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Zebrafish in the Wild: A Review of Natural History and New Notes from the Field

RAYMOND E. ENGESZER, LARISSA B. PATTERSON, ANDREW A. RAO, and DAVID M. PARICHY

ABSTRACT

The zebrafish, *Danio rerio*, has emerged as a major model organism for biomedical research, yet little is known about its natural history. We review the literature pertaining to the geographic range, biotic and abiotic habitats, and life cycle of the zebrafish. We also report our own field study to document several aspects of zebrafish natural history across sites in northeast India. We found zebrafish particularly abundant in silt-bottomed, well-vegetated pools and rice paddies adjacent to slow moving streams at a range of elevations. We further identified co-occurring fishes likely to be zebrafish competitors and predators. Finally, we present observations that indicate substantial habitat degradation and loss, and suggest guidelines for documenting and preserving natural zebrafish populations.

INTRODUCTION

A KNOWLEDGE OF ZEBRAFISH NATURAL HISTORY and ecology is critical for interpreting its behavior and physiology, extant genetic and phenotypic variation, and the evolutionary history of embryonic, larval, and adult traits. Yet we know surprisingly little about the natural environment of the zebrafish or how it interacts with that environment,[1] despite humans and zebrafish having shared waterways around the river Ganges for tens of thousands of years.[2]

Like most other biomedical model organisms, the zebrafish was chosen for particular traits that make it convenient for laboratory study, not for a broad understanding of the organism in its native environment.[3–6] Despite the wealth of information on developmental and genetic mechanisms and the arsenal of resources and techniques for studying zebrafish, this species remains underexploited for organismal research, comprising studies of ecology, evolution, and behavior. More fully realizing the potential of zebrafish for organismal biology, as well as integrative studies spanning multiple levels of organization, requires some knowledge of zebrafish natural history: its geographical distribution, physical habitat, diet, and its competitors and predators.

We review the scant literature on zebrafish in the wild and document our own recent work aimed at developing a deeper understanding of zebrafish natural history. We present a hypothesis for zebrafish life history in the wild, suggest how studies of natural populations can inform research in the laboratory, and make recommendations for future work in this area.

LITERATURE REVIEW

An extensive literature exists on Central Asian fishes and specifically on Indian fishes.[7–13] Yet, these works focus on taxonomy and regional

Department of Biology, University of Washington, Seattle, Washington.

species lists, and contain little or no information on natural history, development and life history, ecology, or behavior, except for some economically important fishes and sport fishes. For zebrafish, there is only meager published information, aside from studies of taxonomy and phylogenetic relationships.[14–21]

Even the geographic range of zebrafish is a matter of conjecture.[22,23] From original collections data, we can reconstruct a range extending from Pakistan in the west to Myanmar (Burma) in the east, and from Nepal in the north to the Indian state of Karnataka in the south (Fig. 1) (Appendix). Nevertheless, records for the extremes of this distribution are mostly outdated. For example, the last recorded collection of zebrafish in Myanmar occurred in 1926, despite successful collections of similar fishes in that country more recently. Given the rapidly expanding human population in Central Asia since the early twentieth century and the concomitant negative anthropogenic effects on freshwater ecosystems, historical records may not reflect the current zebrafish range. Moreover, some specimens are likely to have been misidentified, particularly at the extremes of the range (T. Roberts, personal communication).

What is the typical habitat of wild zebrafish? Several sources agree that zebrafish are found in rivers, small streams and other channels, stagnant or slow-moving pools near streams, and rice paddies.[1,12,23,24] More precise information is mostly lacking. For example, several works on regional Indian fauna include water quality data for river systems that contain zebrafish, yet these measurements cannot be related to the particular microhabitats where zebrafish or other species reside (e.g., fast flowing main river channels *vs*. slow flowing or stagnant streamside pools).[24-26] However, a recently published study presents data collected in September and October 1995 from three sites where zebrafish were found in the northern and northeastern India states of Uttar Pradesh and West Bengal.[27] Although data from individual sites were not presented, pooled data across all three sites associate zebrafish with relatively still water (currents, 0 m^{-sec} to 0.1 m^{-sec}) at 27°C to 34°C and pH 7.9–8.2; widths of water bodies ranged from 1 to 12 m, and depths ranged from 16 to 57 cm; water was relatively clear (transparent to >35 cm), over substrates of clay, silt, cobble, or boulders. Thus, previous works suggest some general features of zebrafish habitats in the wild, though it is not possible to associate data for multiple parameters with specific zebrafish localities.

Biotic features of the zebrafish habitat are similarly undocumented. Extensive collections data exist, yet they are not sufficiently detailed to indicate the extent to which zebrafish co-occurred with particular species at particular sites. Thus, it is virtually impossible to infer which species might be predators or competitors of zebrafish in its native environment. Zebrafish themselves are known to feed on mosquito larvae[28] and, presumably, other insects, though the precise species are not known.[27] Although vegetation can provide cover from terrestrial and aquatic predators, and microhabitats for spawning and foraging, the types and extent of vegetation in the zebrafish environment is not known, although canopy cover ranged from 0% to 50% across the three sites mentioned above.[27]

A final and critical aspect of zebrafish natural history is its behavior and life cycle. The breeding season is reportedly between April and August,[1] presumably varying somewhat by latitude, elevation, and prevailing climatic conditions. Egg laying is thought to occur in small pools adjacent to streams.[23] Spawning behavior itself has been described only in the laboratory.[29,30] Anecdotal comments suggest that individuals hatch within 3 days postfertilization, but may take as long as 5–6 months to reach reproductive maturity in the wild.[1,23]

This brief review indicates that much of zebrafish natural history awaits discovery, and there are exciting opportunities to learn basic features of this species' behavior, ecology, and evolution. Such information is valuable in its own right, and also sets the stage for integrative research programs made feasible by the many resources accompanying this biomedical model organism.

OBSERVATIONS FROM THE FIELD

To document the abiotic and biotic habitat of zebrafish and to better understand its natural

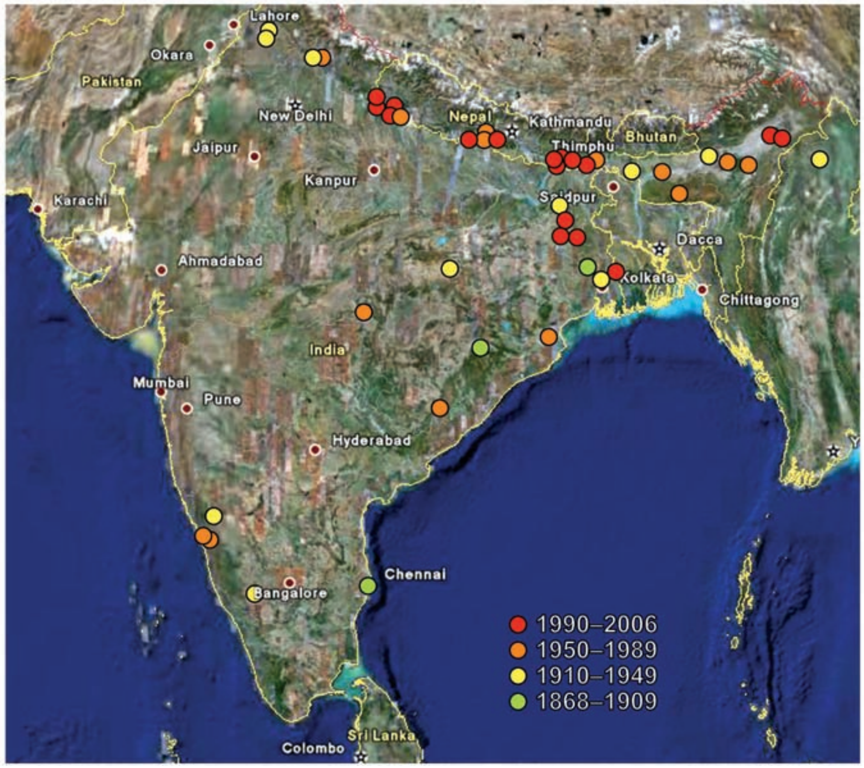

FIG. 1. Historical collections of zebrafish in India and neighboring countries since 1868.

history, we sought wild populations across a range of geographical and elevational localities in the northeast Indian states of West Bengal, Assam, Meghalaya, and Orissa (Table 1). At all localities, we recorded global positioning system (GPS) coordinates and elevation; we took photographs and sketched geographical features; we measured water temperature, pH, and conductivity; and we made qualitative observations of water clarity, substrate type, rate of flow, and extent of vegetation. To assess the fish communities in which zebrafish occurs, we collected and imaged fishes at each locality, and we identified them to genus and species when possible, or to genus alone for species not yet formally described.

Throughout the trip we worked with local fisherman, who typically used box seines. At some sites these were supplemented with larger seines, gill nets, or minnow traps. Different fishing methods bias collections towards particular types of fishes, and our seines and traps frequently were most suitable for smaller species, like zebrafish. Thus, our collections are not exhaustive, although we did often recover juveniles of much larger species that would not otherwise have been trapped using our methods. We employed local fisherman partly for their regional expertise, but primarily for their familiarity with the inhabitants of the local farms and villages. Many sites are used for subsistence fishing of *Barilius barilius*, *Notopterus notopterus*, *Tor progenius*, and other species, and for this reason access is guarded jealously, regardless of the intended catch. Sites were only fished after we received the blessings of local leaders. Fish were returned alive to their sites of capture immediately after imaging and identification.

Itinerary

We began our expedition in Kolkata (formerly Calcutta), the capital of West Bengal, in early July 2006 during the monsoon season. Our goal was visit as wide a range of sites as could be achieved in 17 days.

West Bengal and Assam. After one night in Kolkata, we traveled by air to Bagdogra in the northeast corner of West Bengal (Fig. 2A, B and

Table 1. Site Descriptions[a]

No.	Name	GPS	Elev, m[b]	°C	pH	μS[c]	Clarity	Flow	Substrate	Vegetation	Date, time
Sites with zebrafish											
1	Jorai river (a)[d]	N 26°31.039′ E 89°51.306′	51	24.6	6.8	271	Bottom visible, tea colored	Very slow	Silt	Submerged	7/9/06, 1045
3	Suthimari river (a)	N 26°29.403′ E 89°46.259′	63	34.0	6.5	185	Bottom visible, clear,	Medium	Silt	Abundant submerged	7/9/06, 1422
4	Suthimari river (b)	N 26°29.403′ E 89°46.259′	63	32.7	6.4	168	45 cm, some suspended dirt	Medium	Silt	None	7/9/06, 1422
5	Suthimari river (c)	N 26°29.403′ E 89°46.259′	63	32.0	6.3	—	Bottom visible, clear	Medium	Silt	Abundant submerged and flooded	7/9/06, 1422
6	Suthimari river (d)	N 26°29.403′ E 89°46.259′	63	38.6	7.3	—	Bottom visible, clear	Very low	Silt	Yams in rice paddy, broad leaves, ample shade	7/9/06, 1422
9	Tributary of Rydak I	N 26°31.107′ E 89°43.593′	54	26.7	7.1	171	Bottom visible, clear	Slow to medium	Gravel, cobble, silt	Abundant submerged	7/10/06, 0900
11	Lefraguri swamp	N 26°30.889′ E 89°49.993′	74	26.9	6.3	72	~40 cm	None	Silt	Abundant submerged and flooded	7/11/06, 1130
12	Ghotimari river	N 26°29.825′ E 89°47.127′	50	27.6	6.1	74	Bottom visible	Slow to medium	Silt	Flooded and submerged	7/11/06, 1720
14	Seinpoh stream (a)[e]	N 25°31.361′ E 92°08.270′	1323	27.1	6.5	11	Bottom visible ~100 cm	Medium	Gravel	Some overhanging from bank	7/14/06, 1010
15	Seinpoh stream (b)	N 25°31.361′ E 92°08.270′	1323	26.4	6.2	10	Bottom visible	Very low	Silt	Rice paddy with mature growth of rice	7/14/06, 1010
16	Seinpoh stream (c)	N 25°31.361′ E 92°08.270′	1323	28.4	5.9	10	~50 cm	Low to none	Silt	Flooded	7/14/06, 1010
18	Dukan river	N 25°15.401′ E 91°44.013′	1234	24.7	—	29	Bottom visible	Slow	Gravel, cobble	Some overhanging from banks	7/15/06, 1420
24	Tarania village (b)	N 21°48.473′ E 87°23.276′	14	32.2	8.1	98	Very turbid. <3 cm	None	Silt	Submerged and flooded	7/19/06, 1412
26	Tarania village (b)	N 21°48.473′ E 87°23.276′	14	31.0	7.8	161	Very turbid. <3 cm	None	Silt	Submerged and flooded	7/19/06, 1412

Sites without zebrafish											
2	Jorai river (b)	N 26°31.039′ E 89°51.306′	51	27.8	6.8	198					7/9/06, 1045
7	Rydak I	N 26°31.555′ E 89°43.769′	54	26.1	7.6	226	Bottom visible	Medium to fast	Gravel, cobble	Some submerged near bank	7/10/06, 0945
8	Shipra swamp	N 26°30.234′ E 89°44.095′	54	30.6	6.7	68	Bottom visible	Very low	Silt	Flooded and submerged	7/10/06, 0835
10	Bura-Rydak	N 26°19.407′ E 89′45.105′	35	29.9	6.5	101	~50 cm	Slow to medium	Silt	Flooded rice paddy	7/11/06, 0905
13	Pathriguri river	N 26°30.884′ E 90°43.339′	389	31.0	7.5	154	Bottom visible	Slow to medium	Silt	Some submerged near bank	7/12/06, 1325
17	Umraleng river	N 25°38.854′ E 91°48.855′	1322	26.7	7.5	17	Bottom visible	Medium to fast	Gravel, cobble	Moss and algae	7/14/06, 1531
19	Mawsami Cave stream	—	1759	21.6	—	115	Bottom visible	Medium	Gravel	Some overhanging from banks	7/15/06, 1250
20	Umtyngar river	N 25°27.940′ E 91°49.561′	1682	20.9	7.7	—	Bottom visible	Medium	Gravel	Some overhanging from banks	7/15/06, 0925
21	Lamlyngkot stream	N 25°26.256′ E 91°51.503′	1795	18.6	—	22	Bottom visible	Medium	Gravel	Some overhanging from banks	7/16/06, 1255
22	Pungtung river	N 25°15.337′ E 91°57.284′	1276	23.6	—	29	Bottom visible	Fast	Gravel, cobble	Some overhanging	7/16/06, 1230
23	Tarania village (a)	N 21°48.473′ E 87°23.276′	14	30.7	7.8	23	Very turbid. <3 cm	None	Silt	Submerged and flooded	7/19/06, 1412
25	Tarania village (c)	N 21°48.473′ E 87°23.276′	14	31.0	7.6	168	Very turbid. <3 cm	None	Silt	Submerged and flooded	7/19/06, 1412
27	Andrew's farm (a)	N 22°22.713′ E 88°16.548′	55	28.6	7.4	—	Very turbid. <3 cm	None	Silt	Submerged and flooded	7/20/06, 1255
28	Andrew's farm (b)	N 22°22.713′ E 88°16.548′	5	30.1	7.4	—	Very turbid. <3 cm	None	Silt	Submerged and flooded	7/20/06, 1255

[a]Only sites with zebrafish or other fishes are listed.
[b]Elevation in meters.
[c]Conductivity.
[d]Conditions in January 2006, mid-morning: 20.5°C, pH 7.6 (P. Cottle, personal e-mail communication on 8/16/06).
[e]Conditions in January 2006, mid-morning: 18.9°C, pH 7.9 (P. Cottle, personal e-mail communication on 8/16/06).

Table 1). Localities here were relatively low-lying, with elevations ranging from 39 m to 63 m above sea level. Our first day of field work began in the Buxa Tiger Reserve, about 10 km northeast of Barovisha, a "village" with over 500,000 people, yet too small to appear on our maps. After hiking past stinging nettles larger than dinner plates, we reached the Jorai river (sites 1 and 2). During the monsoon, seasonal streams known as *nalas* feed into larger rivers such as the Jorai, and it was in one of these tributaries that we netted our first zebrafish. At this site we also collected terrestrial leeches, though unintentionally.

The same day we stopped at the Suthimari river (sites 3–6, Fig. 2B), a larger tributary of the Jorai. While recording water quality in the Suthimari (Fig. 3A), we observed runoff from nearby rice paddies that markedly raised the temperature in that area of the river (Fig. 3B). Further examination of these paddies revealed vast numbers of zebrafish juveniles and adults. Despite a water temperature of 38.6°C, six degrees higher than the main river a few meters away, zebrafish were active, seemingly healthy, and showed no obvious signs of stress such as labored gilling or gasping at the surface. Here and elsewhere, we found zebrafish only in rice paddies with crops that were mature or already harvested; fish were not found in freshly planted paddies. The Suthimari also had surprises such as freshwater pufferfish (*Tetraodon cutcutia*) and gravid male pipefish (*Microphis deocata*) (see below). That afternoon we experienced our first, and only, monsoon shower in the field.

Additional sites in West Bengal yielded a wide variety of species, including more zebrafish. A trip to Lefraguri swamp (site 11, Fig. 3E), in the Buxa Tiger Reserve, 8 km north of Barovisha, rewarded us with zebrafish, a previously seen but undescribed loach (*Lepidocephalus*), and an entirely new species of piscivorous snakehead (*Channa*), unusual in its complete lack of pelvic fins. We also collected two larger species related to zebrafish, *D. dangila* and *Devario devario* (see below). Adults of both species are considerably larger than zebrafish and seem likely to compete with one another for food or habitat. Larvae and juveniles of *D. dangila* also are nearly indistinguishable from zebrafish (unpublished data), and might compete with equivalently staged zebrafish; we never found *D. dangila* at sites with abundant zebrafish.

As the sun set on our final day in West Bengal, we visited the Ghotimari river (site 12, Fig. 3C, D), a slow moving, silt-bottomed tributary of Rydak II, 15 km northwest of Barovisha. The Ghotimari and adjacent pools contained zebrafish at higher abundance than any other site we visited in West Bengal, and our catch included both juveniles and adults. This site lacked obvious piscivorous fishes or other *Danio* or *Devario* species.

A single locality in the adjacent state of Assam (site 13, Fig. 2C), 90 km east of Barovisha, was known by local fisherman to harbor zebrafish, though we found none on the occasion we visited. Political unrest in Assam made further exploration unwise and we continued on.

Meghalaya. To assess the elevational distribution of zebrafish, we continued by car to Guwahati, the capital of Assam, and then to the hilltop city of Shillong, capital of the state of Meghalaya. Our localities here ranged from 1234 m to 1795 m above sea level. After stifling heat in the plains, the cooler, mist-shrouded hills of Meghalaya were a welcome respite. Seinpoh stream (sites 14–16, Fig. 2D) yielded tremendous numbers of zebrafish, including juveniles and adults (Fig. 4A). Though close to a road and to farms, the site appeared otherwise relatively free of human disturbance. Seinpoh stream itself had a gentle flow and a rock bottom; we found only small numbers of zebrafish in the stream proper. By contrast, a swampy pool connected to the stream had little or no flow, a silt bottom, and abundant vegetation (Fig. 4C); we observed vast numbers of juvenile and adult zebrafish shoaling in this pool (Fig. 4D). We also found large numbers of zebrafish in nearby rice paddies. Notably, zebrafish in the water were easily distinguishable from other species by their cranial patch of reflective iridophores (Fig. 4D). Although we did not find *Channa* or other piscivorous fishes, we did collect the large zebrafish relative, *D. meghalayensis*.

Despite the abundance of zebrafish at Seinpoh, most of the high elevation streams and

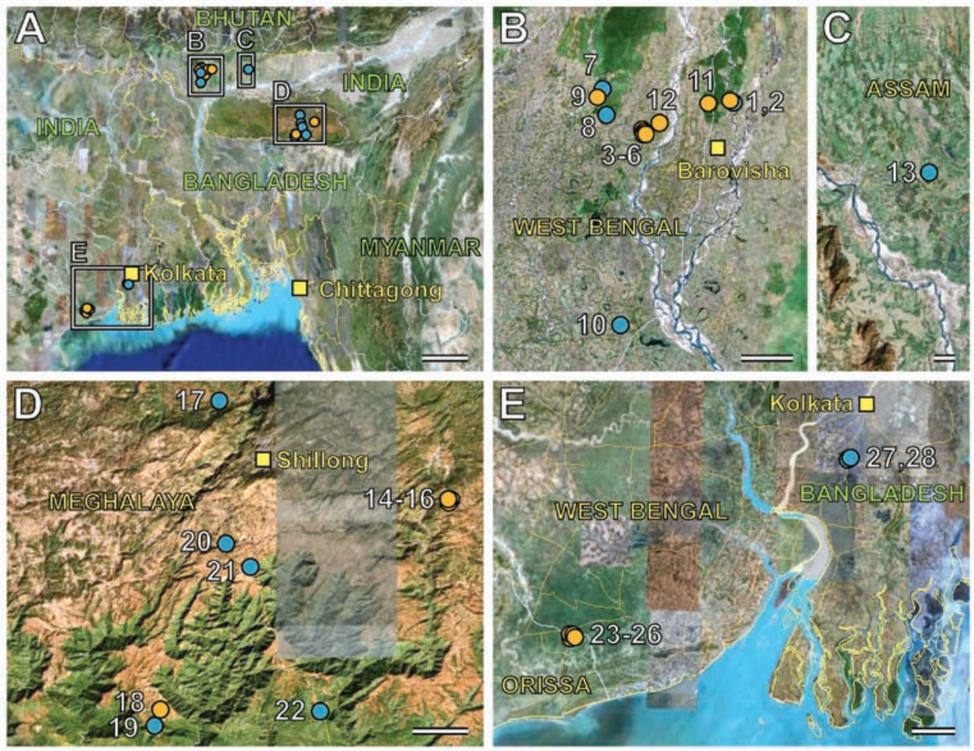

FIG. 2. Localities visited during July 2006 survey of zebrafish habitat and natural history. (**A**) Overview of region in northeast India, bordered by Bhutan, Bangladesh, and Myanmar. Scale bar, 100 km. Details of boxed regions are shown in figures B–E. Legend: Orange circles, sites where zebrafish were found. Blue circles, sites without zebrafish. Site numbers reflect the approximate sequence in which sites were visited; locality details and GPS coordinates are listed in Table 1. (**B**) Northern West Bengal low elevation localities with zebrafish found at 8 of 12 sites. The location of Barovisha is approximate. Scale bar, 5 km. (**C**) Assam low elevation locality in which no zebrafish were found, though local fisherman had found them there within the previous year. Scale bar, 2 km. (**D**) Meghalaya high elevation localities. Zebrafish were found at only 2 of 7 sites visited, probably owing to habitat loss. Scale bar, 10 km. (**E**) Orissa and southern West Bengal low elevation localities. Zebrafish were found in 2 of 4 closely situated sites in Orissa but were not found at sites just outside of Kolkata in West Bengal, where they have been found within the past 30 years. Scale bar, 20 km.

rivers in Meghalaya had faster flows, with rock or gravel bottoms, and relatively little vegetation. We found zebrafish at only one other locality, the Dukan river (site 18, Fig. 4B), which had a gentler flow and some overhanging vegetation, similar to Seinpoh. After several days in Meghalaya, we drove from Shillong back to Guwahati, and then returned by air to Kolkata.

Orissa. To expand our latitudinal range, we traveled by car southwest from Kolkata to Tarania village (sites 23–26, Fig. 2E) in the state of Orissa near the border with West Bengal. Like the areas we visited in West Bengal, Tarania is at low elevation with extensive agriculture and numerous rice paddies. Sites in and around the village included ponds and flooded rice paddies. These ponds were silt bottomed with some submerged vegetation, similar to sites where we had previously found zebrafish. In contrast to other sites, however, visibility in the ponds was very low (<3 cm) owing to algae as well as recent heavy rains (Fig. 5). While zebrafish were caught in 2 of the 4 bodies of water we fished, they were less abundant than at Seinpoh in Meghalaya or Ghotimari in West Bengal. In addition to finding zebrafish, we also caught the largest fish of the trip, an adult piscivore, *Notopterus notopterus*, ~35 cm standard length.

Abiotic environment summary

The 14 of 28 sites where we found zebrafish had slow or still waters at 24.6°C to 38.6°C, pH

5.9–8.1, and conductivities of 10 μS to 271 μS. All but two of these sites had silt-covered bottoms with submerged or overhanging vegetation. On the two occasions we located zebrafish in rocky bottomed streams (sites 14 and 18), they were found in small numbers, shoaling only near the bank under overhanging vegetation. We found zebrafish in relatively clear waters, except for the turbid pools in Orissa.

Biotic environment summary

Zebrafish co-occurred with a wide variety of fishes, from brightly colored barbs to the droll and bewhiskered loaches. The fish species collected at sites with zebrafish are shown in Fig. 6A. Selected fishes collected at sites where zebrafish was absent but other *Danio* or *Devario* were present are shown in Fig. 6B and fishes from all sites are listed in Table 2. While our sampling was not intended to be exhaustive, our observations nevertheless suggest candidate competitors and predators.

The fishes most likely to compete with zebrafish are other minnows of the same family as zebrafish, Cyprinidae. A wide variety of cyprinids co-occur with zebrafish, both small fishes like the barbs, *Puntius*, and larger fishes like the hill trouts, *Barilius*. We regularly found other *Danio* or *Devario* species and, in some cases, both, syntopic with zebrafish. *Esomus danricus* may be a principal competitor with zebrafish for food, as both species are similarly sized, with similar gapes, and occupy similar, high positions in the water column; when we found *E. danricus*, we found them in large numbers. *Aplocheilus panchax*, though slightly larger than zebrafish, also swims high in the water column and could compete with zebrafish for food. Morever, *E. danricus*, *A. panchax*, and zebrafish all feed on insects.[27,28] If there is competition for breeding sites and larval habitats, then *Puntius shalynius* would be an obvious candidate for such a role, as we observed these fish spawning in rice paddies that were swarming with juvenile zebrafish.

A number of species we collected are likely to prey on zebrafish, though analyses of gut contents will be needed to test these inferences. The snakeheads, *Channa*, the needlefish, *Xenentodon cancila*, the catfish, *Mystus bleekeri*, and the knifefish, *N. notopterus*, all co-occur with zebrafish and have gapes sufficient to swallow adults. Previous gut content analyses showed that a large proportion of the diet of these fishes consists of small fishes[31]; at sites with these piscivorous fishes, zebrafish were either absent or occurred at very low abundance. We speculate that tire-track eel, *Mastacembelus armatus*, may feed incidentally on zebrafish embryos or hatchlings: other mastacembelids are oophagous[32] and presumably find eggs while probing the substrate for benthic invertebrates. While the long-tailed catfish, *Olyra longicaudata*, certainly has a gape large enough to take adult zebrafish, this species tends to occupy riffles and faster moving waters where zebrafish are not found. Another potential predator, the swamp eel, *Monopterus cuchia*, has a tiny mouth for its size and would be less likely to feed successfully on zebrafish adults.

We did not find large predatory fishes in rice paddies or shallow seasonal waters where juvenile zebrafish were abundant. Nevertheless, even these apparent refuges harbored aquatic dragonfly larvae. Odonate larvae are voracious, visually oriented predators of larval fish in freshwater environments and could play a significant role in juvenile zebrafish mortality.[33–36] Adult dragonflies were plentiful at every site we visited and we often collected their aquatic larvae with zebrafish. Other aquatic invertebrates may be predatory on larval zebrafish as well.[37,38]

Habitat degradation and loss

An unanticipated finding of our field survey was the degree to which anthropogenic factors have affected zebrafish habitat, both in the distant past and quite recently. An historical influence has surely been rice cultivation. As waterways were dammed to allow irrigation, many seasonal streams and wetlands would have been lost. Fortunately for zebrafish, the species appears to reproduce just as well in rice paddies as in seasonal *nalas*. Nevertheless, these changes probably have altered the biotic community, as some competitors or predators would have been more adept than others at colonizing these habitats. A second agricultural practice that affects zebrafish is the production of jute, *Corchours capsularis*, a crop that is the

FIG. 3. Zebrafish localities in northern West Bengal. (**A**) At the Suthimari river (sites 3–5), a wide variety of fish species were collected from the main portion of the river. (**B**) Adjacent rice paddies that drain into the main waterway of the Suthimari river (site 6). Paddies are in different states of cultivation. Numerous juvenile zebrafish were present in the paddy at the upper left. This shallow water reached 38.6°C in the afternoon sun. (C, D) The Ghotimari river (site 12) had zebrafish in the greatest abundance of all West Bengal sites. (**C**) The main stream had a slow current and extensive vegetation on the banks. Left to right, L. Patterson, local fisherman, R. Engeszer, A. Rao. (**D**) Shallow pools adjacent to the stream harbored juvenile and adult zebrafish in similar or greater abundance. Fisherman "drive" fish towards a triangular box seine. (**E**) Lefraguri swamp (site 11), a swampy habitat with no discernable water flow, yielded small numbers of zebrafish. Images in Figures 3–7 were recorded with a Nikon D200 digital single lens reflex camera equipped with a Nikon 105 mm f/2.8G ED-IF AF-S VR Micro-Nikkor or a Nikon 18–200 f/3.5–5.6G DX VR Zoom-Nikkor, mounted on a Gitzo tripod. Additional images can be seen at http://protist.biology.washington.edu/dparichy.

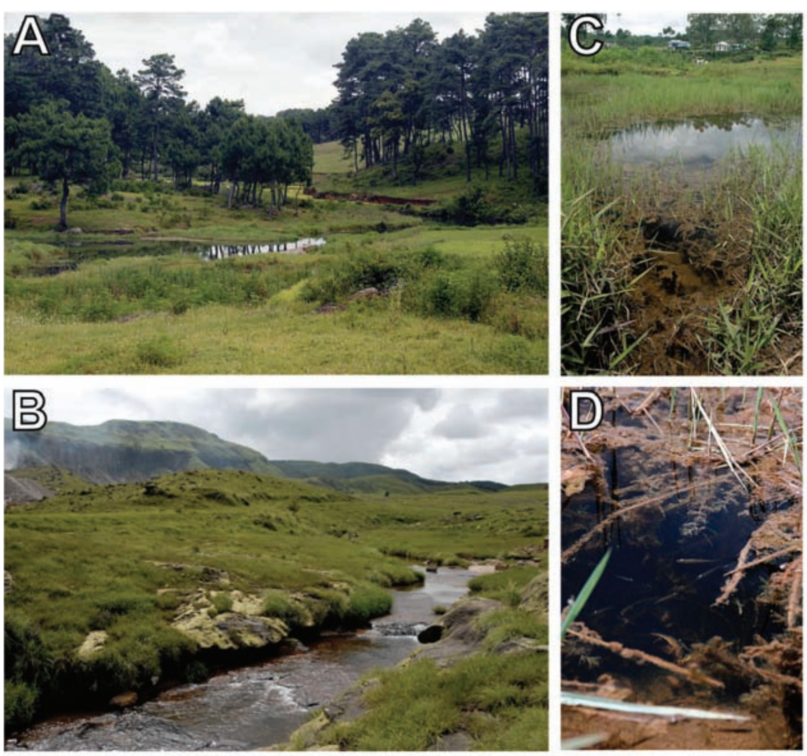

FIG. 4. Zebrafish localities in Meghalaya. (**A**) Seinpoh stream (site 14), a small, slow moving stream in relatively undisturbed habitat of meadow and trees, is a high elevation site in northern Meghalaya. (**B**) Dukan river (site 18) is a high elevation site in the southern hills of Meghalaya, immediately to the north of much lower-lying Bangladesh. Only a few adult zebrafish were collected in this small but swiftly moving stream. (**C**) A pool (site 16) adjacent to the Seinpoh stream in A, with abundant vegetation, virtually stagnant water, and numerous juvenile and adult zebrafish. (**D**) Close-up of shoaling zebrafish in C. A movie of these same fish can be found at http://protist.biology.washington.edu/dparichy.

FIG. 5. Zebrafish localities in Orissa. (**A**) Tarania village (sites 24 and 25): ponds kept flooded by local villagers serve as habitat for food fish such as *N. notopterus*. Zebrafish were found in the pond to the left of the mud walkway. Note the proximity to human habitation. (**B**) Local fisherman using a seine net to catch zebrafish at site 24. One fisherman swims to the far end, and will drive fish back towards the net, moving in the opposite direction. The pond was covered with algae and had essentially no visibility, yet it yielded numerous zebrafish. (**C**) Fishermen examining the catch at site 25.

source of fiber used in clothing, sacks, twine, and other items. Once harvested, jute is cured in small streams, where it acidifies the water, causing fish mortality. More recently, the agricultural lands and native wetlands are themselves being converted rapidly for housing and industrial use around cities such as Kolkata and other population centers.

We observed several other dramatic examples of habitat degradation and loss in Meghalaya. At one site, for example, strip mining for lime resulted in extensive pollution in a neighboring stream (Fig. 7A). Although we had collected zebrafish in an adjoining clear stream (Fig. 4B), we found no animal or plant life downstream of the confluence of the two streams. Coal mining and coal storage appear to have similar detrimental effects, as we found streams black with coal dust and completely free of fish (Fig. 7B). Yet, even seemingly more innocuous practices appear to degrade zebrafish habitat. One example is the introduction into streams of detergents used for washing laundry, a common practice that decimates the local fish fauna (Fig. 7C). Another disturbing practice is the relatively recent and unsustainable method of fishing for food species by poisoning entire stretches of streams with industrial reagents.

Having come to view the zebrafish as a hardy species in the laboratory, we had assumed it would be equally resilient in the field, and many of our observations were consistent with this assumption: we found zebrafish at high temperatures and across a broad pH range. Yet even a fish as robust as zebrafish faces serious challenges from habitat degradation and loss.

→

FIG. 6. Fish species identified across all sites. Species are grouped by family (listed above images). (**A**) Species co-occuring in the same water bodies as zebrafish. (**B**) Selected species not found with zebrafish but co-occurring with other species of *Danio* or *Devario*. Overall, species in the family Cyprinidae were caught most often, although numerous species of loaches (Cobitidae) and snakeheads (Channidae) were found as well. A detail is shown of teeth in the belonid *Xenentodon cancila*, a presumptive predator of zebrafish. The individual shown is a juvenile. Another detail is shown of the syngnathid pipefish *Microphis deocata*, revealing developing embryos in the pouch of a pregnant male. *Devario acuticephela* was obtained in Kolkata and was probably collected in the state of Minapur. Scale bars in all images, 10 mm.


A
Cyprinidae
Barilius barna
Barilius bendelisis
Chela laubuca
Danio dangila
Danio meghalayensis
Devario assamensis
Devario devario
Esomus danricus
Psilorhynchus sucatio
Puntius conchonius
Puntius shalynius
Puntius sp.
Cobitidae
Lepidocephalus annandalei
Lepidocephalus guntea
Lepidocephalus sp.
Lepidocephalus sp.
Channidae
Channa gachua
Channa punctata
Channa sp. "himalayensis"
Channa stewartii
Badidae
Badis blosyrus
Dario dario
Belontidae
Colisa sota
Ctenops nobilis
Cyprinodontidae
Aplocheilus panchax
Oryzias sp.
Mastacembelidae
Mastacembelus armatus
Olyridae
Olyra longicaudata
Synbranchidae
Monopterus cuchia
Tetraodontidae
Tetraodon cutcutia
Belonidae
Xenentodon cancila
Bagridae
Mystus bleekeri
Syngnathidae
Microphis deocata
Notopteridae
Notopterus notopterus
B
Cyprinidae
Devario aequipinnatus
Devario acuticephela
Mastacembelidae
Macrognathus pancalus
Belontidae
Colisa lalia
Parambassidae
Parambassis ranga

Table 2. Species Occurrence by Site

	Sites with zebrafish														*Sites without zebrafish*													
Family/species	*1*	*3*	*4*	*5*	*6*	*9*	*11*	*12*	*14*	*15*	*16*	*18*	*24*	*26*	*2*	*7*	*8*	*10*	*13*	*17*	*19*	*20*	*21*	*22*	*23*	*25*	*27*	*28*
Cyprinidae																												
Danio rerio	X	X	X	X	X	X	X	X	X	X	X	X	X	X														
Amblypharyngodon mola														X														
Barilius barila																X												
Barilius barna		X				X										X												
Barilius bendelisis		X																										
Chela laubuca														X														
Danio dangila	X					X	X																					
Danio meghalayensis									X											X			X					
Danionella sp.															X													
Devario devario							X											X	X									
Devario assamensis												X									X							
Devario aequipinnatus																							X	X				
Esomus danricus								X											X									X
Garra lissorhynchus																				X								
Labeo bata														X														
Labeo rohita																		X										
Oreichthys cosuatis																		X										
Psilorhynchus sucatio						X																						
Puntius chola																			X									
Puntius conchonius		X																										
Puntius phutino																	X	X										
Puntius shalynius									X											X								
Puntius sophore																		X										X
Puntius sp.	X														X													
Puntius ticto																		X										X
Tor progenius																				X								
Adrianichthyidae																												
Oryzias sp.						X									X			X										

Anabantidae																												
Anabas testudineus														X													X	X
Aplocheilidae																												
Aplocheilus panchax	X					X									X			X										
Badidae																												
Badis badis																			X									
Badis blosyrus						X																						
Badis sp.																X												
Dario dario	X						X	X																				
Dario sp.									X																			
Bagridae																												
Mystus bleekeri						X																						
Mystus tengara														X														
Mystus vittatus																		X										
Balatoridae																												
Acanthocobitis botia																X												
Lepidocephalus annandalei		X														X												
Lepidocephalus guntea							X	X																				
Lepidocephalus sp. 1									X								X	X				X						
Lepidocephalus sp. 2						X														X								
Schistura barapaniensis																												
Schistura savona																X												
Schistura sp.																				X								
Belonidae																												
Xenentodon cancila		X																X										
Channidae							X																					
Channa gachua							X							X			X											
Channa punctata							X																					
Channa sp. 1							X																					
Channa sp. 2																							X					
Channa stewartii	X																											
Channa striata																											X	

(continued)

Table 2. Species Occurrence by Site (Cont.)

	Sites with zebrafish														*Sites without zebrafish*													
Family/species	*1*	*3*	*4*	*5*	*6*	*9*	*11*	*12*	*14*	*15*	*16*	*18*	*24*	*26*	*2*	*7*	*8*	*10*	*13*	*17*	*19*	*20*	*21*	*22*	*23*	*25*	*27*	*28*
Gobiidae																												
Glossogobius giuris																	X											
Mastacembelidae																												
Macrognathus aral																			X									
Macrognathus pancalus																	X		X									
Macrognathus sp.																X												
Mastacembelus armatus						X																						
Notopteridae																												
Notopterus notopterus														X														
Olyridae																												
Olyra longicaudata									X																			
Osphronemidae																												
Colisa fasciata																											X	X
Colisa lalia																	X	X									X	X
Colisa sota							X																					
Ctenops nobilis			X																									
Parambassidae																												
Parambassis lala																		X										
Parambassis ranga																		X	X									X
Sisoridae																												
Hara jerdoni																		X										
Syngnathidae																												
Microphis deocata		X				X																						
Synbranchidae																												
Monopterus cuchia							X																					
Tetraodonitidae																												
Tetraodon cutcutia		X																										

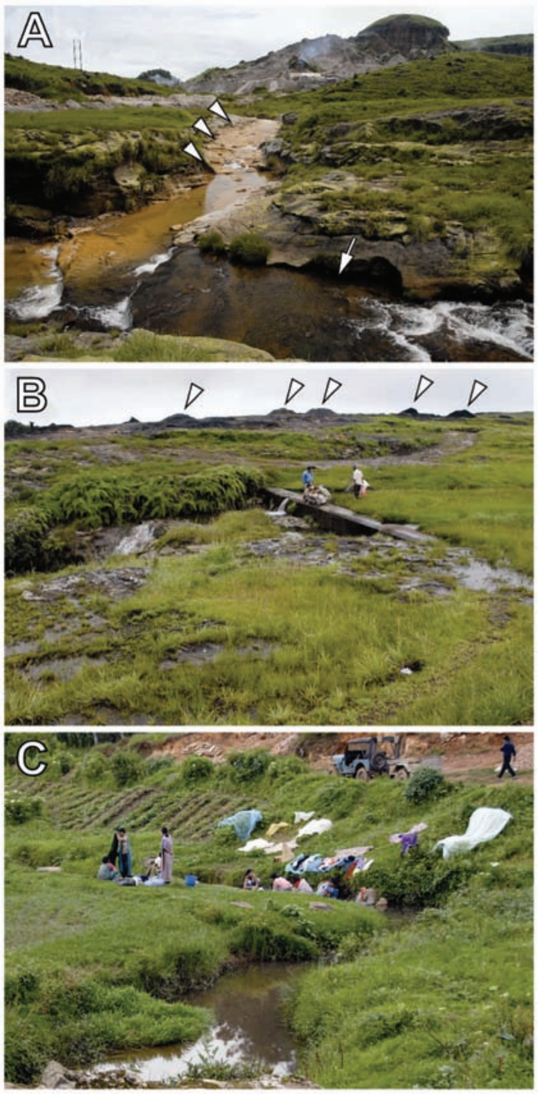

FIG. 7. Pollution and loss of zebrafish habitat. Sites shown here are in Meghalaya. (**A**) Runoff from a lime quarry (arrowheads) joins with clean water (arrow) at the Dukan river (site 18, immediately downstream of Fig. 4B). While zebrafish were found upstream of the confluence, no fish or other species were found in water downstream. (**B**) Coal mining and runoff from piles of coal (arrowheads) at a site that appeared otherwise suitable for zebrafish. No living organisms were found in the water at this location. (**C**) Streams serve many purposes including clothes washing. Numerous streams showed evidence of detergent use or other chemical or waste contamination. Here, otherwise prime zebrafish habitat, where this species was found within the past 10 years, has been denuded of zebrafish and other fauna.

DISCUSSION

Our limited field observations allow us to hypothesize the following life history for zebrafish. During the year, adults occupy shallow vegetated areas and areas shaded by overhanging vegetation in streams proper. While in the streams, they feed primarily on insects and are themselves susceptible to a variety of fishes such as *Channa*. With the onset of the rainy season, adults move into *nalas* and nearby flooded areas, including rice paddies. Spawning then occurs amid flooded vegetation in relatively still, shallow waters, with silt-covered bottoms. Larvae and juveniles remain in these seasonal waters as they develop, likely gaining some refuge from piscivorous fishes, though experiencing predation by invertebrates and particularly odonate larvae. Subsequently, young zebrafish move back into the streams proper as the seasonal waters recede. This model of zebrafish natural history and the zebrafish life cycle should be testable with additional study in the field.

Naturally occurring populations offer a wealth of information and the tools to address a variety of questions in both organismal and biomedical research. For example, genetically distinct populations can provide insights into genes responsible for natural phenotypic variation[39–41] and the effects of domestication.[42,43] Such populations also are a valuable source from which to create inbred lines for genetic mapping. Moreover, natural populations harbor mutant phenotypes (unpublished data) that supplement genetic screens for induced mutations.[44] Indeed, even mutant phenotypes isolated in the laboratory can vary in penetrance and expressivity across genetic backgrounds,[45–49] and such variability allows modifier loci to be identified. Wild populations are an important reservoir for such genetic variation. More generally, any attempt to understand the function or evolution of a phenotypic trait must address the environment in which the organism exists.[50–52]

As developmental genetic studies of zebrafish increasingly address larval and adult phenotypes, an understanding of natural history will provide a critical perspective on the forms taken by such traits within zebrafish, as well as variations in form both within zebrafish and between species.[6] For example, teleost adult pigment patterns influence shoaling, schooling, mate recognition, mate choice, and predator avoidance.[53–57] The form of any particular pigment pattern likely reflects interactions among these biotic factors (e.g., conspicuousness to mates *vs*. predators) as well as the abiotic environment (e.g., ambient light levels and colors).[58,59] The distinctive stripes of zebrafish presumably reflect the relative importance of such selective factors, and should be interpretable with additional reference to the visual ecology of these fish. In turn, the disparate spots, bars, and uniform pigment patterns of closely related danios may reflect species differences in the relative importance of these factors.[14,15,60] Similarly, interspecific and intraspecific variation in the lateral line sensory system might reflect differences in current speeds and predation regimes.[21,61–64] Finally, variation in jaws and teeth may reflect different requirements for capturing and handling particular kinds of prey.[16,65] In this regard it will be especially interesting to see how competition among danios and other syntopic species may have shaped the evolutionary history of these traits. As more is learned about zebrafish natural history, such insights can be combined with developmental and genetic tools available for zebrafish, to allow studies spanning levels of organization from the species level, to selection within populations, to form and variation in form among individuals, to the cell behaviors and gene activities underlying these forms.

Even limited field observations such as ours also have implications for research in the laboratory. For example, the range of temperatures in which we found zebrafish suggests that laboratory screens for temperature-sensitive mutant alleles[66–69] with appropriate stocks could exploit even wider temperature ranges than are typically used. This thermal range also raises questions of how such extremes are tolerated physiologically. Our finding that zebrafish appear to breed in silt-bottomed, well-vegetated pools suggests that spawning some "difficult" stocks, particularly if recently wild-caught, might be facilitated by conditions that mimic those in the field. Finally, our observations suggest that historical anthropogenic factors, such as converting natural water bodies to rice cultivation, should be considered when interpreting zebrafish ecology and behavior; more recent anthropogenic factors, such as habitat degradation by industrial and other pollutants, should be seen as lending a certain urgency to further studies of zebrafish natural history.

Given the importance of natural zebrafish populations, both for their own sake and for research, we suggest a greater emphasis on documentation and preservation. Minimally, future field studies should provide GPS coordinates and other detailed locality data, such as distances to nearest town centers or other landmarks. This information is critical for longitudinal analyses of populations and habitats, but is lacking from most publications to date. Likewise, population isolates of zebrafish should receive specific designations, by analogy with nomenclature conventions for the naming of mutant lines, and the salient data should be deposited in publicly accessible databases such as the Zebrafish Information Network. When possible, living fish or DNA should be made available to the research community through the Zebrafish International Resources Center or through other mechanisms.

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Address reprint requests to:
David M. Parichy, PhD
Department of Biology
University of Washington
Box 351800
Seattle, WA 98195-1800

E-mail: dparichy@u.washington.edu

Appendix. Historical Collections of Zebrafish in India and Neighboring Countries Since 1868[a]

Catalog number	Collector	Year	Locality	Country
BMNH 1868.10.27.51-53	Day F	1868	Madras	India
BMNH1889.2.1.1301-1309		1889	Bengal	India
BMNH 1983.7.11.15-29	Parshall A	1983	Salane River	India
CAS 11063 (SU 19063)	Herre AW	1930	Bisrampur (former Central province), Sheonath river	India
CAS 134558 (SU 34558)	Herre AW	1937	River delta at Pulta (Palta)	India
CAS 141024 (SU 41024)	Herre AW	1940	Bisrampur (former Central province), Sheonath river, Bihar State	India
CAS 141109 (SU 41109)	Herre AW	1940	Bisrampur (former Central province), Sheonath river	India
CAS 44582	Herre AW	1941	Bisrampur (former Central province), Sheonath river, Bihar State	India
CAS 50186	Roberts TR	1975	Chitawan Valley, including Khagari Khola, 45 mi. E and slightly N of Hetaura (Hitaura) and 11 mi. SSE of Narangar	Nepal
CAS 50324	Roberts TR	1975	Chitawan Valley, at Kasa Darbar (Dabar)	Nepal
CAS 50348	Roberts TR	1975	Chitawan Valley, 10 miles west of Narangar	Nepal
CAS 62038	Roberts TR	1985	Karnataka, NW/WNW of Mysore	India
SU 41108	Hora SL	1938	Kalimpong Duars and Siliguri Terai	India
KU 28676	Edds D	1996	Confluence of 3 rivers (Chaudhar, Bahuni, Gobraiya) in the Royal Shuklaa Phantaa Wildlife Reserve	Nepal
KU 28707	Edds D	1996	3 km W of Pipariya, Shuklaa Phataa Wildlife Reserve	Nepal
KU 28726	Edds D	1996	Raj-Marg highway, 9 km E of Mahendranagar	Nepal
KU 28743	Edds D	1996	Waters of Kailali district along Raj-Marg highway	Nepal
KU 28836	Edds D	1996	Tribeni	Nepal
KU 28840	Edds D	1996	Tribeni	Nepal
KU 29055	Edds D	1996	Narayangarh	Nepal
KU 29144	Edds D	1996	Bhadrapur	Nepal
KU 29182	Edds D	1996	At Raj-Marg highway	Nepal
KU 29196	Edds D	1996	Belbari	Nepal
KU 29363	Edds D	1996	Just downstream from irrigation dam at Phattepur	Nepal
NRM 26408	Sundberg H	1934	Cauvery river drainage, Mysore	India
NRM 40441	Fang F, Roos A	1998	Ganga river drainage, about 65 km NNE of Calcutta, Tumapao river close to Duma village	India
NRM 41655-6	Fang F, Roos A	1998	Ganga river drainage, about 65 km NNE of Calcutta, Tumapao river close to Duma village	India
NRM 40446	Fang F, Roos A	1998	Ganga river drainage, crossing stream about 35 km on Dumka-Rampurhat road	India
NRM 40550	Fang F, Roos A	1998	Ganga river drainage, crossing stream about 35 km on Dumka-Rampurhat road	India
NRM 41661	Fang F, Roos A	1998	Ganga river drainage, crossing stream about 35 km on Dumka-Rampurhat road	India
NRM 47184-9	Fang F, Roos A	1998	Ganga river drainage, crossing stream about 35 km on Dumka-Rampurhat road	India

(continued)

Appendix. Historical Collections of Zebrafish in India and Neighboring Countries Since 1868[a] (Cont.)

Catalog number	Collector	Year	Locality	Country
NRM 47434	Fang F, Roos A	1998	Ganga river drainage, crossing stream about 35 km on Dumka-Rampurhat road	India
NRM 40466	Fang F, Roos A	1998	Ganga river drainage, crossing stream about 35 km on Jamtara-Deughar road	India
NRM 41665-9	Fang F, Roos A	1998	Ganga river drainage, crossing stream about 35 km on Jamtara-Deughar road	India
NRM 40509	Fang F, Roos A	1998	Brahmaputra river drainage, about 100 km SSE of Dibrugarh, small stream near Dilli river	India
NRM 40536	Fang F, Roos A	1998	Brahmaputra river drainage, about 22 km on Dibrugarh-Jorhat road, roadside ditch by the Sessa Tinali (Sessa crossing)	India
NRM 40546	Fang F, Roos A	1998	Ganga river drainage, roadside stream about 62 km from Bhagalpur on Deughar-Bhagalpur road	India
NRM 41662	Fang F, Roos A	1998	Brahmaputra river drainage, about 22 km on Dibrugarh-Jorhat road, roadside ditch by the Sessa Tinali (Sessa crossing)	India
NRM 41663	Fang F, Roos A	1998	Brahmaputra river drainage, about 22 km on Dibrugarh-Jorhat road, roadside ditch by the Sessa Tinali (Sessa crossing)	India
ZMH 2103	v. Maydell	1956	Sharavati river	India
ZMH 3196	von Maydell	1956	Umsa, West Assam, Khasi Hills	India
ZMH 3197	von Maydell	1956	Nishangara, Varei stream	India
ZMH 3198	von Maydell	1956	Garampani, Assam, Kopili river	India
ZMH 3199	von Maydell	1956	Dharmawalla (Siwalik), Asan river	India
ZMH 3200	von Maydell	1956	Kaziranga, Mikir Hills	India
ZMH 3201-3	von Maydell	1956	Raimona, Janali river	India
ZMH 3204	von Maydell	1956	Jog-Falls, Sharavati River	India
ZSI F 10957/1	Chopra BN	1926	Mitkyina district, Upper Burma	Myanmar
ZSI F 12008/1	Hora SL	1935	Dehra Dun (Dehradun), Uttar Pradesh	India
ZSI F 12420/1	Rao HS	1937	Stream on Kalurkatte Road, Karnataka	India
ZSI F 2207/2	Hora SL	1939	Darrang district, Assam	India
ZSI F 2273/2	Lamba BS	1961	Balaghat district, Madhya Pradesh	India
ZSI F 2529/2	Mukerji DD	1929	River Ganges, Bhagalpur, Bihar	India
ZSI F 7327/1-7337/1	Annandale N	1911	Kalka Hill stream, Hariana	India
ZSI F 862	Rao KVS	1972	Koraput district, Orissa	India
ZSI F 9353/1	Southwell T	1917	Cooch Behar, West Bengal	India

[a]Collections data shown in Fig. 1 as obtained from on-line databases. Specimens were originally classified as zebrafish, *D. rerio*, and were not physically re-examined here.

BMNH, British Museum of Natural History; CAS, California Academy of Sciences; SU, Standford University Ichthyology Collection, now held at the Caifornia Academy of Sciences; KU, Kansas University; NRM, Swedish Museum of Natural History; ZMH, Zoological Museum of Hamburg; ZSI, Zoological Survey of India. GPS coordinates or their approximations are available through the online databases for each collection.