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ADAPTATION OF RAINBOW FISH TO LAKE AND STREAM HABITATS

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Abstract.—Fish occupy a range of hydrological habitats that exert different demands on locomotor performance. We examined replicate natural populations of the rainbow fishes *Melanotaenia eachamensis* and *M. duboulayi* to determine if colonization of low-velocity (lake) habitats by fish from high-velocity (stream) habitats resulted in adaptation of locomotor morphology and performance. Relative to stream conspecifics, lake fish had more posteriorly positioned first dorsal and pelvic fins, and shorter second dorsal fin bases. Habitat dimorphism observed between wild-caught fish was determined to be heritable as it was retained in *M. eachamensis* offspring raised in a common garden. Repeated evolution of the same heritable phenotype in independently derived populations indicated body shape divergence was a consequence of natural selection. Morphological divergence between hydrological habitats did not support a priori expectations of deeper bodies and caudal peduncles in lake fish. However, observed divergence in fin positioning was consistent with a family-wide association between habitat and morphology, and with empirical studies on other fish species. As predicted, decreased demand for sustained swimming in lakes resulted in a reduction in caudal red muscle area of lake fish relative to their stream counterparts. *Melanotaenia duboulayi* lake fish also had slower sustained swimming speeds (U_{crit}) than stream conspecifics. In *M. eachamensis*, habitat affected U_{crit} of males and females differently. Specifically, females exhibited the pattern observed in *M. duboulayi* (lake fish had faster U_{crit} than stream fish), but the opposite association was observed in males (stream males had slower U_{crit} than lake males). Stream *M. eachamensis* also exhibited a reversed pattern of sexual dimorphism in U_{crit} (males slower than females) relative to all other groups (males faster than females). We suggest that *M. eachamensis* males from streams responded to factors other than water velocity. Although replication of muscle and U_{crit} phenotypes across same habitat populations within and/or among species was suggestive of adaptation, the common garden experiment did not confirm a genetic basis to these associations. Kinematic studies should consider the effect of the position and base length of dorsal fins.

Key words.—Burst speed, common garden experiment, Melanotaeniidae, morphology, natural selection, red muscle, U_{crit}.

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Locomotion is a complex, whole animal function, crucial for activities that have deterministic effects on fitness. For example, differential locomotor performance has been demonstrated to result in both differential survival (e.g., Beamish 1978; Swain 1993) and feeding efficiency (e.g., Schaefer et al. 1999). There is also mounting evidence that locomotor performance has a genetic basis (van Berkum and Tsuji 1987; Garland 1988, 1994; Nicoletto 1995). Therefore, locomotor performance might evolve through natural selection if environments differ in their locomotor demands. Water velocity is one aspect of a fish's environment that is likely to exert selective pressure on locomotor performance. Water velocity influences all aspects of a fish's life, including performance at activities such as feeding, predator avoidance, and social interaction (e.g., Allan 1995; Biro et al. 1997; Schaefer et al. 1999). This suggests the locomotor requirements of fish in flowing water will differ from those of fish in still water. In particular, fish in flowing water will need to avoid downstream displacement as they engage in essential activities.

An extensive array of theoretical and empirical literature underpins our current understanding of fish locomotion (e.g., Weihs 1972; Lighthill 1975, 1977; Webb 1977, 1992; Altringham et al. 1993; Wakeling and Johnston 1999). Many fish swim by lateral oscillation of the body and caudal (tail) fin. This mode of locomotion can be used for both sustained (aerobic) and burst (anaerobic) swimming. However, maximum performance at one can preclude maximum performance at the other (e.g., Reidy et al. 2000). Because of this trade-off, selection to increase sustained performance, such as in response to increased water velocity, could result in a commensurate decrease in burst performance. The basis of this trade-off is both morphological and physiological.

Morphologically, maximum sustained swimming performance depends on a high aspect ratio caudal fin, a narrow caudal peduncle, and an inflexible, streamlined body (Webb 1982). In contrast, burst performance is maximized by a deep, flexible body and a deep caudal peduncle (Webb 1982). These predicted associations between morphology and swimming performances have been observed empirically among closely related intra- (e.g., Williams and Brett 1987; Taylor and Foote 1991) and interspecific (e.g., Taylor and McPhail 1985a, b; Hawkins and Quinn 1996) populations.

Another factor that deterministically affects swimming performance and is of interest in this paper are the muscles that power swimming. Sustained swimming is powered by red (slow twitch, oxidative) muscle and burst swimming by white (fast twitch, glycolytic) muscle (Mosse and Hudson 1977; Jayne and Lauder 1994). Therefore, faster sustained swimming speeds are predicted to be associated with a greater proportion of red muscle, relative to white muscle mass. In contrast, faster burst speeds should be negatively associated with relative red muscle area. These associations have been demonstrated empirically (Boddeke et al. 1959; Broughton et al. 1981; Meyer-Rochaw and Ingram 1993).

The effect of water velocity on swimming performance and associated traits has received limited attention, despite the known ecological effects and potential role of water velocity in natural selection. Morphologically, fish from fast

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versus slow flowing water conform to hydromechanical predictions (fast-water fish have shallower bodies and caudal peduncles); however, these differences have not been correlated with locomotor performance (e.g., Gatz 1979; Riddell and Leggett 1981; McLaughlin and Grant 1994; Sagnes et al. 1997).

The family Melanotaeniidae (rainbow fish) is one of the most species rich (17 described species and subspecies), widespread, and locally abundant fish families in Australia (Allen 1995). Melanotaeniids swim using primarily body-caudal fin oscillation, but are observed to utilize pectoral and median fins during low speed maneuvering. In this study, we examined two nonsister species: *Melanotaenia duboulayi* from southeast Queensland and *M. eachamensis* from northeast Queensland. Several features of this rainbow fish system make it ideal for the investigation of evolutionary responses to water velocity.

First, although found in disjunct geographic regions, *M. duboulayi* and *M. eachamensis* have similar ecologies. They are opportunistic omnivores, utilizing the entire water column to forage on algae, benthic invertebrates, and floating terrestrial insects (Allen and Cross 1982; Allen 1995). Both species occupy a range of hydrological habitats, including those at the extremes of the water velocity distribution: lakes and high-gradient streams. Stream fish are observed in the water column in areas of maximal water flow, indicating they experience faster net velocity than do lake fish. Replication of lake and stream populations within and across species enables dissociation of phenotypic variation due to adaptation to hydrological environment from that due to either the speciation process, or random genetic drift.

Second, occupation of lakes is particular to *M. duboulayi* and *M. eachamensis*, as all their close relatives occupy only streams or, occasionally, recently ($<$60 years) created dams (Allen 1995; McGuigan et al. 2000; McGuigan 2001). Relative to time since speciation ($\geq$1.8 million years; McGuigan et al. 2000), lakes occupied by *M. duboulayi* or *M. eachamensis* formed recently ($<$700 thousand years (Longmore 1997) and $<$1 million years (Jardine 1925) respectively). This suggests postspeciation colonization of lakes by stream fish has occurred independently in each species. Therefore, we can assign the direction of evolution to any phenotypic differences observed between fishes from each habitat. Occupied lakes are closed catchments (i.e., not connected to streams), implying restricted gene flow between hydrological habitats. This restriction was observed at the molecular level using microsatellite and mtDNA data from *M. eachamensis* (Zhu et al. 1998). Low levels of gene flow will facilitate adaptation.

In this study, we assayed replicate lake and stream populations of *M. duboulayi* and *M. eachamensis*, as well as raising each replicate population of *M. eachamensis* in a common-garden experiment. We tested the hypotheses that differences in water velocity have resulted in heritable differences in: (1) body shape, (2) red muscle area and, (3) sustained and burst swimming speeds. We tested the specific hypotheses that rainbow fish from lakes had deeper caudal peduncles, deeper bodies, a smaller area of red muscle caudally, slower sustained swimming speeds, and faster burst swimming speeds relative to their stream counterparts.

Materials and Methods

In this paper, we asked two broad questions: (1) was phenotypic variation associated with water velocity habitats? If yes, (2) was there a heritable basis to the phenotypic divergence between water velocity habitats? The first question was addressed by comparing wild-caught *M. eachamensis* and *M. duboulayi* from lakes and streams. The second question was addressed using *M. eachamensis* to determine if phenotypic divergence between wild-caught stream and lake fish was maintained after one generation in a common garden. Thus, there were two datasets analyzed throughout the paper, referred to as ''wild-caught'' and ''common-garden,'' respectively.

Collection of Fish

Ten males and 10 females were collected (Nov. 1999–June 2000) from each of two lake and two stream populations of *M. eachamensis* (Lake Eacham, see below; Lake Euramoo (17°09′S, 145°37′W), South Johnstone River (17°39′S, 145°42′W), Dirran Creek (17°28′S, 145°32′W)); and of *M. duboulayi* (Ocean Lake (24°55′S, 153°16′W), Lake Boomanjin (25°33′S, 153°04′W), Amamoor Creek (26°21′S, 152°40′W), Kholo Creek (27°31′S, 152°50′W)). *Melanotaenia eachamensis* from Lake Eacham (17°17′S, 145°38′W) were obtained from captive stock (Caughley et al. 1990), collected before the local extinction of *M. eachamensis* (Barlow et al. 1987). For three years prior to experiments described in this paper, Lake Eacham fish were maintained in large, open-air enclosures and foraged exclusively on insects and algae that naturally colonized the tanks. Each of the eight collection sites represented different, independent catchments, and large geographic distances separated all sites. Only Lake Boomanjin had a stream connection (inflow) and fish were collected as far as possible from this point.

Wild-caught fish were held in 72 L still water tanks at 25°C (12 h light: 12 h dark) and fed once daily on Serra Vipa flake food. For the common-garden experiment, one generation of each of the four populations of *M. eachamensis* was reared in the laboratory in still-water tanks. Spawnings were conducted monogamously, with up to 10 (minimum of five) pairs of parents per population contributing to the laboratory populations. Spawning mops were supplied as substrate for egg attachment. Mops were checked daily for eggs, which were placed in 2 L tanks in the same room. After hatching, fry were moved to 72 L tanks and fed Serra Vipa fry food, replaced with flake food when they were large enough to consume it. As with the wild-caught data, 10 males and 10 females were analyzed from each of these four common-garden populations.

Body Shape

Morphometric measurements were made on microscope images of anesthetized (MS 222, 1:10000) fish using Video Trace, computer software that facilitates calibrated measurement directly from a live-video feed. Body shape of each fish was characterized using a truss network with 10 landmarks and 21 interlandmark distances (Strauss and Bookstein 1982: Fig. 1). In addition, we recorded standard length (from tip

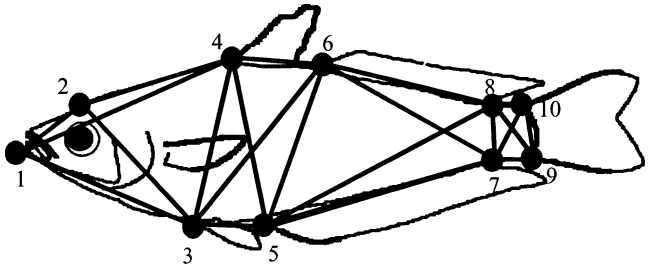

FIG. 1. Truss network with 10 landmarks (see Strauss and Bookstein 1982). Landmarks were: (1) most anterior point of snout; (2) dip above front of eye; (3) origin of pelvic fins; (4) origin of first dorsal fin; (5) origin of anal fin; (6) origin of second dorsal fin; (7) insertion of anal fin; (8) insertion of second dorsal fin; (9) posterior point of the caudal peduncle ventrally; (10) posterior point of the caudal peduncle dorsally. Interlandmark distances were identified with reference to the numbers of the two defining landmarks.

of snout to end of caudal peduncle) and body depth below the insert of the first dorsal fin (perpendicular to standard length). All data were natural log transformed prior to analyses.

Principal component analyses

Principal component analysis (PCA) was conducted on each truss dataset (wild-caught and common-garden) for two reasons. First, a large number of interlandmark distances were measured and some of these were highly correlated with each other. Datasets with a high degree of multicolinearity are not well suited to hypothesis testing using multivariate techniques such as MANOVA. Principal component analysis was useful in both reducing the number of variables and eliminating multicolinearity. Because all variables were measured on the same scale, factors were extracted from the covariance matrix. We retained all Principal Components (PCs) that explained greater than 1% of the variance in the dataset.

Second, PCA allowed the removal of the effect of body size. Hydromechanical theory predicts differences in body shape, but interlandmark distances are measures of the absolute size of regions of the body, and variation in size might obscure variation in shape (Humphries et al. 1981; Reist 1985). Principal component analyses of morphometric data often results in a first principal component (PC1) that explains variation in overall body size, recognizable because all variables contribute strongly and in the same direction (Reist 1985; Jolliffe 1986). If PC1 is determined by variation in size, subsequent PCs can be interpreted as size-free variation in body shape. We tested the hypothesis that PC1 represented size variation in two ways. First, all PCs were subjected to correlation analyses to determine their relationship to standard length, a variable commonly used to describe size in fish. Second, we used linear regression analyses to determine whether a significant portion of variation in PC1 could be explained by variation in standard length.

After PCA eliminated multicolinearity from the dataset, we analyzed the variation described by the PCs to determine the contributions of habitat, species/generation, sex, and population. Individual PC scores were analyzed using analysis of variance (ANOVA) (Pimental 1979), with either Model A1 for analyses of wild-caught fishes:

$$Y_{ijklm} = \mu + H_i + Sp_j + S_k + HSp_{ij} + HS_{ik} + SpS_{jk} + HSpS_{ijk} + P_{l(ij)} + \varepsilon_{m(ijkl)} \quad \text{(Model A1)}$$

or Model A2, for analyses of the common-garden experiment:

$$Y_{ijklm} = \mu + H_i + G_j + S_k + HG_{ij} + HS_{ik} + GS_{jk} + HGS_{ijk} + P_{l(i)} + \varepsilon_{m(ijkl)} \quad \text{(Model A2)}$$

where Y was the PC score for the individual; μ was the grand mean of the population; H_i was the habitat effect for the ith group; Sp_j or G_j were the species or generation effect for the jth group; and S_k was the sex effect for the kth group. These effects were fixed, and their interactions were considered. $P_{l(ij)}$ was the effect of lth population nested within the ith habitat and jth species (Model A1), $P_{l(i)}$ was the effect of lth population nested within the ith habitat (Model A2), and $\varepsilon_{m(ijkl)}$ was the random deviation of individuals from the grand mean.

As separate ANOVAs were conducted on each of the PCs, we applied a sequential Bonferroni correction across PCs for each source of variance (e.g., habitat: see Rice 1989). *P*-values were ranked from smallest to largest, and the first *P*-value (P_1) was considered significant if $P_1 \leq \alpha/k$ where α, the set significance value, was equal to 0.05 and k was the number of comparisons (seven PCs were analyzed, so for P_1, $k = 7$). If P_1 was accepted, then the significance of P_2 was assessed against $\alpha/(k - 1)$ and so on to P_7.

To determine whether PC1 explained variation among treatments (habitat, species/generation, sex, or population) that was not due to variation in size we utilized ANCOVA with standard length as a covariate. We adapted the ANOVA Models A1 and A2 to include a term for the covariate (standard length), and terms to test whether the relationship between PC1 and standard length (i.e., slope) was the same for each habitat, species/generation and sex.

Retention of habitat dimorphism

We addressed the question of whether there was retention of habitat dimorphism in fish raised in the common laboratory environment in two ways. First, we applied the combined PCA/ANOVA analyses described above to determine whether laboratory fish differed from their wild-caught parents. Differences between wild-caught and laboratory-raised fish were assessed both by comparing the structure of PCs across datasets using correlation analyses and by examining the ANOVA (Model A2) results for the common-garden dataset for significance of the generation term, or any interaction terms involving generation (e.g., generation by habitat). Second, we utilized a discriminant functions analysis (DFA) to test the specific hypothesis that traits observed to diverge between habitats in wild-caught fish could discriminate between parental habitats of laboratory-raised fish. Principal component analysis produces a series of orthogonal eigenvectors (PCs) that are in decreasing order with respect to the amount of variation each explains, irrespective of membership to any group (such as habitat). In contrast, DFA produces nonorthogonal eigenvectors (discriminant functions) that maximize among-group variability relative to within-group variability. A lack of correspondence between PCs identified by analyses of wild-caught versus common-garden data would indicate a

difference in the way in which variation was distributed in these datasets, but would not necessarily indicate that habitat dimorphism lacked a genetic basis. Therefore, we used DFA to ask whether traits that contributed strongly to habitat divergence in the wild could be used to assign parental habitat of laboratory-raised fish. We identified traits for use in the DFA from the PCA/ANOVA analyses on wild-caught fish and considered traits to contribute strongly if they had PC coefficients greater than 70% of the maximum contribution (Mardia et al. 1979).

Before the DFA, ln-transformed interlandmark distances were corrected for differences in body size by regression against PC1C (Reist 1985). Size correction by regression analyses relies on the assumption that the relationship between the variable of interest and the size variable is the same across groups. As described above, we tested this assumption using analysis of covariance (ANCOVA). ANCOVA indicated PC1C explained significant portions of variation in each trait, and that there was no heterogeneity in slope among generations, habitats, or sexes for any trait. Therefore, linear regression analyses were conducted and residuals reserved for use in the DFA. Outliers (± 3 standard deviations of the mean) were removed from the dataset.

Differences in body and caudal depth

Based on hydromechanical theory and empirical evidence, we proposed the specific hypotheses that lake fish would be deeper bodied and have deeper caudal peduncles than their stream counterparts. To test these hypotheses we regressed body depth against standard length in the wild-caught and common-garden datasets. Standard length, not PC1C, was used because body depth had not been included in the PCA. ANOVA (Model A1 or A2) were conducted on the size-corrected residuals to determine whether body depth varied between habitats. Caudal peduncle depth (trait 7–8) was size corrected by regression on PC1W or PC1C for wild-caught or common-garden datasets, respectively. ANOVA (Model A1 or A2) was then used to determine the contribution of each experimental source to variation in caudal depth.

Performance and Muscle Morphology

Sustained (aerobic) swimming performance (U_{crit})

Maximum capacity for aerobic activity was characterized by an absolute measure of critical swimming speed (U_{crit}), determined by an increasing velocity test sensu Brett (1964). U_{crit} was characterized in a circular flume in which water flow was generated by an electric motor. Parallel polyvinalchloride pipes and wire mesh functioned as collimators, reducing the circular component of flow that resulted from the use of a rotating propeller, and reducing the turbulence of flow. The voltage applied to the motor was calibrated against a flow meter, and flow was measured throughout the swim chamber to ensure uniformity. Mirrored glass in the viewing wall prevented fish-experimenter interaction.

Each U_{crit}-trial consisted of three fish of the same sex and from the same population. Fish did not swim in close proximity ($\leq$2 cm) to one another, especially not at higher speeds. Nor were fish observed to swim in the same horizontal plane, suggesting they gained no hydrodynamic benefits from sharing the flume (Weihs 1975). Fish swam in a clear perspex tube (15 cm in diameter, 100 cm in length), considerably larger than the fish (cross-sectional area of fish $<$10% cross-sectional area of flume). Therefore, no correction was made for blocking effects (Smit et al. 1971). Fish were fasted for 24 h prior to the trial, and all trials were conducted at 25°C, between 7:00 h and 19:00 h. The trial began immediately the fish were introduced to the flume. A total of seven trials was conducted for each population (four male and three female trials) from each species and generation (total of 84 trials). The order in which same sex individuals of each population swam in a trail was determined randomly.

The U_{crit} trial was designed as a ramp velocity test (Jain et al. 1997). The ramp phase took fish up to approximately 50% of U_{crit} in four steps of one minute duration. The increments approximated 12% ($0.063 ms^{-1}$; approximately 1.3 standard lengths per second) of U_{crit} and the ramp phase was followed by 12% increments held for 20 min. Fish were judged to have fatigued when they no longer actively swam, but were swept against the mesh at the back of the flume. Fish were removed from the flume when they had fatigued, and their standard length recorded. U_{crit} was calculated using Brett's (1964) equation: $U_{crit} = V_{n-1} + (T_{n-1}/T_n)V_n$, where V_{n-1} was the velocity for which the fish swam for the full time period and T_n was the duration of each step (20 min); V_n was the velocity of the step at which the fish fatigued and T_{n-1} was the time for which the fish swam at that step.

Burst (anaerobic) swimming performance

Maximum-burst swimming speed was determined for the each of the fish for which U_{crit} had been recorded. Trials were conducted in a transparent acrylic raceway (100 cm $\times$ 10 cm $\times$ 8 cm). The raceway walls were blacked out except where the two infrared light sources (6.3 cm apart) and their aligned photoelectric receptors were placed. Individual fish were introduced to one end of the raceway and a dark refuge box was placed at the other end to encourage directed swimming. When fish oriented to the covered end, a low amperage electric pulse was administered to elicit swimming. As the fish swam, they interrupted the infrared beam, resulting in a change in output voltage of the photocells and a PowerLab 4 (ADInstruments, Sydney) recorded this voltage change at a sampling rate of 1000 Hz. The time between interruptions and the distance between photocells was used to determine velocity in $msec^{-1}$. Each fish was enticed to burst three times, and the fastest of these trials was retained for analysis.

Muscle morphology

To determine whether stream fish had a greater relative amount of red muscle than lake fish, individuals for which swimming speed had been characterized were euthanaized by severing the spinal cord and sampled for red muscle. As red muscle area increases in the caudal peduncle (e.g., Coughlin and Rome 1996; Devincenti et al. 2000), a block of tissue, 1cm in length (along the anterior-posterior axis), was taken from the posterior edge of the second dorsal fin insertion. This block of tissue was placed in 2-methylbutane and chilled in liquid nitrogen. Sections (10 μm) were cut with a cryostat,

using the notochord/vertebral column and the dorsal/ventral fins as reliable markers of location. The tissue was stained with succinate dehydrogenase, a marker for oxidative metabolism in mitochondria. The high concentration of mitochondria in red muscle results in a dark stain, but white muscle stains very lightly. Sections were mounted in gelatin. Video Trace (Leading Edge, Marion, Australia) was again used to examine the microscope images and record the area of red and white muscle area in one half of each section. Total area in the measured sections was calculated by summing areas of red and white muscle.

Statistical analyses of performance and muscle data

The three fish of each U_{crit} trial were not statistically independent. The mean of each group of was taken for all traits (U_{crit}, burst speed, and muscle area) and all analyses were conducted on those means. Body size can deterministically affect locomotor performance (Jayne and Bennett 1990; McDonald et al. 1998). However, the relationship between size and speed is not constant across taxa (Hammer 1995). Whether there was a relationship between sustained or burst swimming speed and size (standard length), and whether this relationship was constant across species/generations, habitats, or sexes was determined using ANCOVA. ANCOVA was also used to determine whether the area of red muscle was dependent on the total muscle area, and whether regression slopes were the same across groups. When ANCOVA revealed a significant effect of the covariate on the trait of interest, and this effect was the same in each habitat, species/generation, and sex, the variable was regressed against its covariate and the residuals retained as size-corrected data for further analyses. As with the morphological data, wild-caught and common-garden data were analyzed separately.

For variables that had a consistent relationship with the covariate across species/generations, sexes, and habitats, ANOVA (Model A1 for wild-caught data or Model A2 for common-garden data) was used to determine the contribution of each experimental source to variation in the trait. For U_{crit}, the relationship between trait and covariate was not the same for each species in the wild-caught data, or each generation in the common-garden data (see Results). Species and generations were analyzed separately for that trait using ANOVA (Model B):

$$Y_{iklm} = \mu + H_i + S_k + HS_{ik} + P_{l(i)} + \varepsilon_{m(ikl)} \qquad \text{(Model B)}$$

where the terms are as described for Model A2.

Results

Variation in Body Size

Each of the first seven PCs individually explained greater than 1% of the variance in morphology in both the wild-caught and common-garden datasets, with PC1W and PC1C explaining 74% of the variance in their respective datasets (Table 1). In both wild-caught and common-garden data, only PC1 was significantly correlated with standard length (wild-caught: Pearsons correlation = 0.96; $P < 0.001$; common-garden: Pearsons correlation = 0.77; $P < 0.001$). Standard length explained 59% or 60% of the variation in PC1W and PC1C, respectively, and this was a significant amount of the variation (df = 1,148; PC1W: $F = 207.4$, $P < 0.001$; PC1C: $F = 220.0$, $P < 0.001$). ANCOVA, conducted on PC1 with standard length as a covariate, did not attribute significant variation in PC1W or PC1C to any source (Table 1). However, ANOVA (Model A1) determined that variation in PC1W was significantly attributable to a habitat x species x sex interaction, and to populations nested within habitat and species (Table 1; Fig. 2). Variation in PC1C (Model A2) was attributable to sex, populations nested within habitat, and a marginally insignificant interaction between habitat and generation (Table 1; Fig. 2). Failure of ANCOVA to attribute variation in PC1W or PC1C to any source contrasted with the ANOVA results (which did not take size into account) and confirmed that all variation in PC1 that was attributed to experimental sources of variance was due to variation in body size, not body shape.

All traits contributed to PC1W and PC1C in the same direction, increasing as PC scores increased and vice versa (Table 2). Apparent similarity of trait contributions to PC1W and PC1C was substantiated by the correlation analysis, which indicated that the two were highly correlated (Table 1). Greatest contribution to PC1W was from traits describing body depth and the length of median fin bases (Table 2). Likewise, PC1C was strongly influenced by body depth and median fin traits, as well as caudal peduncle depth (Table 2). *Melanotaenia duboulayi* was slightly smaller (shallower bodied with shorter median fin) than *M. eachamensis* (Fig. 2A). All wild-caught females had lower PC1W scores than their male counterparts (Fig. 2A), indicating that they were smaller, particularly being shallower bodied and having shorter median fin bases. Sexual dimorphism was pronounced in *M. eachamensis*, but there was no discernable difference in PC1W scores between habitats (Fig. 2A). Interhabitat divergence in PC1W was apparent in *M. duboulayi*, with stream males smaller (especially shallower bodied, with shorter median fins) than lake males and stream females larger (deeper bodied, with longer median fins) than lake females (Fig. 2A). The habitat x sex x species interaction was further contributed to by reduced sexual dimorphism in *M. duboulayi* from streams relative to those from lakes (Fig. 2A). There was a marginally insignificant difference between wild-caught and laboratory-raised fish in their response of body size (PC1C) to habitat (Table 1). Compared with their wild-caught parents, laboratory-raised *M. eachamensis* exhibited reduced sexual dimorphism, but greater habitat divergence, with lake fish larger (deeper bodied, deeper in the caudal peduncle, and with longer median fins) than their stream counterparts (Fig. 2B). Overall, these results indicated that although habitat affected body size, the effect was not consistent across species or sexes and that habitat differences were not heritable.

Variation in Body Shape

Wild-caught fishes

ANOVA identified significant contributions from interaction terms involving habitat for PC4W (habitat by species) and PC6W (habitat by sex), and a significant habitat effect for PC7W (Table 1). Together, these three factors explained

TABLE 1. Results of ANOVA conducted on PC scores for individuals using either Model A1 (wild-caught data: PC1W to PC7W) or Model A2 (common-garden data: PC1C to PC7C). PC1# reports the results of ANCOVA conducted with standard length as the covariate. The percentage of morphological variance explained by each PC is indicated below the name. Pearsons correlation between corresponding PCs from analyses of wild-caught and common-garden data are reported. Mean Squares (MS) and F-ratios (F_{df}) are reported and significant results are indicated with an asterisk (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Wild-caught		PC1W 73.5%	PC1#	PC2W 12.1%	PC3W 3.7%	PC4W 2.5%	PC5W 2.2%	PC6W 1.6%	PC7W 1.3%
H	MS	0.01	0.09	8.01	6.86	0.12	0.12	0.24	37.32
	$F_{1,4}$	0.0	0.2	2.6	1.5	0.2	0.1	0.1	26.0**
Sp	MS	16.47	0.11	38.66	16.24	3.76	2.96	12.88	12.62
	$F_{1,4}$	3.7	0.7	12.6	3.5	7.4	3.1	7.5	8.8
S	MS	25.86	0.02	10.79	12.78	3.49	1.38	0.17	15.74
	$F_{1,132}$	44.5***	1.0	19.7***	19.4***	5.2	1.4	0.2	31.8***
P(H*Sp)	MS	4.47	0.33	3.07	4.67	0.51	0.95	1.71	1.44
	$F_{4,132}$	7.7***	0.2	5.6***	7.1***	0.8	1.0	2.2	2.9
H*Sp	MS	0.29	1.29	1.90	0.31	37.00	0.00	12.75	1.93
	$F_{1,4}$	0.1	5.6	0.6	0.1	73.2***	<0.0	7.5	1.3
H*S	MS	0.55	0.00	0.43	0.65	1.93	0.37	6.18	0.01
	$F_{1,132}$	1.0	<0.0	0.8	1.0	2.9	0.4	7.9	<0.0**
Sp*S	MS	0.31	0.00	0.25	0.84	4.59	0.20	0.68	3.58
	$F_{1,132}$	0.5	<0.0	0.4	1.3	6.9	0.2	0.9	7.2
H*Sp*S	MS	5.34	0.00	2.59	1.31	0.09	0.30	2.22	1.24
	$F_{1,132}$	9.2**	<0.0	4.7	2.0	0.1	0.3	2.8	2.5
Pearsons Correlation		0.84***		0.92***	0.34	0.50	0.23	0.85***	−0.41
Common-Garden		PC1C 73.6%	PC1#	PC2C 10.7%	PC3C 4.3%	PC4C 3.6%	PC5C 2.2%	PC6C 1.5%	PC7C 1.1%
H	MS	1.67	0.76	26.62	10.14	7.07	1.04	4.31	9.13
	$F_{1,2}$	0.3	0.12	4.8	2.7	6.1	0.9	0.5	1.11
G	MS	39.23	2.55	2.96	18.83	0.13	2.51	4.39	3.48
	$F_{1,2}$	7.5	0.41	0.5	5.1	0.1	2.1	0.5	0.4
S	MS	9.05	0.2	18.53	0.04	5.51	0.00	5.14	4.35
	$F_{1,133}$	20.1***	0.43	30.5***	0.0	6.3*	0.0	7.4**	5.5
P(H)	MS	5.23	6.21	5.58	3.69	1.17	1.19	8.18	8.59
	$F_{2,133}$	11.6***	13.76	9.2***	5.0**	1.3	1.2	11.8***	10.8***
H*G	MS	2.86	0.21	0.28	2.94	5.86	2.34	0.03	1.21
	$F_{1,133}$	6.3	0.46	0.5	4.0	6.7	2.4	0.0	1.5
H*S	MS	0.17	0.54	1.78	0.55	0.64	1.00	12.68	0.06
	$F_{1,133}$	0.4	1.2	2.9	0.7	0.7	1.0	18.3	0.1***
G*S	MS	1.50	0.1	1.27	0.24	0.01	1.10	0.00	2.37
	$F_{1,133}$	3.3	0.23	2.1	0.3	<0.0	1.1	<0.0	3.0
H*G*S	MS	0.15	0.12	0.41	0.08	0.11	0.00	2.05	2.21
	$F_{1,133}$	3.3	0.27	2.1	0.3	0.0	1.1	<0.0	3.0

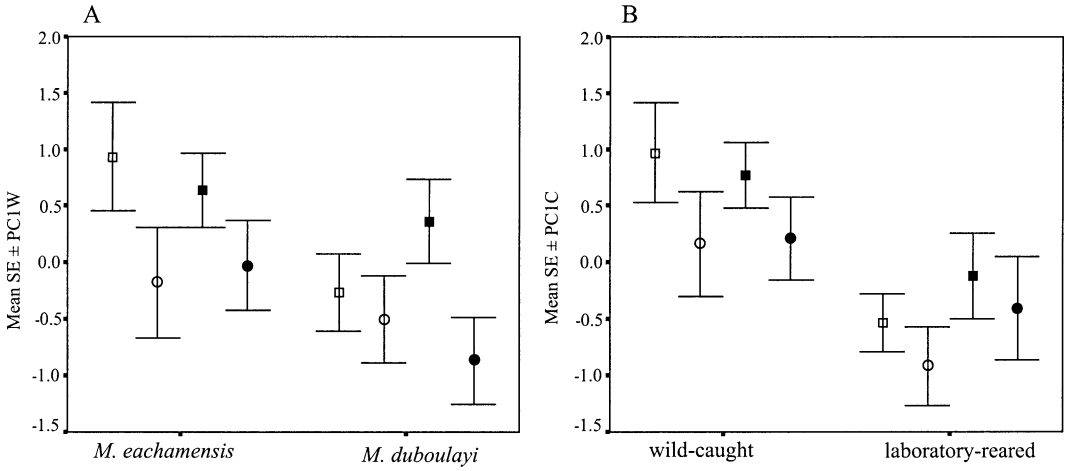

FIG. 2. Mean (± SE) of individual PC scores for males (squares) and females (circles) from lakes (solid symbols) and streams (open symbols) for (A) PC1W and (B) PC1C.

TABLE 2. Standardized contributions of traits to PCs that described significant variation due to habitat or an interaction involving habitat (shown beneath heading: see Table 1) and PC1C. PC1W to PC7W were extracted from analysis of wild-caught fishes and PC1C and PC6C from analysis of common-garden data. Coefficients for each vector, in bold, were ≥ 0.7 times the largest coefficient for that vector (Mardia et al. 1979). Refer to Figure 1 for trait definitions.

Trait	PC1W H*SP*S	PC4W H*Sp	PC6W H*S	PC7W H	PC1C	PC6C H*S
1–2	0.09	0.08	0.00	0.14	0.16	−0.32
1–3	0.11	0.01	0.06	**0.31**	0.16	−0.07
1–4	0.12	−0.01	0.02	**0.32**	0.16	0.02
2–3	0.14	−0.02	0.00	0.26	0.17	−0.05
2–4	0.14	−0.03	0.04	**0.41**	0.18	0.17
3–4	**0.32**	−0.19	−0.30	−0.02	**0.33**	−0.23
3–5	0.22	**0.61**	0.09	−0.13	0.21	0.05
3–6	**0.27**	0.06	−0.14	0.10	**0.24**	−0.05
4–5	**0.34**	−0.20	−0.31	−0.21	**0.32**	−0.30
4–6	0.23	**0.47**	0.02	0.01	0.14	−0.11
5–6	**0.35**	−0.22	−0.27	−0.01	**0.32**	−0.12
5–7	**0.28**	0.20	0.19	0.00	0.19	0.28
5–8	**0.27**	−0.04	0.20	−0.03	**0.23**	0.22
6–7	**0.26**	0.02	0.08	**−0.37**	0.22	0.14
6–8	**0.25**	−0.03	**0.53**	**−0.43**	**0.27**	**0.50**
7–8	0.23	−0.32	−0.11	−0.27	**0.24**	−0.16
7–9	0.05	−0.23	**0.40**	0.05	0.14	**0.41**
7–10	0.10	−0.13	0.17	−0.04	0.16	0.18
8–9	0.11	0.04	−0.16	−0.05	0.13	−0.07
8–10	0.08	0.22	−0.34	−0.26	0.10	−0.09
9–10	0.21	−0.10	−0.05	0.04	**0.30**	−0.20

20.3% of the size-free shape variation (9.4%, 6.0%, and 4.9%, respectively). PC4W, describing a habitat x species interaction, was primarily determined by positive contributions from distances between insertion points of the dorsal fins (4–6) and insertion points of pelvic and anal fins (3–5) (Table 2). In *M. eachamensis*, interfin distance was reduced in lake fish relative to their stream counterparts and, conversely, lake *M. duboulayi* had longer interfin distances than did stream fishes (Fig. 3A; Table 3).

PC6W described a significant portion of variation that could be attributed to a habitat by sex interaction (Table 1). PC6W was influenced by a strong, positive contribution from caudal peduncle length (7–9) and by the length of the base of the second dorsal fin (6–8) (Table 2). Stream males, of both species, had higher scores than their female cohabiters on PC6W, due to their longer caudal peduncles and longer median fin base (Fig. 3B; Table 3). This pattern was reversed in lake fish, where males had lower scores, thus shorter caudal peduncles and median fin bases, than did lake females (Fig. 3B, Table 3).

PC7W was the only PC that described variation between habitats that was not confounded by interactions with species and/or sex (Table 1). Therefore, this PC was interpreted as describing a generalized response to changes in water velocity. PC7W was contributed to strongly by positive loadings from predorsal and prepelvic length traits (1–4 and 2–4; 1–3), and negative loadings from traits describing the length of median fin bases (6–7 and 6–8) (Table 2). Irrespective of species or sex, stream fishes had shorter predorsal/prepelvic lengths and longer median fins than did lake fish (Fig. 3C; Table 3). PC7W also described significant variation attributable to sex (Table 1). Sexual dimorphism occurred in the same direction in both species and both habitats: females had longer predorsals/prepelvics and shorter median fins than did males (Fig. 3C; Table 3).

Common-garden experiment

PC6C, like PC6W, described a significant habitat by sex interaction (Table 1). This was the only PC from the analysis of the common-garden data that was attributable to a habitat level interaction, and no PCs were described as a significant habitat effect. PC6C was tightly correlated to PC6W (Table 1), as these PCs were due to variation in the same traits (Table 2). As observed for PC6W, males from streams had longer caudal peduncles and longer second dorsal fin bases than did stream females (compare Fig. 3B to Fig. 3D). In wild-caught fish, lake females had longer caudal peduncles and second dorsal fin bases than did lake males, but there was little sexual dimorphism in laboratory-raised lake fish (Fig. 3D; Table 3).

Failure of the analyses of PCs of the common-garden data to identify variation due to habitat indicated either that observed wild-caught habitat divergence was attributable to environmental, rather than genetic variation, or that the structure of the PCs had changed. This second explanation was supported by the limited correlation between wild-caught and common-garden PCs. In particular, no common-garden PC was correlated with PC7W, the PC that described a general response to water velocity. Therefore, we used traits that contributed most strongly to PCW7 to specifically test the hypothesis that habitat dimorphism was retained in fish raised in the common laboratory environment. Traits 1–3, 1–4, 2–4, 6–7, and 6–8 had coefficients greater than 70% of maximum coefficient to PCW7 (Table 2) and these traits were entered into a DFA of laboratory raised *M. eachamensis* to determine whether parental habitat could be correctly assigned in laboratory-reared fishes. Males and females were analyzed separately to avoid the potentially confounding effects of sex (PC7W also described significant variation between sexes). Laboratory-raised males and females were correctly assigned to parental habitats with a crossvalidation classification success of 73.7% (df = 5, Wilks' $\lambda = 0.617$, $P = 0.006$) and 71.1% (df = 5, Wilks' $\lambda = 0.557$, $P = 0.001$), respectively. This result indicated a heritable basis to the divergence between habitats in these traits in both sexes.

Differences in Body and Caudal Depth

Despite our prediction that lake fish would have evolved deeper bodies and deeper caudal peduncles than their stream counterparts, neither body nor caudal depth made strong contributions to any PC other than those describing size (PC1W and PC1C: Table 2). ANOVA (Model A1 or A2) conducted on size-corrected caudal peduncle depth revealed only population (nested within habitat and species for the wild-caught data, and within habitat for the common-garden data) contributed significantly to variation (Table 4).

Population-level variation also contributed significantly to size-corrected body depth in wild-caught fish, as did species and sex (Model A1) (Table 4). *Melanotaenia eachamensis* was relatively deeper bodied than *M. duboulayi*, and males were relatively deeper bodied than females (Fig. 4A). There

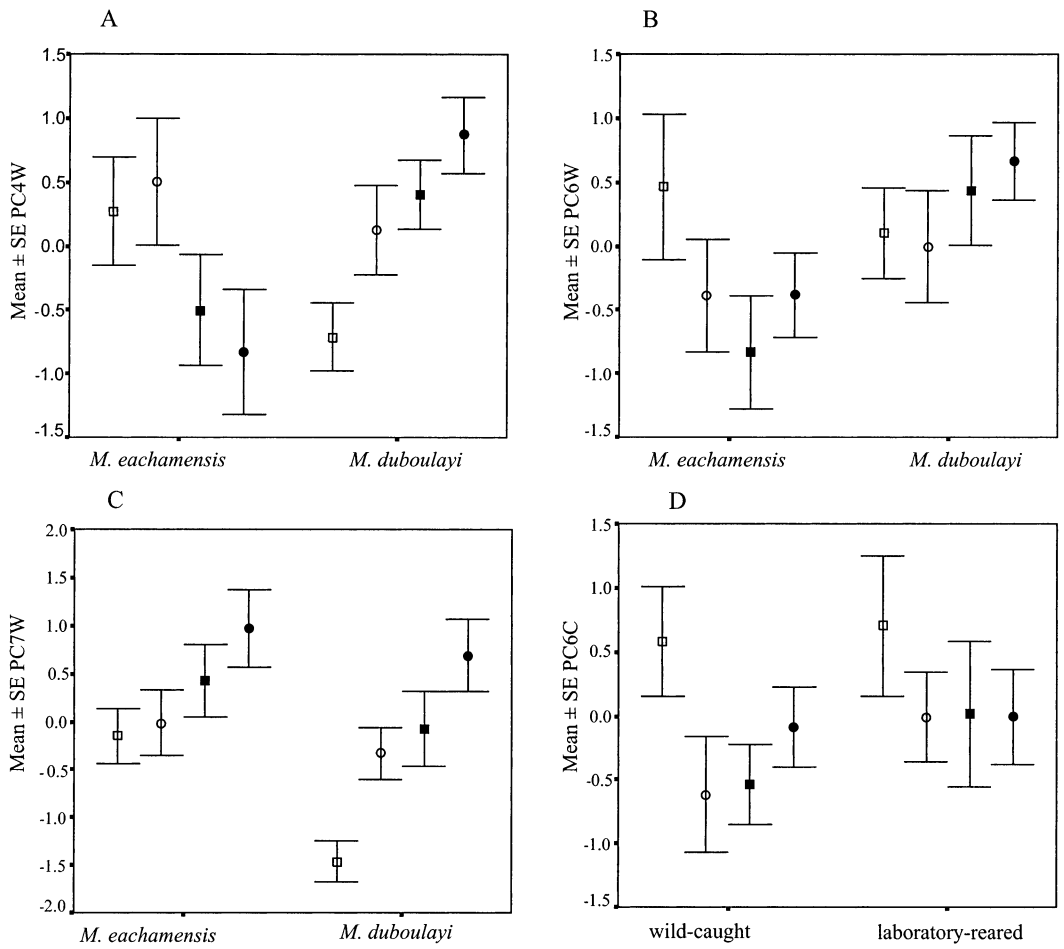

FIG. 3. Mean (± SE) of individual PC scores for males (squares) and females (circles) from lakes (solid symbols) and streams (open symbols) for (A) PC4W, (B) PC6W, (C) PC7W, and (D) PC6C.

was significant variation in size-corrected body depth among populations nested within habitat in the common-garden dataset also (Model A2) (Table 4). For this dataset, the only other significant contribution to body depth was a habitat x sex interaction (Table 4). Similarity of generations (lack of significant interactions between generation and habitat or sex: Table 4) indicated that the habitat x sex interaction was the same for wild-caught and laboratory-reared fish. However, failure to observe a habitat x sex interaction in the wild-caught dataset indicated the effect was specific to *M. eachamensis*. Male *M. eachamensis* were deeper bodied in lakes than in streams, whereas females were deeper in streams than in lakes (Fig. 4B).

Performance and Muscle Morphology

Sustained (aerobic) swimming performance (U_{crit})

Wild-caught fish.—ANCOVA revealed no significant differences in the relationship between U_{crit} and size between *M. eachamensis* and *M. duboulayi* (df = 1,38; $F = 0.21$; $P = 0.646$). However, visual comparison of the regression slope for each species suggested differences (standardized slopes were 0.75 and 0.68 for *M. eachamensis* and *M. duboulayi*, respectively). ANCOVA was conducted a second time on data from each species separately. This revealed that although in *M. eachamensis* significant amounts of variation in U_{crit} were explained by variation in standard length (df = 1,20; $F = 5.63$; $P = 0.028$) in *M. duboulayi* this was not true (df = 1,18; $F = 1.57$; $P = 0.227$). Therefore, U_{crit} was size-corrected by regression against standard length for *M. eachamensis* ($\beta = 0.748$; $t = 5.75$; $P < 0.001$), but not for *M. duboulayi*.

Nested ANOVA (Model B) of U_{crit}, conducted for each species separately, revealed a significant habitat effect (df = 1,2; $F = 43.49$; $P = 0.022$) and a significant sex effect (df = 1,18; $F = 13.91$; $P = 0.002$) in *M. duboulayi* (Fig. 5A). No other terms were significant. *M. duboulayi* from streams had faster sustained swimming speeds than fish from lakes, in accordance with predictions (Fig. 5A; Table 5). Within each habitat, males were faster sustained swimmers than their female counterparts (Fig. 5A). ANOVA (Model B) indicated a significant habitat by sex interaction in *M. eachamensis* (df = 1,20; $F = 6.45$; $P = 0.020$: Fig. 5B), but did not attribute significant amounts of variation to any other source. Female *M. eachamensis* from streams had faster U_{crit} than did lake females, but lake males were faster than stream males (Fig. 5B). Also, stream females had faster U_{crit} than their male

TABLE 3. Morphological variation in lake and stream *Melanotaenia eachamensis* and *M. duboulayi*, wild caught and laboratory reared. Traits were size corrected by regression of ln-tranformed interlandmark distance on PC1. To return traits to the original units of measurement (mm), the mean was calculated within each habitat for each species/generation and added to the regression residual score. The mean ± SE reported here was calculated from these size-corrected traits. See Figure 1 for definition of traits.

	Wild-caught *M. eachamensis*		Laboratory-reared *M. eachamensis*		Wild-caught *M. duboulayi*	
Trait	Stream	Lake	Stream	Lake	Stream	Lake
1–2	7.3 ± 0.11	7.1 ± 0.08	5.3 ± 0.07	5.8 ± 0.09	6.8 ± 0.05	7.5 ± 0.08
1–3	20.0 ± 0.15	20.7 ± 0.17	15.8 ± 0.10	17.8 ± 0.15	17.9 ± 0.11	20.1 ± 0.14
1–4	23.0 ± 0.16	25.4 ± 0.16	19.5 ± 0.11	21.6 ± 0.19	21.2 ± 0.11	23.6 ± 0.12
2–3	18.4 ± 0.12	17.7 ± 0.16	14.6 ± 0.07	15.6 ± 0.13	15.4 ± 0.08	16.1 ± 0.10
2–4	16.7 ± 0.18	18.5 ± 0.19	14.9 ± 0.15	16.1 ± 0.19	15.3 ± 0.12	16.8 ± 0.12
3–4	17.3 ± 0.10	16.4 ± 0.12	11.7 ± 0.11	13.5 ± 0.13	12.9 ± 0.12	11.5 ± 0.11
3–5	8.3 ± 0.17	6.3 ± 0.18	6.7 ± 0.12	6.3 ± 0.16	6.2 ± 0.12	6.5 ± 0.10
3–6	20.9 ± 0.14	19.4 ± 0.12	16.3 ± 0.12	17.0 ± 0.12	15.3 ± 0.10	15.5 ± 0.12
4–5	16.7 ± 0.13	15.5 ± 0.11	11.2 ± 0.10	13.1 ± 0.12	12.6 ± 0.13	11.2 ± 0.12
4–6	8.8 ± 0.19	7.9 ± 0.19	8.8 ± 0.15	7.8 ± 0.18	6.6 ± 0.10	7.7 ± 0.11
5–6	16.0 ± 0.13	16.0 ± 0.12	11.8 ± 0.10	13.3 ± 0.11	12.1 ± 0.11	11.5 ± 0.13
5–7	17.5 ± 0.28	16.2 ± 0.33	17.0 ± 0.22	14.6 ± 0.27	13.9 ± 0.16	16.9 ± 0.18
5–8	21.5 ± 0.22	19.9 ± 0.23	17.2 ± 0.15	17.1 ± 0.18	16.7 ± 0.14	18.0 ± 0.15
6–7	15.6 ± 0.17	13.9 ± 0.17	12.9 ± 0.16	12.1 ± 0.14	13.7 ± 0.15	13.4 ± 0.15
6–8	13.6 ± 0.19	10.8 ± 0.17	9.7 ± 0.19	8.9 ± 0.16	11.0 ± 0.16	10.6 ± 0.13
7–8	6.3 ± 0.08	7.0 ± 0.08	5.2 ± 0.06	6.2 ± 0.06	6.5 ± 0.06	5.7 ± 0.04
7–9	6.2 ± 0.18	7.6 ± 0.22	5.9 ± 0.20	7.6 ± 0.14	8.9 ± 0.16	9.0 ± 0.16
7–10	8.9 ± 0.16	9.6 ± 0.18	7.3 ± 0.15	9.1 ± 0.11	10.2 ± 0.12	10.1 ± 0.10
8–9	7.5 ± 0.13	9.1 ± 0.16	8.1 ± 0.16	8.8 ± 0.13	10.3 ± 0.14	10.5 ± 0.12
8–10	5.1 ± 0.18	6.5 ± 0.19	6.8 ± 0.20	6.8 ± 0.14	8.2 ± 0.17	8.9 ± 0.13
9–10	5.4 ± 0.06	5.6 ± 0.05	3.6 ± 0.05	4.5 ± 0.05	4.8 ± 0.04	4.8 ± 0.03

counterparts, whereas in lakes the reverse was true, with males swimming faster than females (Fig. 5B).

Common-garden experiment.—ANCOVA did not reveal any intergeneration slope heterogeneity (df = 1,40; $F = 0.18$; $P = 0.674$), but again, visual examination of slopes between wild-caught and laboratory-reared fishes suggested a difference (standardized slopes were 0.75 and 0.49 for wild-caught and laboratory reared, respectively). As with wild-caught fishes, separate ANCOVA on each generation revealed a significant effect of standard length in wild-caught *M. eachamensis* (see above), but not in their laboratory-reared offspring (df = 1,20; $F = 1.40$; $P = 0.251$). Therefore, laboratory-raised fish were not sizecorrected. Results for wild-caught fish were presented above. ANOVA (Model B) on U_{crit} of laboratory-reared fish revealed significantly faster sustained speeds in males than in females (sex effect: df = 1,20; $F = 8.80$; $P = 0.008$), but no difference among habitats (df = 1,2; $F = 0.39$; $P = 0.597$), nor variation due to any other source (Fig. 5C). U_{crit} of wild-caught and laboratory-reared *M. eachamensis* were not directly compared due to differences in the relationship with size. This difference in scaling, and the differences in the sources of variance contributing to U_{crit} variation in each dataset, suggested this trait was not heritable.

TABLE 4. Results of ANOVA conducted on size-corrected residuals of body depth (BD) and caudal peduncle depth (CPD) for wild-caught (Model A1) and common-garden (Model A2) datasets. Mean Squares (MS) and *F*-ratios (F_{df}) are reported, and significant results are indicated with an asterix (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Wild-caught				Common-garden			
Source		BD	CPD	Source		BD	CPD
H	MS	34.91	<0.00	H	MS	<0.00	0.14
	$F_{1,4}$	9.03	0.01		$F_{1,2}$	<0.00	2.24
Sp	MS	172.26	0.01	Sp	MS	1.04	0.01
	$F_{1,4}$	44.54**	0.41	(1,2)	$F_{1,2}$	2.58	0.09
S	MS	42.88	<0.00	S	MS	0.20	<0.00
	$F_{1,139}$	55.04***	0.51		$F_{1,140}$	18.00**	0.09
P(H*Sp)	MS	3.87	0.03	P(H*Sp)	MS	0.4	0.06
	$F_{4,139}$	4.96***	6.78***		$F_{2,140}$	36.90***	15.95***
H*Sp	MS	36.36	0.20	H*G	MS	<0.00	0.01
	$F_{1,4}$	9.40	8.01		$F_{1,140}$	0.41	1.57
H*S	MS	0.28	0.01	H*S	MS	0.11	<0.00
	$F_{1,139}$	0.35	1.34		$F_{1,140}$	9.79**	0.33
Sp*S	MS	0.06	0.02	G*S	MS	<0.00	0.01
	$F_{1,139}$	0.08	4.74		$F_{1,140}$	0.27	3.58
H*Sp*S	MS	1.98	<0.00	H*G*S	MS	0.04	0.06
	$F_{1,139}$	2.54	0.55		$F_{1,140}$	3.64	1.05

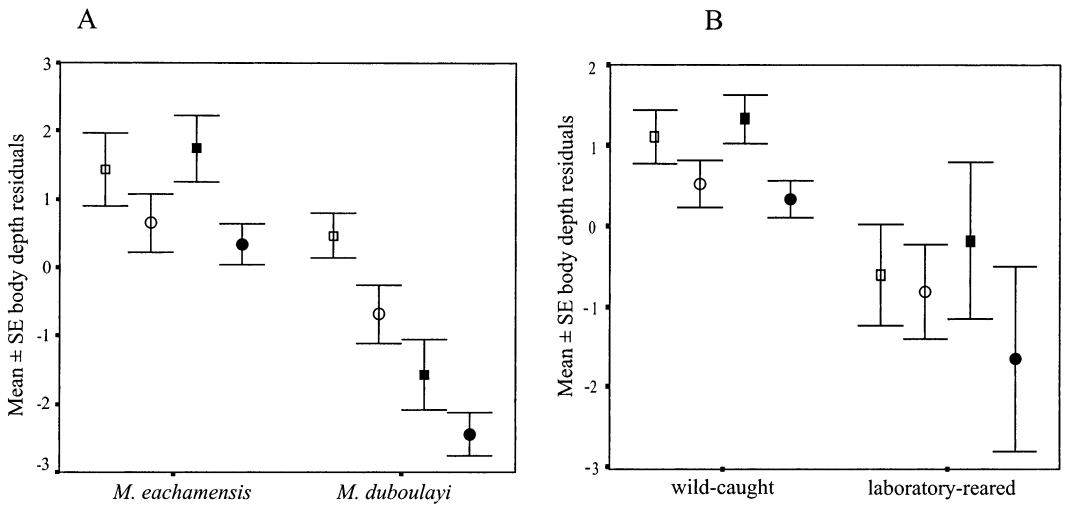

FIG. 4. Mean (± SE) of body depth of males (squares) and females (circles) from lakes (solid symbols) and streams (open symbols) of (A) wild-caught fish and (B) common-garden fish.

Burst-swimming speed

Wild-caught fish.—ANCOVA revealed no interspecific heterogeneity in slope, but a significant contribution of standard length to burst swimming speed (df = 1,33; $F = 4.24$; $P = 0.047$). This relationship, in contrast to that observed for U_{crit}, was negative with smaller fish having faster burst speeds ($\beta = -0.584$; $t = -4.94$; $P < 0.001$). ANOVA (Model A1) conducted on regression residuals revealed no significant contribution to burst speed from any source.

Common-garden experiment.—ANCOVA did not reveal any intergeneration slope heterogeneity, but did indicate that variation in burst speed was significantly, negatively associated with variation in standard length (df = 1,39; $F = 9.46$; $P = 0.004$: $\beta = -0.469$; $t = -3.87$; $P < 0.001$). ANOVA (Model A2) conducted on regression residuals did not identify any significant contributions to burst speed.

Red muscle area

Wild-caught fish.—ANCOVA determined no heterogeneity in slope between species, habitats, or sexes, and that variation in total muscle area contributed significantly and positively to variation in red muscle area (df = 1,36; $F = 6.69$; $P = 0.014$: $\beta = 0.597$; $t = 5.26$; $P < 0.001$). ANOVA (Model A1) indicated that only two sources contributed significantly to variation in size-corrected red muscle area. Fish from streams had a significantly greater relative area of red muscle than did fish from lakes (habitat effect: df = 1,4; $F = 19.69$; $P = 0.011$; Fig. 6A). Females had relatively less red muscle

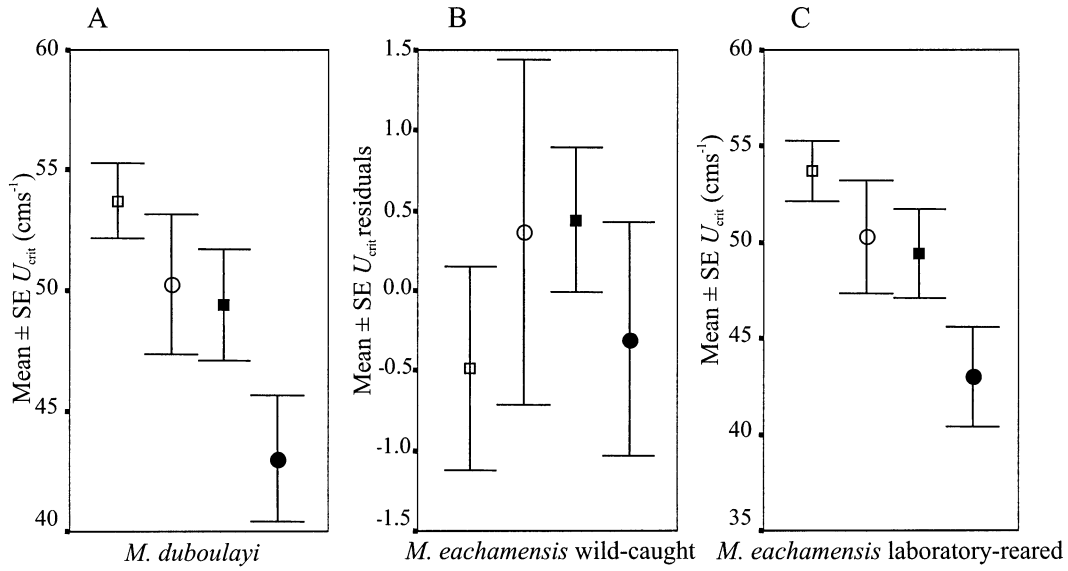

FIG. 5. Mean (± SE) U_{crit} for (A) wild-caught *Melanotaenia duboulayi* (cms^{-1}), (B) wild-caught *M. eachamensis* (residuals of regression of U_{crit} on standard length) and (C) laboratory raised *M. eachamensis* (cms^{-1}) males (squares) and females (circles) from lakes (solid symbols) and streams (open symbols).

TABLE 5. Variation in swimming performance and muscle area for fish of *Melanotaenia eachamensis* and *M. duboulayi* (wild-caught and laboratory reared) from lakes and streams. For wild-caught *M. eachamensis* U_{crit}, and for all groups for burst speed and red muscle area, means were calculated from regression residuals to which the species/generation intrahabitat mean had been added. U_{crit} in laboratory-raised *M. eachamensis* and in *M. duboulayi* were not sized corrected.

	Trait	Stream	Lake
Wild-caught	u_{crit} (ms^{-1})	0.45 ± 0.01	0.46 ± 0.01
M. eachamensis	Burst (ms^{-1})	0.89 ± 0.03	0.94 ± 0.03
	Red (cm^2)	0.44 ± 0.02	0.27 ± 0.02
Laboratory-reared	U_{crit} (ms^{-1})	0.41 ± 0.01	0.45 ± 0.02
M. eachamensis	Burst (ms^{-1})	0.81 ± 0.02	0.85 ± 0.03
	Red (cm^2)	0.50 ± 0.03	0.35 ± 0.03
M. duboulayi	U_{crit} (ms^{-1})	0.53 ± 0.01	0.47 ± 0.01
	Burst (ms^{-1})	0.82 ± 0.03	0.70 ± 0.02
	Red (cm^2)	0.61 ± 0.05	0.28 ± 0.03

area than males (sex effect: df = 1,36; $F = 4.45$; $P = 0.042$: Fig. 6A).

Common-garden experiment.—There was no difference between wild-caught and laboratory-reared fishes in their relationship between red muscle area and total muscle area, which was positive and significant (df = 1,39; $F = 8.98$; $P = 0.005$: $\beta = 0.590$; $t = 5.30$; $P < 0.001$). ANOVA (Model A2) determined males had relatively more red muscle than females (df = 1,39; $F = 4.20$; $P = 0.047$) (Fig. 6B). The pattern of interhabitat differences observed in wild-caught fishes was reflected in laboratory-raised fishes (Fig. 6B), but was not statistically significant (habitat effect: df = 1,2; $F = 3.00$; $P = 0.226$). ANOVA failed to identify any difference between wild-caught and laboratory-reared fishes (generation effect: df = 1,2; $F = 0.08$; $P = 0.799$), or any difference in their reaction to habitat (generation by habitat effect: df = 1,39; $F = 0.66$; $P = 0.421$).

DISCUSSION

In this study we exploited natural replication of lake and stream populations of rainbow fish, in conjunction with a common-garden experiment, to demonstrate that a shift from flowing to still water has resulted in phenotypic evolution through natural selection. Although independently derived from different stream ancestors, all populations of lake fish had more posteriorly positioned first dorsal and pelvic fins, and shorter median fin bases than did stream fish. This habitat divergence in body shape was maintained in lake- and stream-derived *M. eachamensis* raised in a common environment, demonstrating that divergence had a genetic basis. Independent evolution of the same heritable body shape in replicate populations strongly implicated that natural selection was responsible for the divergence between lakes and streams.

Muscle morphology and swimming performance provided weaker evidence of adaptation to water velocity habitat. We accepted our a priori hypothesis that lake fish had a relatively smaller area of red muscle in their caudal peduncle. Despite this reduction in red muscle area between wild-caught lake and stream fish, evidence of a genetic basis to the divergence was equivocal. Variation between parental habitats was in the same direction in laboratory reared fish (i.e., streams greater than lakes), but the difference was no longer statistically significant. Fish muscle is known to be highly plastic and fish can respond to enforced swimming by increasing red muscle area (reviewed by Sänger and Stoiber 2001). No difference in burst swimming speed was observed between lake and stream fish of either species. *Melanotaenia duboulayi* lake fish exhibited decreased sustained swimming speed. In *M. eachamensis*, there was an effect of habitat on U_{crit}, but the relationship was complicated by an interaction with sex. The common-garden experiment did not support a genetic basis to the divergence between habitats in sustained swimming performance. Overall, the results of this study failed to support many of our a priori hypotheses, which were based on existing empirical evidence and simplistic interpretations of hydromechanical theory. We highlight below three major departures of our results from expectations and discuss possible reasons behind these unexpected results.

No Increase in Body or Caudal Depth in Lakes, or with Decreasing U_{crit}

Previous studies examining differences in body shape between fish experiencing different hydrological demands (e.g., Gatz 1979; Taylor and McPhail 1985a,b; McLaughlin and Grant 1994) and theoretical studies of the hydromechanical effects of body shape (e.g., Webb 1982, 1984) led to the expectation that stream rainbow fish would have shallower bodies and shallower caudal peduncles than their lake counterparts, and that this would be deterministically associated with faster sustained swimming speeds. There was no variation in caudal peduncle depth with water velocity habitat in this study. Body depth varied with habitat in a sex-specific way, but only in *M. eachamensis*, indicating that it was not a generalized response to water velocity. Despite similarity to lake fish in body and caudal depth, *M. duboulayi* stream fish sustained a faster swimming speed than did lake fish. This suggested body and caudal depth did not deterministically affect sustained swimming speed. Schaefer et al. (1999), in a comparison between two species of *Lepomis*, observed a positive association between body depth and prolonged swimming speed, but a negative association between those factors and hydrodynamic drag. Increased body depth was proposed to result in an increased cost of sustained swimming through increased drag (see Webb 1984). The results of Schaefer et al. (1999) highlight the potential dissociation of body depth and frictional drag. In addition, Webb (1992) provided evidence that the cost of frictional drag associated with deeper bodies might be offset by the benefits deep bodies provide in terms of reduced recoil. Pakkasmaa and Piironen (2000) examined the response of *Salmo salar* and *S. trutta* to different rearing water velocities and observed *S. salar* to increase their body depth with increased velocity, but no change in *S. trutta*. The relationship between body depth, water velocity, and sustained swimming performance does not appear to be deterministic. Further data on variables such as drag is required to explore these relationships, but an a priori expectation that body depth will decrease with increased sustained swimming ability does not appear to be appropriate.

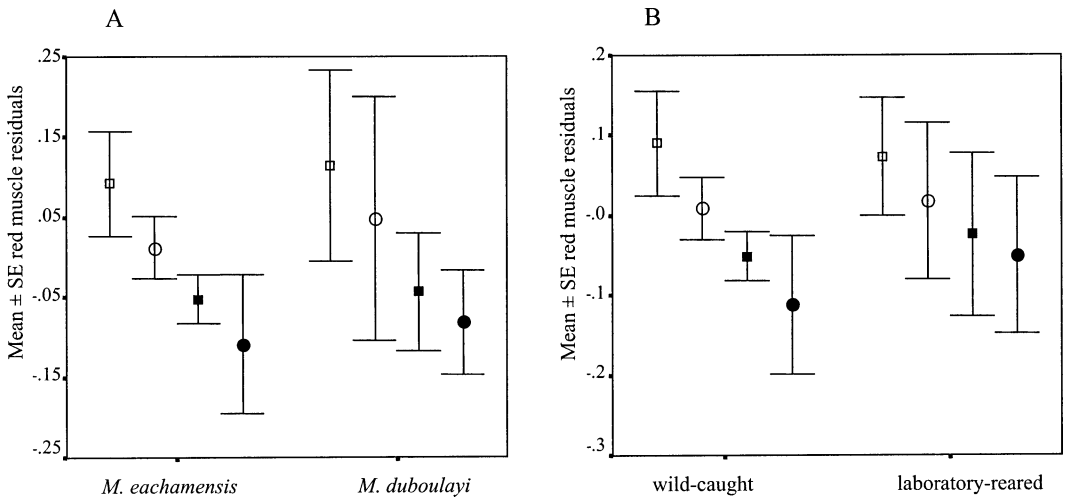

FIG. 6. Mean (± SE) red muscle area (residuals of regression of red muscle area on total muscle area) for (A) wild-caught fishes and (B) common-garden males (squares) and females (circles) from lakes (solid symbols) and streams (open symbols).

Selection for Increased Predorsal and Prepelvic Length and Decreased Second Dorsal Fin Length

Divergence between lake and stream fish in position of the first dorsal and pelvic fins and in the length of the second dorsal fin was observed in both species and both sexes. It was also retained in fish raised in the common-garden environment. We proposed no a priori hypotheses of divergence in these traits. However, as we discuss below, changes in these traits appear to be commonly associated with habitat divergence in fish. Although five traits were highlighted by our analyses as playing important roles in habitat divergence, these interlandmark distances were not independent of one another. All head-length traits were significantly correlated with one another (traits 1–3, 1–4, and 2–4), predorsal traits were negatively correlated with second dorsal traits (i.e., 1–4, and 2–4 were negatively correlated with 6–7 and 6–8), but prepelvic length was independent of the second dorsal length traits (1–3 versus 6–7 and 6–8). Thus, colonization of lakes resulted in a concomitant posterior shift in both landmarks 4 and 6, and that this affected four traits (1–4, 2–4, 6–7, and 6–8). Landmark 3 also shifted posteriorly following lake colonization, but this did not affect the position of landmark 5. The magnitude of shift in landmark 3 was also independent of the magnitude of shift in landmark 6, but was related to the shift in landmark 4.

McGuigan et al. (2000) determined (through phylogenetic analyses of mtDNA) that the northern New Guinea rainbow fish genera *Melanotaenia* and *Glossolepis* were polyphyletic. These genera were described and distinguished on the basis of morphological characteristics (see Allen 1980). Although most *Melanotaenia* species are stream dwelling, most species of *Glossolepis* inhabit either lakes or swamps, where water velocity is slow (Allen 1995). Comparison of predorsal length traits between replicate lake (*Glossolepis*) and stream (*Melanotaenia*) species of a monophyletic northern New Guinea clade (Clade F of McGuigan et al. 2000) revealed lake-dwelling fish had significantly more scale rows anterior of the first dorsal fin (one-way ANOVA: df = 1,2; $F = 76.55$; $P = 0.013$), and marginally insignificantly longer predorsal length (one-way ANOVA: df = 1,2; $F = 13.52$; $P = 0.067$) than stream fish. This evidence further corroborates the hypothesis that selection has driven a posterior shift in the first dorsal fin with colonization of still water environments. Data on length of the second dorsal fin base or prepelvic length were not available.

The morphology of lake rainbow fish was markedly similar to that observed by Walker (1997) in sticklebacks (*Gasterosteus aculeatus*) from lakes without native piscivorous fish (NPF): relative to sticklebacks from lakes with NPF (and to the ancestral marine form), the first dorsal spine was shifted posteriorly and the base length of the dorsal (and anal) fin decreased. Walker (1997) proposed that the anterior positioning of dorsal spines was maintained in lakes with NPF as a selected response to predation by gape-limited predators, and that longer median (dorsal and anal) fins in these same sticklebacks was due to selection for increased thrust (longer median fins increase caudal depth without the cost of increased drag). Walker (1997) was unable to explain why these trait combinations were lost in the absence of NPF.

There is strong evidence that *M. eachamensis* is completely naive with respect to predatory fish (Barlow et al. 1997; Brown and Warburton 1997). The absence of predatory fish rules out piscivory as a causal factor either in the maintenance of the anterior positioning of first dorsal fins in stream rainbow fish, or in the evolution of longer predorsals in lake rainbow fish. We had no expectation that stream fish would have a greater requirement for burst swimming, nor did we observe any difference between habitats in burst speed. Rainbow fish were not observed to erect their median fins during burst swimming. They erected their median fins only during low speed maneuvering.

The apparent similarity of morphological divergence in sticklebacks and rainbow fish is striking, as is the apparent dissimilarity of selective pressures in the different systems. Similarity of morphological divergence might be due to constraints acting to limit the traits that can diverge and the directions in which divergence can occur (see below), irrespective of the selective force acting. Alternatively, stick-

lebacks and rainbow fish might both be experiencing the same, as yet unidentified, selection pressures. This question can only be resolved through further ecological and kinematic study of both groups.

Altogether, due to the inconsistency of our results with expectations of hydromechanical evolution we cannot conclude that the selection applied by water velocity on swimming performance drove evolution in our system. Both Melanotaeniidae and Gasterosteidae belong to the Class Acanthopterygii, which is characterized by differentiated first (bony-rayed) and second (soft-rayed) dorsal fins. The role of these fins in swimming is not well understood. Median fins might play a role in maintaining stability while maneuvering. Recent work by Drucker and Lauder (2001) studying swimming in *Lepomis macrochirus* (another Acanthopterygian) determined that the second dorsal fin was responsible for generating a substantial portion of thrust during steady swimming at speeds greater than 1 L sec^{-1} (the speed at which *L. macrochirus* shifts from pectoral fin swimming to combined paired- and median-fin locomotion). Also, kinematic interactions between the dorsal and caudal fins were observed (Drucker and Lauder 2001). The impact on swimming performance of shifting the origin of the first and/or second dorsal fin is not known. Kinematic analyses of fish that differ in position and/or length of dorsal fins (such as in the rainbow fish system described here) will further elucidate the role of these fins in swimming, and allow us to determine whether divergence between water velocity habitats in rainbow fish was driven directly by water velocity acting on swimming performance or by some other factor.

Swimming Speed Variation

Results of our experimental estimation of swimming performances were more ambiguous than the results of the analysis of body shape. No difference between lake and stream fish was observed in burst swimming speed. Ours is not the first study that has failed to detect differences in burst swimming speed between populations that differ in body shape and locomotor demands (e.g., Law and Blake 1996). This suggests that similar burst speeds might be achieved through different mechanisms. Burst speeds in rainbow fish were within the range of those observed in other fish (Domenici and Blake 1997).

Stream *M. duboulayi* had faster sustained swimming speeds than lake *M. duboulayi*. In *M. eachamensis*, stream females were faster than their lake counterparts, but lake males were faster than stream males. With the exception of *M. eachamensis* males, our hypothesis of faster sustained speeds in stream dwelling fishes was accepted. The peculiarity of *M. eachamensis* stream males was further highlighted by the pattern of sexual dimorphism in U_{crit} of wild-caught fish and the habitat divergence in laboratory-raised fish. In *M. duboulayi*, males had faster U_{crit} than females from the same habitat. *Melanotaenia eachamensis* lake males were likewise faster than lake females. It was only in *M. eachamensis* from streams that this pattern of sexual dimorphism in sustained swimming speed was reversed (females faster than males). In laboratory-raised *M. eachamensis*, although there was no significant habitat effect on U_{crit}, the pattern of sexual dimorphism and habitat divergence was in the same direction as that observed in *M. duboulayi*: stream fish were faster than lake fish and males were faster than females. We propose that some factor other than water velocity has acted to depress the sustained swimming performance of *M. eachamensis* males in streams. We are unable to predict the nature of this factor, but note that *M. eachamensis* stream males exhibit body shape and red muscle area phenotypes consistent with other stream fish, suggesting sustained swimming performance was probably depressed through behavioral or physiological mechanisms. The reversal in laboratory-raised fish of the pattern observed in wild-caught *M. eachamensis* suggested that the effect on male *M. eachamensis* in streams is of environmental, rather than genetic, origin.

Repeated evolution of the same phenotype is not an uncommon phenomenon across fish taxa (e.g., Robinson and Wilson 1994; Pigeon et al. 1997; Bernatchez et al. 1996; Rundle et al. 2000). Repetition of form suggests a constraint on the direction in which evolution proceeds. Recent empirical analyses have supported a hypothesis that genetic variances/covariances might influence the direction in which habitat-based differences evolve (Schluter 1996; Arnold and Phillips 1999). A greater understanding of the genetic basis of body shape and the traits underlying swimming performance is necessary to determine the precise nature of the evolutionary constraints acting in rainbow fish.

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