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Notes on the natural diet and habitat of eight danionin fishes, including the zebrafish *Danio rerio*

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The diet and habitat of eight danionin species (*Danio* and *Devario*) from 18 sites in India and Thailand are reported. At every site, habitat characteristics, including pH, light penetration, water temperature, current speed, stream dimensions, canopy cover and substratum type, were recorded. Danionins were found primarily in warm (24–35° C), moving water of moderate clarity and pH (6.6–8.2) and there were significant differences among species in water temperature, pH and current speed. Deep-bodied *Devario* species generally were associated with faster water currents than more slender-bodied *Danio* species. Gut content analyses of 327 individuals representing 17 populations showed that insects were the primary food resource for the eight *Danio* and *Devario* species. Crustaceans, fish scales, algae and detritus were also important supplements for particular species. Stable isotope data from syntopic species indicated long-term differences in consumption of terrestrial v. aquatic insects in one of two population pairs.

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Key words: *Danio*; *Devario*; diet; habitat; stable isotopes.

INTRODUCTION

Over the past two decades, the zebrafish *Danio rerio* (Hamilton) has become an important model organism in genetics, developmental biology and toxicology (Barinaga, 1990, 1994; Lele & Krone, 1996). Well-established protocols for laboratory use of this species abound, ranging from basic care and breeding to sophisticated genetic, genomic and developmental techniques (Westerfield, 1993; Detrich *et al.*, 1999; Nusslein-Volhard & Dahm, 2002). The complete genome has been sequenced and thousands of laboratory studies have been done on this species (Zfin, 2005). Progress in understanding the phylogeny of the danionin fishes, which include the zebrafish (Meyer *et al.*, 1993, 1995; Zardoya *et al.*, 1996; Fang, 2001; Parichy & Johnson, 2001; Sanger & McCune, 2002), increasingly enables comparative work. These diverse studies offer an opportunity to integrate developmental, genetic, behavioural and ecological information

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in order to better understand the intrinsic and extrinsic factors that have shaped the phenotypes of these fishes.

In marked contrast to the rapid progress in understanding the genetics and development of zebrafish, even basic ecological data such as diet and habitat preferences are lacking for most danios. Detailed data are available only for the giant danio *Devario malabaricus* (Jerdon), which has been studied in several Sri Lankan streams (Moyle & Senanayake, 1984; Wikramanayake & Moyle, 1989). For zebrafish and other danios, the available information on habitat use and diet is either very general or restricted to specific localities. Danionin fishes are found through much of South and Southeast Asia, where they typically inhabit streams (Shaw & Shebbeare, 1937; Smith, 1945) but have been reported from a variety of habitats (Axelrod *et al.*, 1985; Barman, 1991). Yadav *et al.* (1992) found that *D. rerio* readily consume mosquito larvae, and suggested that they could be useful for controlling malaria. Dietary studies of both *D. malabaricus* in Sri Lanka and *Devario devario* (Hamilton) in Nepal and India (Edds, 1987; Dutta & Malhotra, 1991) indicated that these species are primarily insectivorous.

The present study reports the results of a field survey of *Danio* and *Devario* at 18 sites in India and Thailand. Data are presented for the following eight species: *Danio albolineatus* (Blyth), *Devario aequipinnatus* (McClelland), *D. devario*, *Danio kerri* Smith, *Danio pulcher* Smith, *Devario regina* (Fowler), *D. rerio*, and *Devario browni* (Regan). In order to increase understanding of the ecology of these species, habitat use was recorded and diet was quantified using both gut contents and stable isotope analyses.

TAXONOMIC NOTE

At the time the fishes in this study were collected, all were regarded as members of the genus *Danio* (McClure, 1998). Early molecular phylogenies of danios, based on limited numbers of species, depicted a monophyletic *Danio* with two subclades: the '*Danio devario* clade' of relatively large species and the '*Danio rerio* clade' of generally small species (Meyer *et al.*, 1995). Subsequent molecular phylogenetic studies have all detected these same two subclades (McClure, 1998, 1999; Parichy & Johnson, 2001; Sanger & McCune, 2002). These authors have informally referred to the *D. devario* clade as 'large-bodied' or 'deep-bodied' and the *D. rerio* clade as 'small-bodied' or 'slender-bodied,' though Parichy & Johnson (2001) noted that the large, deep-bodied *Danio dangila* (Hamilton) is a member of the *D. rerio* clade.

Recently, Fang (2001, 2003) presented a phylogenetic analysis, based on morphological data, suggesting that *Danio sensu lato* (including all the species discussed herein) is paraphyletic when additional taxa (*e.g.* *Esomus*, *Chela* and *Inlecyrpris*) are included in the analysis. Consequently, Fang (2001, 2003) restricted *Danio* to *D. rerio*, *D. dangila*, *Danio nigrofasciatus* (Day), *D. albolineatus* and *D. kerri* and she has resurrected the genus *Devario* Hamilton for the clade which includes four of the species studied here (*D. aequipinnatus*, *D. browni*, *D. devario* and *D. regina*). Fang's (2001, 2003) restricted *Danio* and *Devario* generally correspond to the two subclades identified in earlier molecular studies. What is interesting is that *Danio sensu stricto* + *Devario* appears not

to be monophyletic, a result stemming from Fang's (2001, 2003) broader sampling of taxa. Thus, while all species of *Danio sensu stricto* and *Devario* are danionins (Roberts, 1986), it should be understood that *Danio* and *Devario* are not sister taxa and that the species studied here are a small, non-monophyletic sub-sample of danionins.

Danio pulcher has been synonymized with the widespread *D. albolineatus* (Fang, 2000), however, ecological data for *D. pulcher* are reported separately from *D. albolineatus* because differences in several osteological characters have been described (Sanger & McCune, 2002). Further study is required to distinguish whether these differences should be considered intraspecific geographic variation or interspecific differentiation. Until this issue is resolved, it is probably advantageous to report ecological data from the two groups separately.

MATERIALS AND METHODS

COLLECTION SITES AND HABITAT DATA

All species of *Danio* and *Devario* encountered were collected by seining (1.22 × 3.05 m, 5 mm mesh) from six sites in the states of West Bengal and Uttar Pradesh, India (September to October 1995), and 12 sites in the provinces of Mae Hong Son, Chantaburi and Phuket, Thailand (October to November 1995). Collection sites were clustered within several regions (Fig. 1), but they were on different waterways and

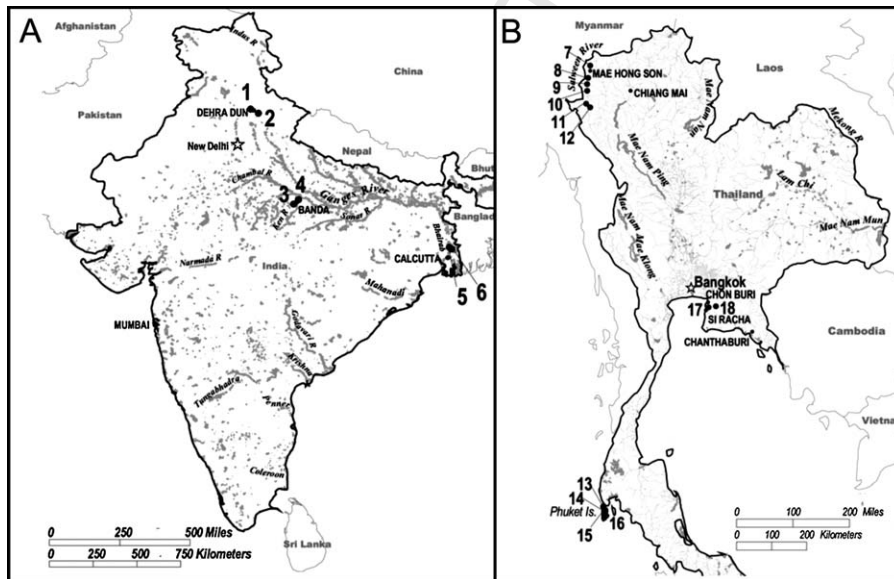


FIG. 1. Localities in (a) India and (b) Thailand where danionin species were collected. Sites 1–6 are in the Ganges River drainage; sites 7–12 are in the Salween River drainage; sites 13–16 are small streams that flow into the Andaman Sea (at the Strait of Malacca); sites 17–18 are small streams that ultimately flow into the Gulf of Thailand, in the Bight of Bangkok. Species were found at the following sites: *Danio albolineatus*, 11; *Danio kerri*, 14, 15 and 16; *Danio pulcher*, 17 and 18; *Danio rerio*, 2, 4 and 5; *Devario aequipinnatus*, 4; *Devario browni*, 7, 8, 9, 10 and 12; *Devario devario*, 1, 2, 3 and 6; *Devario regina*, 13.

1] generally separated by at least 2 km. Details for each collecting site are given in Table I. Sampling in India was conducted at sites where *Danio* and *Devario* species had been collected previously, including small rivers and streams, rice paddies, ponds and irrigation ditches. In Thailand, streams and small rivers were sampled in regions from which *Danio* and *Devario* were known to occur. Specimens were anaesthetized in MS-222, fixed using 10% formalin, stored in ethanol or isopropanol and deposited at the Cornell University Museum of Vertebrates. Catalogue numbers are given in Table I. Preserved fishes were measured for standard length (L_S) using Fowler digital calipers.

At each location where *Danio* or *Devario* were collected, eight habitat characteristics were documented (Table II). Habitat dimensions were described by the depth and width of the water body at specific places where fishes were found. The dominant substratum was categorized visually as organic matter, silt, sand, gravel, cobble, boulders or bedrock. Water temperature and pH were measured with hand-held metres. Water transparency was measured using a modification of the Secchi disc method: a one l graduated cylinder (35 cm in height) was filled with water until the colour contrast of a small Secchi disc at the bottom of the cylinder could no longer be distinguished. This height was recorded, or at sites where the disc was not obscured by filling the cylinder completely, a score of >35 cm was recorded. Current speed was estimated from the travel time of a floating marker moving 5 m through the microhabitat where a species was observed. At the same locations, the proportion of vegetative canopy cover was estimated by holding a metre stick overhead at arm's length and determining whether sky or vegetation was visible at ten 10 cm intervals.

One-way ANOVA was used to compare species in terms of the current speed, pH and temperature at sites where they were found. These analyses included only species that were collected at multiple sites; sites served as independent observations for inter-specific comparisons. Significant *F*-tests were followed by pair-wise comparisons using Tukey's honestly significant difference tests. Habitat dimensions, substrata, water transparency and vegetative canopy cover were compared qualitatively among species.

2]

DIET ANALYSIS

Gut contents were recorded for between 10 and 20 individuals from each of 17 populations (Table III). The stomach and intestine were removed from preserved specimens and examined under a dissecting microscope. Gut fullness was scored on a subjective scale from 0 (entirely empty) to 5 (extremely full). Due to prey processing and digestion, it was impossible to identify contents in detail. Contents were therefore assigned to the following categories: (1) terrestrial insects (based on conclusive identification or presence of wings), (2) aquatic insects and water mites, (3) unidentifiable insects, (4) cladocerans, (5) detritus (unidentifiable organic and inorganic materials), (6) benthic crustaceans (decapods, isopods), (7) fishes (scales and bones), (8) nematodes, (9) terrestrial plant material (leaves, seeds and rice grains) and (10) algae (filaments, diatoms and mosses). The proportional contribution of each category to total gut volume was estimated by eye for each individual. The mean proportion of total gut volume occupied by each food type was calculated for each species at each site.

Morisita's index of dietary overlap was used to assess dietary similarity among the eight danionin species examined. This index was endorsed by Krebs (1999) for its low bias and was calculated for each pair of species in two ways. The first calculation tested differences in overall insectivory by lumping unidentified insects into a single category along with identifiable terrestrial and aquatic insects. The second excluded the 'unidentified insects' in order to distinguish the relative importance of aquatic insects, terrestrial insects and other food resources. The mean volume of each food type calculated across all populations of a species was used in these calculations. To test whether dietary overlap was explained by body size, the second overlap index was regressed against the difference in mean L_S for every possible pairing of species.

To supplement comparisons of gut contents between syntopic species, stable isotope ratios of carbon (C) and nitrogen (N) in muscle tissue were analysed. Stable isotopes reflect the dietary materials assimilated by the fishes, and integrate diet over periods

TABLE I. Localities from which *Danio* and *Devario* were collected

Site number (see Fig. 1)	Date	Species name and museum catalogue number (number of specimens in parentheses)	Locality description (with site number used in McClure, 1998)
1	7 October 1995	<i>Devario devario</i> CU 77862 (20)	India, state of Uttar Pradesh, Dehra Dun district, 2 km from Herbertpur; Ganges Drainage, Assan River bridge. Assan River (site 1).
2	8 October 1995	<i>Devario devario</i> CU 77861 (32)* <i>Danio rerio</i> CU 77867 (37)*	India, state of Uttar Pradesh, Dehra Dun district, Lachhiwala; Ganges drainage, tributary of Song River (site 2).
3	2 October 1995	<i>Devario devario</i> CU 77863 (13)	India, state of Uttar Pradesh, Banda district, 2 km W of Banda city; Ganges Drainage, canal from Ken River (site 3).
4	3 October 1995	<i>Devario aequipinnatus</i> CU 77873 (27)* <i>Danio rerio</i> CU 77872 (33)*	India, state of Uttar Pradesh, Banda district, Janakikund; Ganges Drainage, side channel of Pasuni River (site 4).
5	14 September 1995	<i>Danio rerio</i> CU 78295 (3), CU 77866 (23), CU 77865 (4)	India, state of West Bengal, N. 24-Parganas District, lower Ganges Drainage, rice paddies connected with Bhairab River near Bak Bungalow (site 7).
6	14 September 1995	<i>Devario devario</i> CU 77864 (33)	India, near border with Bangladesh, state of West Bengal, N. 24-Parganas District, near Bak Bungalow, lower Ganges Drainage (site 6).
7	4 November 1995	<i>Devario brownii</i> CU77892 (38)	Thailand, Mae Hong Son Province, c. 35 km S of town of Mae Hon Son; Salween (= Salawin) Drainage, in small stream (site 10).

TABLE I. Continued

Site number (see Fig. 1)	Date	Species name and museum catalogue number (number of specimens in parentheses)	Locality description (with site number used in McClure, 1998)
8	4 November 1995	<i>Devario browni</i> CU77893 (38)	Thailand, Mae Hong Son Province, c. 25 km S of town of Mae Hong Son, Pong Kan stream (site 11).
9	2 November 1995	<i>Devario browni</i> CU 76969 (31)	Thailand, Mae Hong Son Province, c. 8 km SW of Mae Hong Son town; Salween Drainage, Huey Sertao (Old Tiger River), tributary to Pai River (site 8).
10	3 November 1995	<i>Devario browni</i> CU 76970 (31)	Thailand, Mae Hong Son Province, c. 25 km N of Mae Hong Son town, on road to Chiang Mai; Salween Drainage, Huey Mae Salat, tributary to Mai Sanga River (site 9).
11	20 November 1995	<i>Danio albolineatus</i> CU 77841(28)*, CU 78296 (3)	Thailand, Mae Hong Son Province, Mae Sariang district; Mae Sam Laep village, Salween Drainage, stream flowing into Huey Khong Kha, upstream from village, just above confluence of two streams that empty into Salween River (site 12).
12	21 November 1995	<i>Devario browni</i> CU 76960 (32)*	Thailand; Mae Hong Son Province, Mae Sariang district, 20 km SE of Mae Sariang village, above highway department house on road to Mae Sam Laep; Salween River Drainage, tributary of Yuam River (site 13).

TABLE I. Continued

Site number (see Fig. 1)	Date	Species name and museum catalogue number (number of specimens in parentheses)	Locality description (with site number used in McClure, 1998)
13	16 November 1995	<i>Devario regina</i> CU 78300 (3)	Thailand, 4.2 km E of Rt. 4, S of Thai Muang village (site 17).
14	15 November 1995	<i>Danio kerri</i> CU 77860 (31)	Thailand, Phuket Island, 6 km E of Rt. 402, on loop road; stream flowing out of Kha Phra Taew National Park (site 15).
15	14 November 1995	<i>Danio kerri</i> CU 77869 (30)	Thailand, Phuket Island, c. 3 km E of Thalang; stream flowing out of Kha Phra Taew National Park, downstream from Ton Sai waterfall (site 14).
16	16 November 1995	<i>Danio kerri</i> CU 77868 (35)	Thailand, Phuket Island, Kathu District Kathu Waterfall (site 16).
17	23 November 1995	<i>Danio pulcher</i> CU 76967 (1)	Thailand, Chantaburi Province, town of Si Racha, 2 km W Kae Khao Open Zoo; stream flowing out of Khae Khao Golf Course and Country Club (site 18).
18	23 November 1995	<i>Danio pulcher</i> CU 77840 (28)	Thailand, Chantaburi Province, town of Si Racha, 6 km E off Highway 3, on road to Chantaten Waterfall, 10 km from Chantaten Waterfall (site 19).

*, lots sampled for stable isotopes.

TABLE II. Physico-chemical characteristics for *Danio and Devario* in India and Thailand (see Fig. 1 for site numbers). For pH, transparency, temperature and current speed, the mean \pm s.d. and range (in parentheses) are given for the microhabitats where each species was found. Note that characteristics may be different for different species at the same sites because species occupy different microhabitats within sites. For stream width, depth and per cent cover of overhanging vegetation, the minimum and maximum values observed at any site are presented

Species/sites	pH	Transparency (cm)	Temperature ($^{\circ}$ C)	Current speed ($m\ s^{-1}$)	Width (m)	Depth (cm)	Per cent canopy	Substratum
ALB 11	8.0	>35	27	0.33	2-3	15-50	0	Silt, gravel, boulders
KER 14, 15, 16	7.1 \pm 0.3 (6.6-7.4)	33.3 \pm 3.5 (28 to >35)	27.0 \pm 0.8 (26-28)	0.2 \pm 0.12 (0.1-0.33)	1-3	5-70	40-90	Silt, sand, gravel, boulders
PUL 17, 18	7.2 \pm 0.1 (7.1-7.3)	34.3 \pm 1.0 (33.5 to >35)	27.3 \pm 4.6 (24-30.5)	0.4 \pm 0.2 (0.25-0.50)	1-2	1-22	15-40	Sand, gravel
RER 2, 4, 5	8.0 \pm 0.2 (7.9-8.2)	>35	31.0 \pm 3.6 (27-34)	0.07 \pm 0.05 (0-0.1)	1-12	16-57	0-50	Clay, silt, cobble, boulders
AEQ 4	8.0	>35	32	0.25	1-6	54	85	Gravel, boulders
BRO 7, 8, 9, 10, 12	8.0 \pm 0.2 (7.7-8.2)	30.4 \pm 6.3 (22.5 to >35)	26.1 \pm 1.7 (24-29)	0.4 \pm 0.2 (0.22-0.67)	1-5	1-50	10-100	Sand, gravel, cobble, boulders
DEV 1, 2, 3, 6	8.2 \pm 0.3 (7.9-8.2)	26.7 \pm 11 (10.5 to >35)	31.3 \pm 3.4 (27-35)	0.2 \pm 0.1 (0.05-0.33)	1-50	21-100	0-85	Clay, silt, sand, cobble, boulders
REG 13	7.4	35	27	0.5	1-2	10-60	70	Sand, boulders

ALB, *Danio albolineatus*; AEQ, *Devario aequipinnatus*; BRO, *Devario brownii*; DEV, *Devario devario*; KER, *Danio kerri*; PUL, *Danio pulcher*; REG, *Devario regina*; RER, *Danio rerio*.

TABLE III. Mean \pm S.D. standard length, gut 'fullness' index measurements and number of food categories found in sample for *Danio* and *Devario* by site (see Fig. 1). Fullness index is a subjective scale from 0 (completely empty) to 5 ('bulging')

Species	Site	<i>n</i>	per cent guts empty	Mean L_S (mm)	'Fullness' index	Number of food types
<i>Danio</i> <i>albolineatus</i>	11	20	5	30.5 \pm 2.5	3.2 \pm 1.2	4
<i>Danio kerri</i>	14	20	0	20.8 \pm 1.9	1.5 \pm 1.0	5
<i>Danio kerri</i>	15	20	5	15.9 \pm 1.1	1.2 \pm 0.7	2
<i>Danio kerri</i>	16	20	0	28.9 \pm 3.5	1.5 \pm 0.6	3
<i>Danio pulcher</i>	18	20	0	23.8 \pm 3.3	1.7 \pm 0.7	4
<i>Danio rerio</i> *	2	20	0	18.4 \pm 1.1	1.6 \pm 0.9	3
<i>Danio rerio</i> *	4	20	20	21.9 \pm 1.4	1.1 \pm 0.9	3
<i>Danio rerio</i>	5	10	70	16.4 \pm 1.2	0.2 \pm 0.2	2
<i>Devario</i> <i>aequipinnatus</i> *	4	20	0	40.2 \pm 3.9	2.2 \pm 0.7	4
<i>Devario browni</i>	7	20	0	39.7 \pm 7.3	2.6 \pm 1.1	4
<i>Devario browni</i>	8	20	0	40.5 \pm 5.9	3.0 \pm 0.9	5
<i>Devario browni</i>	10	20	0	37.8 \pm 6.9	2.2 \pm 1.2	2
<i>Devario browni</i>	12	20	0	41.3 \pm 5.5	1.6 \pm 0.9	3
<i>Devario devario</i>	1	20	0	36.3 \pm 5.9	3.2 \pm 1.0	4
<i>Devario devario</i> *	2	20	0	39.0 \pm 7.0	3.7 \pm 0.7	5
<i>Devario devario</i>	6	20	0	39.1 \pm 2.7	2.5 \pm 0.8	3
<i>Devario regina</i>	13	17	0	45.2 \pm 6.5	3.1 \pm 1.1	7

*, populations sampled for stable isotopes.

of weeks to years (Hesslein *et al.*, 1993). Museum specimens collected for this study were sampled after formalin-alcohol preservation, which may have minor quantitative effects on isotope ratios but does not change relative patterns (Hobson *et al.*, 1997; Bosley & Wainright, 1999; Arrington & Winemiller, 2002; Edwards *et al.*, 2002). Correction factors for preservative effects were not applied because the appropriate values are unknown, and fishes and food items compared here were all preserved in the same way. Some fishes were stored in ethanol and others in isopropanol, however, these alcohols do not differ in their effects on isotope ratios (Fabian, 1998).

Stable isotope ratios of food resources often vary widely between ecosystems (Jepson & Winemiller, 2002), therefore only comparisons between syntopic populations offer a strong test of dietary differences. To interpret isotope data from fishes, composite samples of terrestrial (primarily ants) and aquatic (Ephemeroptera, Trichoptera and Megaloptera) insects were taken from gut contents of fishes. Digestive processes do not significantly change isotope ratios of food items (Sterner & George, 2000).

Dorsal muscle tissue was sampled from preserved fishes ($n = 4$ per species per site). Fish muscle and insects were desiccated to constant mass (60° C, 48 h), pounded into a fine powder, and analysed on a Finnigan Delta-Plus mass spectrometer at the Cornell Stable Isotope Laboratory. Data are expressed in the typical δ notation (Peterson & Fry, 1987), which standardizes the ratio of heavy to light isotopes in the sample relative to an international standard (atmospheric N₂ for N; Pee Dee Belemnite for C) using units of parts per thousand (‰). Analytical precision of replicate analyses was 0.14‰ (S.D., $n = 6$) for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. MANOVA was used to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between each pair of syntopic populations. When differences were significant, univariate ANOVAs were used to evaluate patterns in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ separately.

RESULTS

Danio and *Devario* species were found in small streams and rivers with shallow, clearwater and a variety of substrata (Table II). Where present, they were usually the most abundant fishes. *Devario devario* and *D. rerio*, the two species most commonly collected in India, were found in syntopy only at site 2 (Table I). *Danio rerio* also co-occurred with *D. aequipinnatus* at site 4 in India. In Thailand, no more than one danionin species was collected per site.

Analysis of environmental variables for all populations indicated that species differed significantly in the characteristic temperature ($F_{4,15}$, $P < 0.05$), pH (F_4 , $P < 0.001$) and current speed ($F_{4,15}$, $P < 0.05$) of their habitats. There were pair-wise differences among some species in pH, however, no pair-wise differences temperature or current speed were significant.

Insects were the dominant items in the diet of all species, but most insect material could not be clearly identified as being terrestrial or aquatic in origin (Fig. 2). The abundance of other food categories varied between species and sites, and there was a significant positive relationship between mean gut fullness and diversity of food types consumed across all populations ($r^2 = 0.41$, $P < 0.01$; Table III). Within populations, all individuals had similar gut contents; food types consumed by only a few individuals seldom made up $>5\%$ of the mean for the population.

Species comparisons showed moderate to high (0.55–0.93) values of Morisita's index of dietary overlap when all insect material was lumped into a single food category (Table IV). Overlap values decreased, and showed a substantially

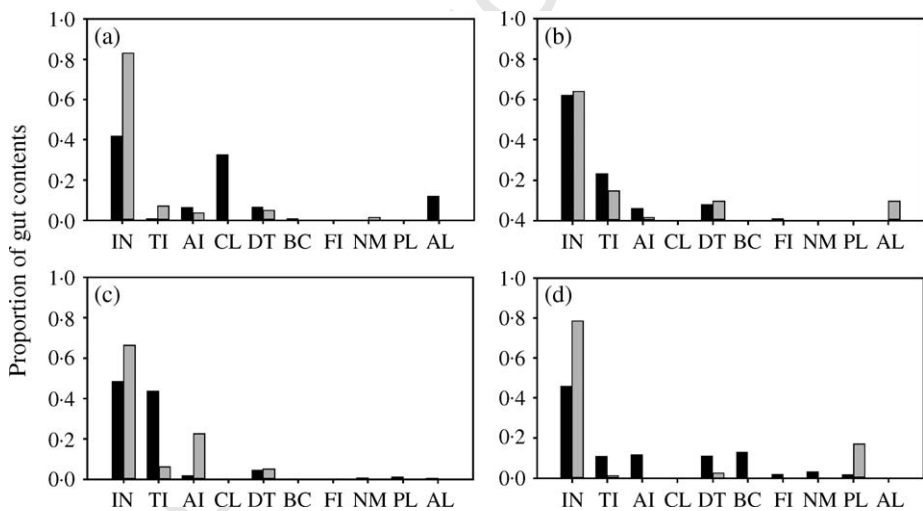


FIG. 2. Proportion of occurrence of dietary items in (a) *Devario devario* (■) and *Danio rerio* (□), (b) *Danio pulcher* (■) and *Danio Kerri* (□), (c) *Devario browni* (■) and *Danio albolineatus* (□) and (d) *Davario regina* (■) and *Devario aequipinnatus* (□). IN, unidentified insects; TI, terrestrial insects; AI, aquatic insects and water mites; CL, cladocerans; DT, detritus; BC, benthic crustaceans; FI, fishes; NM, nematodes; PL, terrestrial plant material; AL, algae and moss. Note that the greatest diversity of food types was found in *Devario devario* (see text), although the volume of NM and FI was so small that they are not visible in this figure.

TABLE IV. Values of Morisita's index of dietary overlap (Krebs, 1999) for eight species (see Table II) of *Danio* and *Devario*. Index values below the diagonal were calculated using all dietary categories except for unidentified insects. Index values above the diagonal were calculated using all dietary categories, with terrestrial, aquatic and unidentified insects lumped into 'total insects'. High values indicate high degree of overlap, with the maximum potential overlap = 1

	REG	KER	PUL	RER	DEV	ALB	BRO	AEQ
REG	X	0.69	0.74	0.76	0.55	0.76	0.75	0.66
KER	0.36	X	0.82	0.84	0.62	0.84	0.84	0.73
PUL	0.45	0.56	X	0.91	0.63	0.91	0.90	0.78
RER	0.60	0.69	0.80	X	0.65	0.93	0.93	0.81
DEV	0.27	0.36	0.23	0.36	X	0.65	0.65	0.56
ALB	0.44	0.38	0.49	0.65	0.43	X	0.93	0.81
BRO	0.39	0.52	0.68	0.67	0.10	0.32	X	0.81
AEQ	0.13	0.09	0.11	0.13	0.03	0.07	0.13	X

greater range (0.07–0.80) when the 'unidentified insects' category was removed from the analysis. Though overall patterns of relative dietary overlap between pairs of species were similar in both analyses, apparent similarity was strongly reduced by excluding unidentified insects (Table IV). Patterns of interspecific dietary overlap were unrelated to differences among species in mean L_S ($r^2 = 0.02$, $P > 0.05$).

There was no evidence of lower dietary overlap (excluding unidentified insects) between syntopic populations than for the same species as a whole. Only one strong comparison, however, was possible; overlap between *D. rerio* and *D. aequipinnatus* was 0.13 overall compared to 0.12 in syntopy at site 4. *Danio rerio* and *D. devario* from site 2 could not be compared reasonably because 90% of *D. rerio* gut contents were unidentifiable insects.

Stable isotope data indicated dietary differences between syntopic species in one of two comparisons (Fig. 3). *Danio rerio* and *D. devario* at site 2 were significantly different (Wilks' $\lambda = 0.27$, $F_{2,5}$, $P < 0.05$); *D. rerio* was relatively enriched in ^{15}N ($F_{1,6}$, $P < 0.01$). *Danio rerio* and *D. aequipinnatus* were indistinguishable in $\delta^{15}\text{N}$ (mean \pm s.d. 7.4 ± 0.4 v. 7.8 ± 0.4 , respectively) and $\delta^{13}\text{C}$ (-24.5 ± 0.2 v. -24.7 ± 0.2) at site 4. At site 2, terrestrial insects had higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ than aquatic insects (Fig. 3). No aquatic insects could be identified from gut contents of *D. rerio* and *D. aequipinnatus* at site 4, so isotopic reference values were obtained only from terrestrial insects ($\delta^{15}\text{N} = 4.36$, $\delta^{13}\text{C} = -26.95$).

SPECIES ECOLOGY

Danio albolineatus was collected only from a clearwater, open-canopy stream in the Salween River drainage of north-western Thailand. Insects comprised an average of 95% of gut volume, and were found in every individual examined. Ants and mayfly larvae were the most common identifiable taxa, and water mites and nematodes were also found inside some individuals.

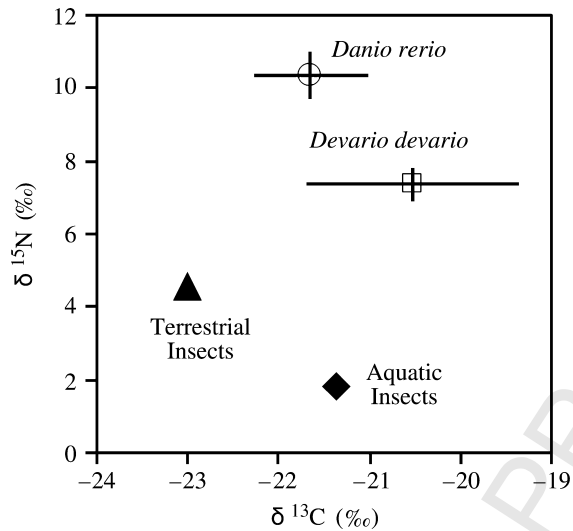


FIG. 3. Stable isotope data from insects and syntopic danionin species at site 2 (see Fig. 1). Mean \pm s.e. of four individuals is shown for fishes. Identifiable terrestrial and aquatic insects were taken from gut contents of fishes and are represented by one composite sample per site.

Danio kerri was collected at three sites in southern Thailand. The pH was significantly lower at these sites than elsewhere, and the water was slow moving. Insects made up the majority of gut volume at all sites, and the supplemental contribution of diatoms, multicellular algae, insect eggs and benthic crustaceans varied widely among localities.

Danio pulcher was found in two small, shallow streams in central Thailand. Current speed and temperature at these sites were relatively high, and pH was among the lowest. Insects were the primary food items, but detritus was also present in 45% of the individuals examined.

Danio rerio was found in relatively warm, slow-moving waters in India, including a rice paddy (site 5) and slower reaches of streams. Water transparency was generally high at these sites. Insects had been eaten by every individual examined, and nematodes were the only other identifiable category of gut contents. In general, the guts of *D. rerio* were less full than those of other species (Table III). Dietary overlap with other *Danio* species (Fang, 2001: *D. albolineatus*, *D. pulcher* and *D. kerri*) was higher than with *Devario* species.

Devario aequipinnatus was collected only from site 4, a side channel of the Paisuni River in India. Water temperature at the site was high and current speed was moderate. Gut contents comprised mostly insects, but also more plant material than any other species in the study.

Devario browni was collected from five fast-flowing, shaded streams in the Salween River drainage of Thailand. Terrestrial insects, including numerous ants as well as other hymenopterans and flies, formed a large portion of the diet of this species. Diatoms, multicellular algae, fish scales, water mites and rice (probably household waste) were also found in the guts of some individuals.

Devario devario was found usually in streams with a moderate current in the Ganges drainage of India, but also was collected in nearly still water above

a small dam at site 6. Water at sites occupied by this species had the lowest mean transparency and highest mean temperature. *Devario devario* guts contained a total of eight food types, which is the greatest diversity of any species (Fig. 2). This high diversity of food types explains its relatively low dietary overlap with other species (Table IV). Aquatic and terrestrial insects formed a large part of its diet at most sites, but substantial amounts of filamentous algae and diatoms were consumed by some individuals. Water mites, fish scales, isopods, nematodes and detritus were also eaten occasionally. An exception to these patterns was the dammed site (site 6), where the gut contents consisted almost entirely of cladocerans.

Devario regina was collected only from a fast-flowing, clearwater stream with substantial canopy coverage in southern Thailand. Insects made up over half of the gut contents, and decapod claws were present in 35% of individuals. *Devario regina* also had many nematodes in their intestines, as well as gill arches and teeth from fishes (probably an unidentified syntopic pufferfish).

DISCUSSION

This paper presents the first quantitative survey of habitat and diet for six danionin species (*D. albolineatus*, *D. kerri*, *D. pulcher*, *D. aequipinnatus*, *D. browni* and *D. regina*) and expands the information available for two species (*D. rerio* and *D. devario*) that have been studied previously. This additional information will enable more detailed comparative studies in the laboratory and field for this important group of fishes.

All *Danio* and *Devario* species were found primarily in warm, moving water (Table II). *Danio rerio* was the only species found in the still water of rice paddies, though this species was also abundant in foothill streams. Overall, species collected in India (*D. rerio*, *D. devario* and *D. aequipinnatus*) were found in warmer water than those from Thailand. Species from southern Thailand (*D. kerri*, *D. pulcher* and *D. regina*) were found in relatively acidic waters compared to counterparts from northern Thailand (*D. browni* and *D. albolineatus*) and India (*D. rerio*, *D. devario* and *D. aequipinnatus*). These patterns could reflect differences in temperature and pH preferences, or simply coincidences of biogeographical history and regional biogeochemistry. These hypotheses could be tested using either laboratory experiments or field data from streams in each region that lack these species.

Typical current speed also varied for different species (Table II). *Danio rerio* was found in the slowest water, though no interspecific pair-wise comparison was significant. When two danionins were collected at the same site (*D. devario* and *D. rerio* at site 2 and *D. aequipinnatus* and *D. rerio* at site 4), the deeper bodied species (*D. devario* and *D. aequipinnatus*) were generally collected in faster moving microhabitats, while the more slender-bodied *D. rerio* was collected in pools and stream margins. In Sri Lanka, *D. malabaricus* is another deep-bodied *Devario* that uses swift-moving areas in streams (Moyle & Senanayake, 1984; Wikramanayake & Moyle, 1989). This pattern contradicts the classic generalization that relatively deeper bodied species are found in slow-moving water while more slender fishes are found in faster water (Hubbs, 1941; Keast & Webb, 1966; Matthews, 1998). Body depth, however, was not independent of overall body

size among the species in the present study and *Devario* species were all larger than *Danio* species (Table III). Hence large body size may also facilitate use of fast-flowing areas.

Gut content data suggest differences among danionin species in dietary flexibility. At one extreme, diets of *D. rerio* and *D. browni* remained constant across sites. In contrast, *D. devario* in the present study consumed insects, zooplankton, algae and detritus, whereas work in Nepal indicated their reliance exclusively on insects (Edds, 1987) and another study in India reported omnivorous surface feeding (Dutta & Malhotra, 1991). *Danio kerri* also differed widely between sites in the relative contributions of zooplankton, algae and insects to gut contents. Data on prey availability are required to test whether these differences reflect short-term responses to prey abundance or long-term divergence among populations in trophic specializations.

Dietary overlap between species, as indicated by gut contents, was often high (Table IV). In particular, *Danio* species (*D. rerio*, *D. kerri*, *D. albolineatus* and *D. pulcher*) showed high overlap with each other relative to most *Danio-Devario* and *Devario-Devario* comparisons. *Devario* species usually overlapped little with congeners, and the reliance of *D. devario* on cladocerans at one site resulted in low overlap with all other species. Interspecific comparisons did not show any relationship between similarities in diet and L_S .

There was no evidence that dietary differences between species were enhanced in syntopy, which would be expected due to competitive displacement. Unfortunately, gut contents could only be conclusively compared for one pair of syntopic populations (*D. rerio* and *D. aequipinnatus* at site 4), hence this conclusion must be interpreted cautiously. It is also interesting that both pairs of syntopic populations were from species with low overall dietary overlap (*D. rerio* and *D. devario*, and *D. rerio* and *D. aequipinnatus*). This could suggest that coexistence among danionin species is facilitated by pre-existing differences in diet.

Stable isotope data indicated long-term dietary differences between syntopic species at one of two sites. $\delta^{13}\text{C}$ is used as a tracer of aquatic v. terrestrial carbon sources at the base of the food web. The insect samples reflected the general pattern of ^{13}C -enrichment of aquatic primary producers in tropical streams relative to terrestrial plants (Salas & Dudgeon, 2001; March & Pringle, 2003), and comparing them to danionin muscle indicates minor trophic enrichment (McCutchan *et al.*, 2003). $\delta^{15}\text{N}$ is typically interpreted as a reflection of trophic position due to the predictable, large enrichment in ^{15}N with each trophic transfer (McCutchan *et al.*, 2003). That interpretation is complicated by the high $\delta^{15}\text{N}$ of terrestrial insects compared to aquatic insects at the sites (Fig. 3), thus fish $\delta^{15}\text{N}$ was used only to infer the relative contributions of terrestrial and aquatic food resources. The enrichment in ^{15}N between insects and insectivorous danionins was well above average though within the total range observed previously (McCutchan *et al.*, 2003).

The isotope data support and expand upon the inferences possible from gut contents at site 2. $\delta^{15}\text{N}$ values of *D. devario* and *D. rerio* were statistically distinct (Fig. 3), and $\delta^{13}\text{C}$ values differed suggestively though not significantly. The lower $\delta^{15}\text{N}$ and higher $\delta^{13}\text{C}$ of *D. devario* indicate that aquatic insects were its primary food resource, in agreement with the fact that aquatic insects made up of an average of 74% of identifiable items in its guts. Though 91% of the

gut contents of *D. rerio* were unidentifiable insects, the higher $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ of its muscle reveal that a majority of the insects it eats are of terrestrial origin (Fig. 3).

At site 4, neither isotope nor gut content data were definitive. *Danio rerio* and *D. aequipinnatus* were isotopically similar, suggesting that they consumed similar proportions of aquatic and terrestrial insects. No conclusions can be drawn, however, about relative contributions due to the lack of an isotopic reference value for aquatic insects. Similarly, gut contents from both populations comprised >75% unidentified insects, preventing comparisons with the isotope data.

The dietary analyses suggest a phylogenetic association between body shape and diet. Dietary overlap between the shallow-bodied *Danio* species studied here greatly exceeded the overlap between *Danio* and deeper bodied *Devario* species (Table IV). This pattern of dietary differences is consistent with the field observations of microhabitat preferences in syntopy. At sites 2 and 4, *D. rerio* were found primarily in pools and stream margins, where terrestrial insects were most likely to fall into the water, whereas *Devario* species were found in faster water where aquatic insects might have been more accessible. Comparisons of gut contents and stable isotopes between *D. rerio* and *D. devario* found in syntopy at site 2 also support the conclusion that *Danio* rely more heavily on terrestrial insects and *Devario* on aquatic insects (Fig. 3). Taken together, these ecological patterns suggest that associations among relative body depth, diet and microhabitat use may have been conserved during the evolution of danionin fishes. It would be interesting to know whether deep-bodied *D. dangila* depend most heavily on terrestrial insects, like its congeners, or instead feed primarily on aquatic insects, like the deep-bodied *Devario* species.

The naturally occurring ecological variation within danionin fishes revealed by this survey offers many opportunities to integrate genetic, developmental and ecological research. For example, field data show that *D. devario* can feed at the surface, on zooplankton in the water column and in the benthos. Research on developmental patterns in this species could resolve whether morphological plasticity (e.g. gill rakers and snout length; Schluter, 1994) facilitates these switches, and current understanding of jaw development in zebrafish (Piotrowski *et al.*, 1996) could elucidate the genetic basis for producing such adaptive shifts. Subsequent field surveys could test the agreement between genotypic and phenotypic distributions in nature.

Similarly, the apparent preferences of deep-bodied species for fast-moving water and shallow-bodied species for slower currents suggest that there may be functional differences in body shape, muscle morphology or muscle physiology among species. Many mutants affecting muscle structure, motility, somitogenesis and heart function have been identified in zebrafish (Haffter *et al.*, 1996). Combining knowledge of the functional effects of such genes with genetic surveys of wild populations and assays of performance could enhance the understanding of the origin and maintenance of phenotypic variation. Further phylogenetic studies could test whether the hypothesized functional association of body depth, diet and microhabitat use is indeed conserved during diversification.

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