ruiz-Campos 2000). The largely saltwater *F. persimilis* has been captured infrequently since its description in 1955 and is thought to be rare (Miller et al. 2005). For example, in the hypersaline Rio Lagartos, Yucatan, Mexico, *F. persimilis* occurred only during the windy season, and its occurrence was correlated positively with dissolved oxygen and negatively with water temperature. Bimonthly seine and trawl sampling of 28 sites from 1992–1993 yielded only 20 *F. persimilis* (0.38%) of 16,000 specimens captured (Vega-Cendejas & Hernández de Santillana 2004). Little is known about population or range sizes in *F. grandissimus* or *L. interioris*. Their conservation status is based on presumed limited distributions and general threats to habitats in the region. *Fundulus philpisteri* is only known from a few localities (García-Ramírez et al. 2007). This species likely faces similar threats and will eventually be considered imperiled as well.

COMMERCIAL IMPORTANCE

The genus *Fundulus* is one of the most thoroughly studied groups of fishes and a valuable resource to science. Thirty years ago, DiMichele et al. (1986) reported >3,000 papers had been written on members of the genus since the mid-1800s. Other reviews (Atz 1986; Burnett et al. 2007) noted the value members of the group offer to the research enterprise. Their abundance in highly variable environments, high physiological tolerances, ease of capture and captive propagation, and broad distribution and dominance in some of the most rapidly developing coastal communities all make Topminnows desirable for a number of basic and applied research questions.

Fundulus heteroclitus is sometimes referred to as the white rat of aquatic vertebrate research. A search of the literature in November 2013 yielded 859 published articles containing *Fundulus*, *Lucania*, or *Leptolucania* in the title, abstract, or as a keyword from 2003–2013. Of those publications, 54% dealt specifically with *F. heteroclitus* in some capacity. The species has served as a model for our understanding of responses to environmental perturbation (most notably salinity, temperature, and hypoxia), development and sensory systems, and evolutionary responses to toxicants (Burnett et al. 2007). An active group of researchers are currently working toward a complete genome for *F. heteroclitus* that will increase the value of Topminnows in addressing basic and applied research questions by providing a robust set of genomics tools.

Some of the other common and accessible species (*Lucania* spp. and the *F. notatus* species complex) are also proven, excellent experimental models in evolutionary ecology (Fuller et al. 2007; Schaefer et al. 2011b; Duvernell & Schaefer 2013; Kozak et al. 2014). *Fundulus xenicus* is a possible model for embryological studies because of its hardiness, small size, long period of egg production, and egg characteristics (Koenig & Livingston 1976).

Their small size means Topminnows are rarely consumed by humans, but high local abundance and prominence in some piscivore diets (McGrath et al. 2013) renders them a prime baitfish. The only species consumed by humans is the relatively large *Fundulus grandissimus* (Miller et al. 2005), but many species of *Fundulus* are used as bait in fresh and salt water. Seining in either environment yields a mixture of species that conform to the angler's view as minnows. Along the Gulf and Atlantic Coasts of the United States, *Fundulus heteroclitus* and *F. grandis* are often the commercial bait of choice and are sold under the names Mummichogs, mud minnows, or cocahoe minnows (Fig. 30.49). Many ichthyologists doing field work in the southeast have witnessed anglers using nuptial male Topminnows as bait. Other species, such as *F. xenicus* (Ross 2001), are also occasionally used as bait.

Almost all of the extant Topminnows, even some of those of conservation concern, enter the aquarium trade (Goldstein et al. 2000). Most species are small, not overly aggressive and will spawn in aquaria, making them popular with aquarists (Riehl & Baensch 1996). The males of many species (e.g., *Fundulus catenatus*) are brightly colored, characterized by various brightly colored dots and short dashes on the body and fins (e.g., Figs. 30.2–30.4, 30.20, 30.24, 30.31, and 30.33).

Figure 30.49. Several species of Topminnows, Fundulidae, are popular as baitfishes, particularly some of the abundant, easy to trap or seine, hardy, coastal marsh species, where fishermen collectively call them cocahoe minnows or mudminnows. They are so popular as bait, reputedly being effective on a variety of gamefishes, that lure companies produce and sell artificial, soft-bodied versions of the cocahoe minnow in a variety of natural and quite dazzling, unnatural colors (courtesy of the H&H Lure Company, Baton Rouge, Louisiana).

As generalist feeders specializing in shallow, sometimes ephemeral habitats, Topminnows likely play an important role in regulating mosquito populations (Boschung & Mayden 2004). At some times of the year, the stomach contents of coastal Topminnows (e.g., *Fundulus confluentus*, *F. heteroclitus*, *F. pulvereus*, and *Lucania parva* along the Atlantic Coast) contain ≤90% larval mosquitos (Harrington & Harrington 1961).

LITERATURE GUIDE

Although Topminnows as a group are generally well studied, *Fundulus heteroclitus* is arguably the best-studied North American fish (DiMichele et al. 1986; Atz 1986). The literature addressing the biology of *F. heteroclitus* is vast and covers all aspects, including genomics (Duvernell et al. 2004; Cossins & Crawford 2005; Whitehead & Crawford 2006; Burnett et al. 2007; Whitehead et al. 2011a), population genetics (Powers et al. 1986; Gonzalez-Vilasenor & Powers 1990; Powers & Schulte 1998; Oleksiak et al. 2002; Adams et al. 2006; Duvernell et al. 2008; Strand et al. 2012), toxicology and environmental genomics (Fisher & Oleksiak 2007; Whitehead et al. 2011b; Dubansky et al. 2013), ecosystem ecology (Kneib 1986, 1997), behavior (Able & Hata 1984; Weis & Weis 1998), physiology (Oleksiak et al. 2004; Whitehead et al. 2011b; Brown et al. 2012; Bozinovic et al. 2013), and development (Oppenheimer 1937; Armstrong & Child 1965; Bozinovic et al. 2011; Brown et al. 2012).

The relationships of the family to other closely related groups and interspecific relationships are available using a variety of techniques. The evolutionary relationship of Fundulidae to other cyprinodontiforms was reviewed using morphological data (Parenti 1981; Costa 1998b) and sequences from the X-src gene (Meyer & Lydeard 1993). Relationships within Fundulidae were studied using morphology (Gosline 1949; Foster 1967b; Farris 1968; Wiley 1986), ethology (Foster 1967b), karyology (Setzer 1968; Chen 1971), hybridization (Hubbs & Drewry 1959; Setzer 1970), development (Hubbs & Burnside 1972), electrophoresis of serum proteins (Griffith 1968, 1972), allozyme data (Cashner et al. 1992), mitochondrial cytochrome *b* sequences (Bernardi 1997; Grady et al. 2001), and sequences from one mitochondrial and two nuclear genes (Whitehead 2010). Ghedotti & Davis (2013) analyzed four gene sequences (two nuclear, two mitochondrial) combined with morphological and behavioral data along with a number of non-fundulid taxa for rooting (see also Near et al. 2012b, 2013; Betancur-R. et al. 2013ab).

Evolution and geographic variation of Topminnows is covered by many contributions. Geographic variation at the morphological or genetic level is reviewed by Brown (1956, 1958), Braasch & Smith (1965), Thomerson (1966, 1969; Fig. 30.50), Fahy (1972), Suttkus & Cashner (1981), Poss & Miller (1983), Able & Felley (1986), and Schaefer et al. (2012). Morphological evolution of Topminnow species is reviewed by Krabbenhoft et al. (2009), Schaefer et al. (2011a), Welsh et al. (2013), and Ghedotti & Davis (2013). The phylogeographic history of several fundulid species is reviewed in Powers & Schulte (1998), Kreiser et al. (2001), April & Turgeon (2006), Haney et al. (2009), and Duvernell et al. (2013).

Physiological aspects of Topminnows are addressed by several authors. Salinity tolerance was examined by Griffith (1972, 1974ab), Nordlie (1987, 2000b, 2006), and Kozak et al. (2014). Schaefer & Walters (2010) and Schaefer (2012) examined thermal optima and metabolic rates of populations at different temperatures or from different latitudes. Egg and embryo development times in and out of water as well as reproductive synchrony with tidal cycles is addressed by Harrington & Haeger (1958), Harrington (1959), Taylor et al. (1977, 1979), and DiMichele & Taylor (1980, 1981). Early development is described for *Fundulus xenicus* (Koenig & Livingston 1976; Cunningham & Balon 1985, 1986) and *Lucania goodei* and *L. parva* (Crawford & Balon 1994ac).

Research has touched on several topics regarding the ecology and biology of Topminnows. The diet and ecological interactions of coexisting Topminnows is reviewed by Harrington & Harrington (1961), Thomerson & Woolridge (1970), Arndt (1971), Kneib (1978), and Champagne (2011). Information on life history and reproductive biology can be found in Hastings & Yerger (1971), Byrne (1978), Rakes (1989), Blanchard (1996), Fuller & McEachran (2001), Edberg & Powers (2010), and Lopez et al. (2010). Reproductive isolation and hybridization among species is discussed in Duggins et al. (1995), Fuller et al. (2007), Hernandez Chaves & Turgeon (2007), Vigueira et al. (2008), Berdan & Fuller (2012), Gregorio et al. (2012), and Duvernell & Schaefer (2013).

Figure 30.50. Jamie E. Thomerson (1935–2016) researched hybridization, geographic variation, distribution, and aspects of life history of Topminnows and studfishes in eastern North America. He later became an expert on New World Rivulines (Rivulidae), especially those found in northern South America (courtesy of Leo Nico).

Acknowledgments

We thank Gayle Henderson for redrawing figures and Gordon McWhirter and Zanethia Barnett for proofing and style editing the literature cited, figures, and tables. Richard L. Mayden and Timothy Schmidt cleared and stained and dissected most of the photographed skeletal specimens.