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Intra-annual genetic variation in the downstream larval drift of sutchi catfish (*Pangasianodon hypophthalmus*) in the Mekong river

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Larvae of the sutchi catfish *Pangasianodon hypophthalmus* were collected during peak downstream drift in the Lower Mekong river on four occasions over an 8-week period during the 2003 spawning season, and genotyped using seven microsatellite loci. We provide evidence for several heterogeneous groups within and among the temporally discrete larval peak samples. Strong evidence for a significant deficit of heterozygotes was observed for each larval sample and the pooled sample, possibly due to population admixture. Although individual-based assignment tests suggested that each larval peak sample was admixed, significant but low genetic differentiation was observed among larval samples ($F_{ST} = 0.0052$, $P < 0.01$). The lack of significant relatedness confirms the multifamily composition of each larval group, excluding family bias to explain the observed genetic heterogeneity. Both the entire larval peak and each temporally separated larval peak originated from spawning groups with heterogeneous allelic composition involving several distinct spawning events. We propose three explanations to account for our findings: (1) the ecological match/mismatch hypothesis; (2) the genetic 'sweepstakes' selection hypothesis; and (3) life-history-specific characteristics of the spawning populations. Finally, an intra-annual shift in the contribution of the spawning populations to the larval drift was detected on successive occasions. *Biological Journal of the Linnean Society*, 2006, **89**, 719–728.

ADDITIONAL KEYWORDS: fish – individual assignment – match/ mismatch theory – population subdivision – recruitment – spawning waves – sweepstakes hypothesis.

INTRODUCTION

The population structure of freshwater and marine fish species is determined, in part, by recruitment variation in time and space. Such variation may be generated by biotic processes (Cushing, 1972, 1975), environmental physical processes linked to the ecology (Lambert, 1984; Sinclair, 1988; Taggart & Frank, 1990; Hedgecock, 1994), or climatic/meteorological processes (Levitan & Petersen, 1995). Any of the above-described processes affecting gamete quality and larval survival have the potential to affect the offspring (potential recruits) of some families, groups or populations (Levitan & Petersen, 1995; Ruzzante, Taggart & Cook, 1996). Timing differences in the production of distinct groups may result in a large variance in recruitment and, if there are differences in allelic composition among spawning groups, then there should be detectable genetic heterogeneity in larvae (Lambert, 1987; Sinclair, 1988; Ruzzante *et al*., 1996). Hedgecock (1994) proposed that temporal genetic variation in marine species may result from a large variance in reproductive success among adults (i.e. major discrepancies between observed and effective population sizes or 'sweepstakes' hypothesis) such that resulting cohorts comprise the progeny of a small proportion of the spawning population. The major con-

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sequence may be subsequent differences in the genetic composition of recruits over time to the extent that separate proportions of adult populations contribute successful progeny at different times. Alternatively, if different assemblages of larvae, originating from different spawning events involving heterogeneous groups of spawning individuals, are collected over an extended period, then collections may be drawn from distinct spawning groups. Hence, perceived temporal instability of genetic patterns may represent comparisons between sympatric groups sharing the same spatially defined, but not temporally defined, spawning location. Temporally distinct spawning (between May/June and July/August) of sutchi catfish *Pangasianodon hypophthalmus* (Pangasiidae, Teleostei) has been proposed in the Mekong river (Van Zalinge *et al*., 2002; N. So, pers. observ.). As such, the presence of sympatric groups (So, Maes & Volckaert, 2006), whose spawning times are separated by periods of 3 months or less, appears to be possible (Van Zalinge *et al*., 2002).

Sutchi catfish, *P. hypophthalmus* (Sauvage, 1878), is a prolific species (Van Zalinge *et al*., 2002), spawning in temporally discrete groups or populations separated by several days to weeks (So *et al.*, 2006). Up to three peaks of drifting larvae, each separated by a flood-pulse, are recorded in each spawning season at the onset of the rainy season in the Lower Mekong river. Larval drift is concentrated in June and July, but extends into August according to climatic (i.e. water temperature and rainfall) and hydrological conditions (i.e. water level and flow: flood-pulse) (Van Zalinge *et al*., 2002; N. So, pers. observ.). Each larval peak takes place for a few days and its intensity varies within and among years (N. So, pers. observ.). Sutchi catfish adults may exhibit natal spawning-site fidelity (So *et al.*, 2006). They spawn upstream between the town of Kratie and the Khone Falls on the Cambodian-Lao border, and are concentrated in three major spawning habitats: Prek Kempi (near Kratie town, Kratie province), Sambo district (Kratie province), and Siembok district (Strung Treng province) (Van Zalinge *et al*., 2002; N. So, pers. observ.) (Fig. 1). Their spawning habitats consist of rapids and sandbanks interspersed with deep rocky channels and pools (Van Zalinge *et al*., 2002). Commercial harvests of sutchi catfish have decreased dramatically over the last few decades in the Lower Mekong river basin (Van Zalinge *et al*., 2002). Estimated numbers of sutchi catfish larvae collected from *dai* (bag-net) fishery in Cambodia dropped from 165 billion in 1981 to 2.1 billion in 1998, and in Vietnam from 800 million in 1977 to 0.4 million in 2000 (Van Zalinge *et al*., 2002).

In the present study, we aimed to characterize genetically intra-annual larval peaks of sutchi catfish from the Lower Mekong river channel (Van Zalinge *et al*., 2002), to test the hypothesis of reproductive variance in adults and confirm cryptic population structure in their progeny. First, we examined the intra-annual genetic variation and differentiation at seven microsatellite loci among larvae sampled repeatedly on the lower Mekong main channel on four occasions over 8 weeks between June and August 2003. We quantified the amount of reproductive variance in adults by comparing the relatedness of larvae, possibly enabling the detection of a finite set of more successful spawning families. Furthermore, we assessed the source population(s) of each larval peak, to detect unequal contributions in the spawning stock. Finally, we compared the intra-annual population proportions in early and late larval peaks.

MATERIAL AND METHODS

Field collections

Larval sample

Larval sutchi catfish were collected from the province of Prey Veng (Cambodia) and Ving Xuong (Vietnam) on the Mekong proper (lower Mekong or Mekong *Krom* in Cambodian) during the 2003 spawning season (Fig. 1). Sampling was conducted on four occasions: 8 June 2003 (PV1, $N = 60$), 11 June 2003 (VX1, $N = 50$), 5 July 2003 (PV2, $N = 60$), and 1 August 2003 (PV3, $N = 50$). They represent all peaks of sutchi larvae drifting downstream through the Mekong proper towards the nursery areas on the floodplain in 2003. The samples PV1 and VX1 belong to the same temporally discrete larval peak (i.e. the first peak), but collected at two sites (separated by 45 km) and two different occasions on the same river branch, whereas the samples PV2 and PV3 belong to the second and third larval peak, respectively. Hence, all four larval samples were considered as temporal samples collected from the same river branch (Fig. 1, Table 1).

Sutchi larvae were collected using a bag-net (*dai kone trey pra* in Cambodian), which is made of mosquito netting (mesh size: 3–7 mm) and has the shape of a trawl net with a 5-m wide mouth and a length of 10–15 m (Van Zalinge *et al*., 2002; N.T. Tung, pers. comm.). The bag net is installed at a distance of 5–40 m from the river bank in an area sheltered from strong water currents and wind or wave action (L. Haing, pers. comm.). Water can drift from Kratie to Chaktomouk, Phnom Penh (Fig. 1) in 3 days and to the Mekong delta in 4 days; hence, the age of larvae is 1 week at most.

Baseline sample

We used a data set comprising several spawning populations from two different years (So, 2005; Table 1) as

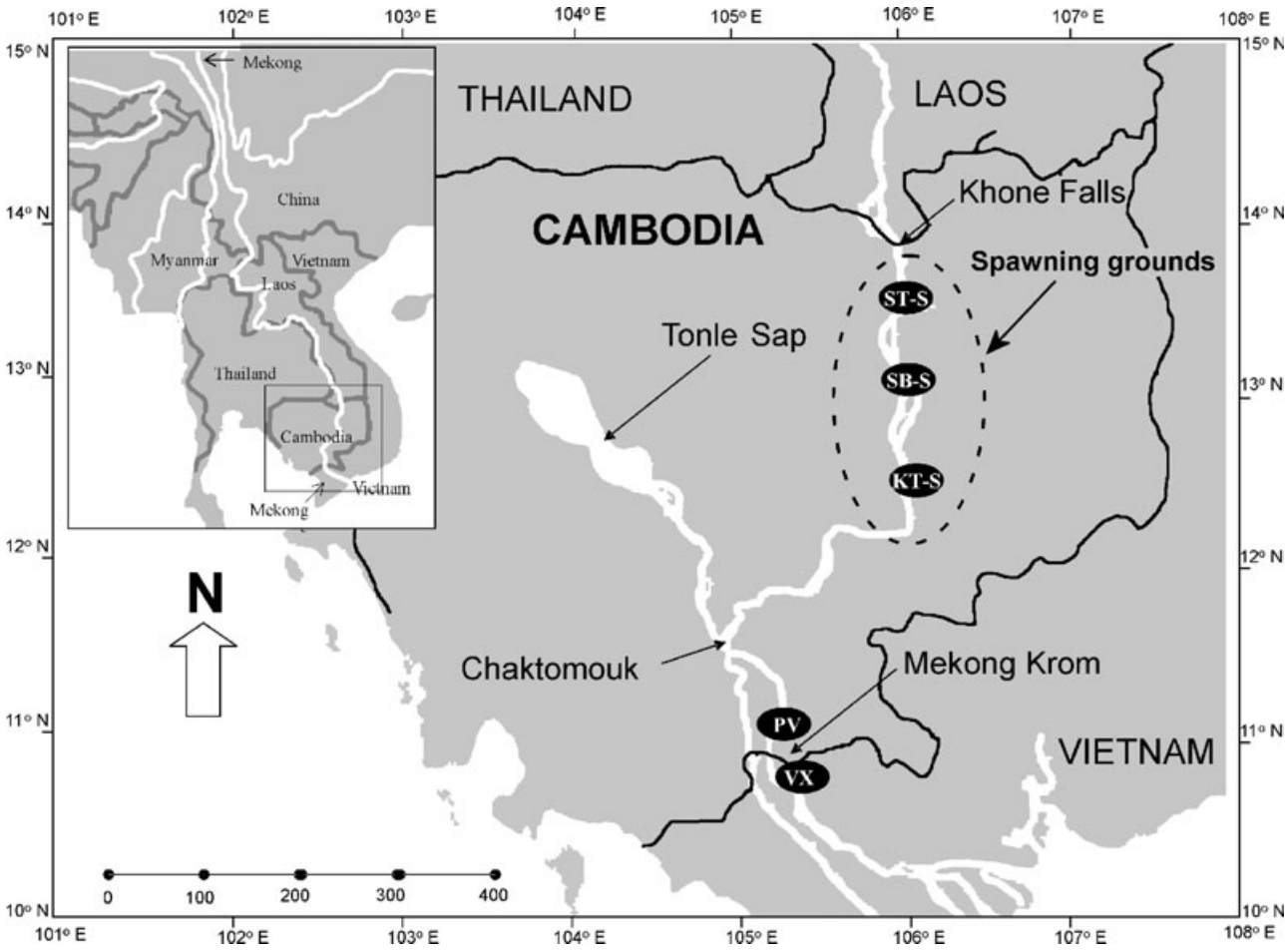

Figure 1. Location map of the Lower Mekong river basin (upper left corner), including the three major spawning habitats (ST, Stung Treng, Siembok district, Stung Treng province; SB, Sambo district, Kratie province; KT, Kratie, Kampi, Kratie district, Kratie province) and the larval sampling sites on the lower Mekong proper (Mekong Krom) (PV, Prey Veng province, Cambodia; VX, Vinh Xuong, Vietnam) (for details, see Table 1).

a putative source of populations of larvae. In that study, a total of five groups were distinguished at the spawning grounds using the same microsatellites used in the present study. These 'populations' were moderately differentiated (So, 2005) and hence can be used as reference populations for individual assignment analysis of sutchi catfish larvae.

Microsatellite analysis

Approximately 100 mg of fin tissue was digested and purified DNA was amplified by the polymerase chain reaction for seven microsatellite loci in accordance with So *et al.* (2006). Three loci (Phy01-KUL, Phy03-KUL, and Phy05-KUL) contained dinucleotide repeats and were isolated from *P. hypophthalmus* (Volckaert, Hellemans & Pouyaud, 1999); two loci (PSP-G 505 and PSP-G 579) contained dinucleotide repeats and two loci (PSP-G 509 and PSP-G 576) contained tetranucleotide repeats and were isolated from a genomic bank including three pangasiid catfishes, but not *P. hypophthalmus* (Hogan & May, 2002).

The resulting amplified fragments were run on an automated sequencer (LI-COR, model 4200) with the appropriate size standards using the software E-seq, version 2.0 (LI-COR Inc.). Products were scored using the software Gene ImagIR, version 4.03 (Scanalytics Inc., 2001, Fairfax, VA).

Statistical analysis

The number of alleles (A) and observed (H_o) and expected (H_e) heterozygosity were calculated for each locus individually and as a multilocus estimate for each of the four temporal larval samples. Single and multilocus F_{IS} values were estimated according to

Table 1. Spawning and larval samples of *Pangasianodon hypophthalmus*, including sampling location, abbreviation, sampling year and life stage

Sampling location	Sampling year/date	Abbreviation	Life stage: Larvae (0 years)	Life stage: Subadult (1–2 years old; 15–50 cm)	Life stage: Adult (3 years or older > 50 cm)	Total
Stung Treng, Cambodia	2002	ST-S1	0	6	54	60
	2003	ST-S2	0	0	70	70
Sambo, Kratie, Cambodia	2002	SB-S1	0	0	60	60
	2003	SB-S2	0	0	43	43
Kratie, Kratie, Cambodia	2002	KT-S1	0	15	45	60
	2003	KT-S2	0	10	50	60
Prey Veng, Cambodia	8 June 2003	PV1	60			60
	5 July 2003	PV2	60			60
	1 August 2003	PV3	50			50
Ving Xuong, Vietnam	11 June 2003	VX1	50			50

Subadults were removed for the cluster and assignment analysis.

Weir & Cockerham (1984); the *P*-value for each test was calculated by a permutation with 1000 iterations. Linkage disequilibrium was tested among all pairs of loci and for all temporal samples using the LinkDis procedure (permutation method, 1000 iterations). All computations were performed using the statistical software package GENETIX, version 4.01 (Belkhir, 2000).

Estimates of global and pairwise F_{ST}, θ, following Weir & Cockerham (1984), and the R_{ST} analogue statistic, ρ (Rousset, 1996), were calculated using GENETIX and RST-CALC, version 2.2 (Goodman, 1997), respectively. Significance of those estimates was assessed using 1000 data permutations. In all cases of multiple simultaneous tests, significance levels were adjusted with the sequential Bonferroni correction (Rice, 1989).

Mean identity, corresponding to the relatedness, defined as the expected proportion of loci that are homozygous in the offspring of the chosen pair of individuals, was estimated for each temporal sample following Mathieu *et al*. (1990), as implemented in IDENTIX, version 1.0 (Belkhir, Castric & Bonhomme, 2002). Identity was chosen over other available measures of genetic relatedness (Queller & Goodnight, 1989; Lynch & Ritland, 1999) because of its substantially reduced variance (Belkhir *et al*., 2002). We tested whether the relatedness pattern of fish in each temporal sample significantly differed from its null expectation of a genetic homogenous population (panmictic population). The mean identity among all pairs of fish in a temporal sample was compared with its expected distribution under the hypothesis of no relatedness obtained by random permutation of genotypes (1000 iterations).

Finally, individual assignment proportions (q) in each of the four temporal larval samples and their 90% posterior probability intervals were calculated by also assuming a no-admixture model (i.e. each individual purely originates from one of the inferred populations) as an admixture model (where each individual genotype can originate from multiple source populations that cross-hybridized) using the program STRUCTURE, version 2.0 (Pritchard, Stephens & Donnelly, 2000). The outcome records the posterior probability that one individual genotype originates from one or more populations. The model considers the individuals from the baseline samples as 'pure' (i.e. non-admixed individuals). We repeated the cluster analysis of the spawning samples (from 2002 and 2003) to define putative baseline populations as a source for the sampled larvae. Due to the expected sympatry of populations and a likely recent differentiation, we assumed allele frequencies in populations to be correlated. In practice, all four temporal samples of larvae (i.e. PV1, VX1, PV2, and PV3) were assigned to each putative source population (termed 'baseline samples') to test for intra-annual heterogeneity in distribution. Individuals having high assignment scores (> 60–70%) were considered to belong to one of the populations for further statistical analyses. Finally, relative proportions of the baseline populations between temporally separated larval samples were compared using chi-square tests, associated with probability values as implemented in the STATISTICA software, version 6.0.

RESULTS

All four temporally separated larval samples (PV1, $N = 60$; VX1, $N = 50$; PV2, $N = 60$; PV3, $N = 50$) showed a high genetic diversity (i.e. mean $A = 9.4$, $H_o = 0.74$, $H_e = 0.76$) (Table 2). Globally, there was a strong and significant deficit of heterozygotes among sutchi catfish larvae ($F_{IS} = 0.071$, $P < 0.001$). Ten single-locus analyses among a total of 28 tests (36%) showed deviations from Hardy–Weinberg equilibrium (HWE) (Table 2). After sequential Bonferroni correction for multiple comparisons (initial $k = 4$), only seven tests exhibited significant divergence from HWE. When the results were combined across all loci, all four (but three after sequential Bonferroni correction) temporal larval samples were inconsistent with HWE (Table 2). The PV2 sample showed a lower heterozygosity and higher F_{IS} than the other three samples. By contrast, the VX1 sample exhibited the highest heterozygosity and the lowest F_{IS}.

We compared the genetic structure of the four temporally separated larval samples. Based on multilocus F_{ST} estimates, samples differed significantly when analysed together ($F_{ST} = 0.0052$, $P < 0.01$). When analysed separately, only one (VX1-PV2) out of six sample pairs showed significant differentiation after sequential Bonferroni correction (Table 3). Based on multilocus R_{ST} estimates, a similar pattern of genetic differentiation was observed (global $R_{ST} = 0.0135$, $P < 0.05$; Table 3).

No evidence for increased relatedness was found in any of the four temporal larval samples. The mean pairwise identity coefficient is in agreement with its expected distribution under the null hypothesis of random association of single locus genotypes within individuals (two-tailed test, $P = 0.542$ for PV1, $P = 0.489$ for VX1, $P = 0.918$ for PV2, and $P = 0.406$ for PV3; Fig. 2).

We used an individual assignment test to estimate the presence and contribution of each reproductively separated population within the larval peaks. An initial analysis of spawning samples yielded the highest likelihood of $k = 5$, namely five putatively divergent spawning populations. Subsequent analysis of the larvae, using the five newly-defined 'populations' as baseline, indicated that sutchi catfish from populations 1 and 2 were predominantly present in all four larval samples; whereas population 3 was absent in all samples (Fig. 3). Population 4 was equally underrepresented (< 5%) in all larval samples. Finally, population 5 was intermediately present in three samples (PV1, PV2, and PV3) and absent in one sample (VX1).

The distribution of the five populations among the four larval peaks differed significantly. This was due to differences between VX1 and the other two larval samples PV1 ($\chi^2 = 15.09$, $P < 0.005$) and PV2 ($\chi^2 = 21.28$, $P < 0.001$), and between the second (PV2) and third (PV3) larval peaks ($\chi^2 = 0.65$, $P < 0.05$). There was a shift in population proportions between the first (PV1, 08 June 2003) and the second sampling (VX1, 11 June 2003), 3 days apart, with a significant lower proportion of the populations populations 1 and 5, and a significant higher proportion of the population 2 (Fig. 3). The third larval sample (PV2, 5 July 2003), collected 25 days later, showed a significant higher proportion of populations 1 and 5 and a lower proportion in population 2 between the second and third sampling. The fourth sample (PV3, 1 August 2003), collected 26 days later, also showed a significant variation, with a lower proportion of populations 1 and 5 and a higher proportion of population 2.

DISCUSSION

The results obtained in the present study clearly demonstrate intra-annual changes in the genetic composition of drifting larvae of sutchi catfish collected over a period of approximately 8 weeks from the Lower Mekong river. We found evidence for population admixture within the larval peaks and a temporal shift in the overall proportion of various spawning populations.

Genetic heterogeneity in larval sutchi catfish

Genetic heterogeneity was detected within and between temporal larval samples, possibly due to stock admixture (i.e. the Wahlund effect) and reproductive variance, respectively. This pattern has already been observed in adult fish (i.e. population admixture and the spatial and temporal genetic differentiation; So *et al.*, 2006) using the same microsatellite loci in the same river basin.

HWE equilibrium is expected for large, randomly mating populations. Departures from HWE may be attributed to several factors such as the presence of null alleles, inbreeding and/or admixture of discrete populations/families (Allendorf & Phelps, 1981; Brookfield, 1996). We believe that null alleles and inbreeding are unlikely to cause a heterozygote deficiency at several loci simultaneously because the heterozygote deficiency was similar at each locus, and genetic diversity and census population size ($N > 20 \times 10^6$; So & Nao, 1999) were high. Hence, another explanation must be sought, such as an admixture of genetically discrete groups (i.e. the Wahlund effect).

A previous study (So *et al.*, 2006) reported that population admixture is the most likely explanation for a deficit of heterozygotes in adult sutchi catfish. There may be two explanations for this deficit: (1) the joint sampling of several randomly mating populations or

Table 2. *Pangasianodon hypophthalmus*: single-locus statistics of the four larval samples from Prey Veng (PV1, 2, and 3) in Cambodia and Vinh Xuong (VX1) in Vietnam

Locus		PV1 (8 June 2003)	VX1 (11 June 2003)	PV2 (5 July 2003)	PV3 (1 August 2003)	Total
PhyOl-KUL	*N*	59	49	59	49	216
	A	6	7	6	6	8
	H_e	0.638	0.674	0.615	0.684	0.653
	H_e	0.555	0.669	0.533	0.591	0.587
	F_{IS}	0.126	0.003	0.112	0.171	0.143
	P (HW)	0.000	0.460	0.000	0.000	0.000
PhyO3-KUL	*N*	59	50	59	50	218
	A	19	15	15	16	23
	H_e	0.880	0.829	0.860	0.849	0.855
	H_o	0.797	0.720	0.864	0.800	0.795
	F_{IS}	0.103	0.142	0.004	0.068	0.076
	P (HW)	0.008	0.006	0.381	0.072	0.001
Phy05-KUL	*N*	60	49	59	49	217
	A	6	5	6	5	7
	H_e	0.714	0.673	0.700	0.716	0.701
	H_o	0.683	0.614	0.593	0.776	0.666
	F_{IS}	0.051	0.092	0.152	–0.072	0.076
	P (HW)	0.221	0.044	0.000	0.799	0.026
PSP-G 505	*N*	60	50	59	50	219
	A	10	9	10	10	15
	H_e	0.774	0.773	0.672	0.759	0.744
	H_o	0.850	0.760	0.610	0.780	0.750
	F_{IS}	–0.090	0.027	0.101	–0.018	0.001
	P (HW)	0.919	0.248	0.041	0.519	0.443
PSP-G 509	*N*	*60*	50	59	50	219
	A	10	10	10	10	10
	H_e	0.823	0.833	0.848	0.852	0.839
	H_o	0.733	0.940	0.848	0.800	0.830
	F_{IS}	0.118	–0.119	0.009	0.071	0.024
	P (HW)	0.012	0.967	0.352	0.081	0.167
PSP-G 576	*N*	60	50	59	50	219
	A	11	11	10	10	11
	H_e	0.821	0.846	0.795	0.825	0.822
	H_o	0.800	0.920	0.763	0.920	0.851
	F_{IS}	0.034	–0.077	0.049	–0.106	–0.021
	P (HW)	0.197	0.900	0.172	0.016	0.720
PSP-G 579	*N*	60	50	59	49	218
	A	9	8	6	8	13
	H_e	0.684	0.712	0.667	0.697	0.690
	H_o	0.700	0.700	0.610	0.694	0.676
	F_{IS}	–0.015	0.026	0.094	0.014	0.029
	P (HW)	0.506	0.277	0.091	0.357	0.232
Total	*N*	59.7	49.7	59.0	49.6	218
	A	71	65	63	65	87
	H_e	0.762	0.762	0.737	0.769	0.757
	H_o	0.705	0.752	0.651	0.728	0.738
	F_{IS}	0.083	0.029	0.124	0.063	0.071
	P (HW)	0.000	0.041	0.000	0.008	0.000

N, number of genotyped fish; A, number of alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{IS}, estimate of inbreeding coefficient; *P* (HW), permutation test probability of Hardy–Weinberg equilibrium. Numbers 1, 2 and 3 refer to the first, second, and third peak of downstream larval drifts during the spawning season (see also Fig. 1).

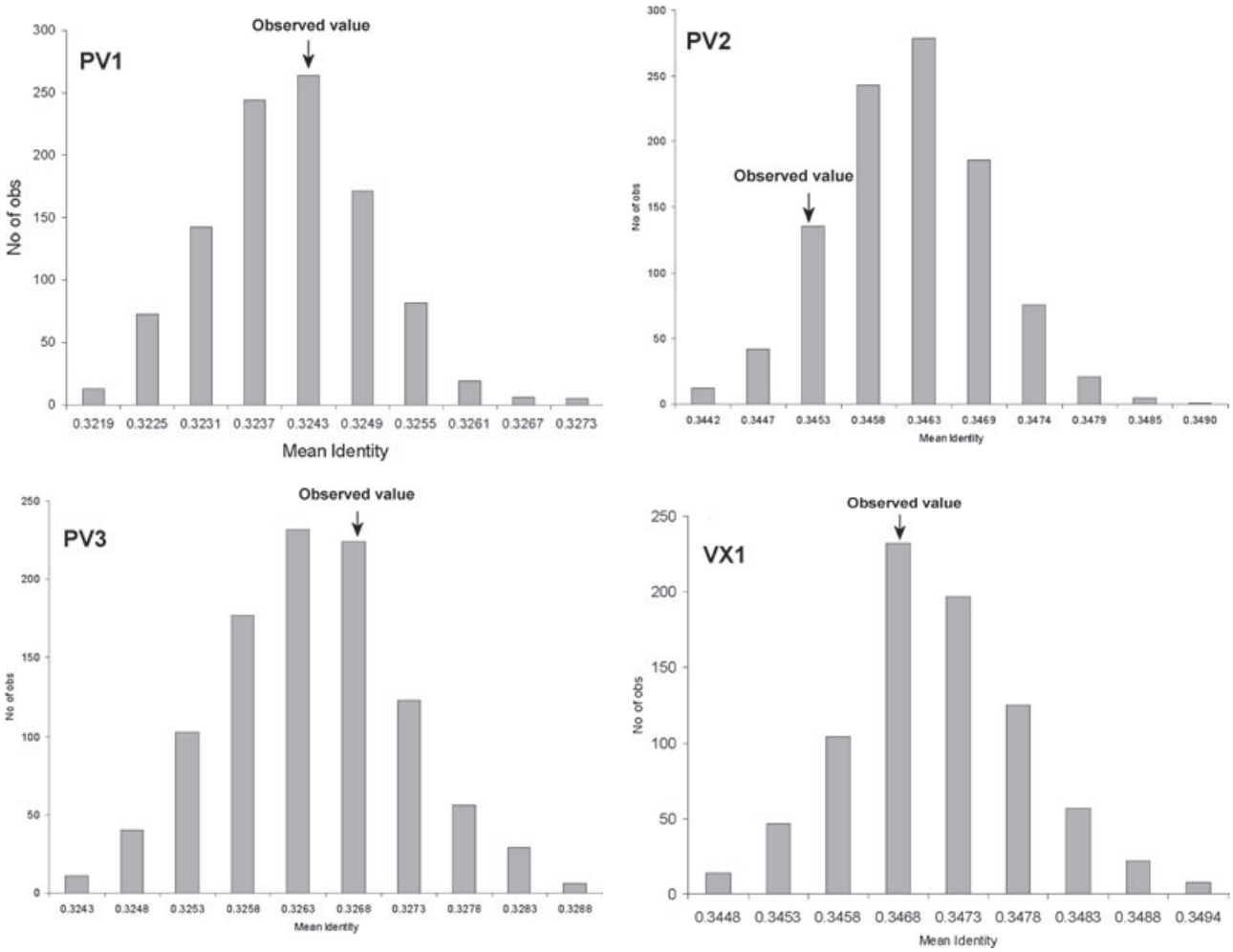

Figure 2. Distribution of pairwise relatedness coefficients among larval sutchi catfish peaks (PV1, PV2, PV3, and VX1) according to the identity coefficient defined by Mathieu *et al*. (1990), assuming random association of single locus genotypes. Two-tailed test: $P = 0.542$ for PV1, $P = 0.489$ for VX1, $P = 0.918$ for PV2, and $P = 0.406$ for PV3. The arrows indicate the observed mean identity.

(2) the sampling of offspring originating from a finite number of successful families. Evidence for the first scenario is provided by the lack of significant departure from the expected relatedness (i.e. no relatedness) under random mating in all larval samples, indicating that the larvae originated from a large number of families (but see also Allendorf & Phelps, 1981; Hansen, Nielsen & Mensberg, 1997) or groups of spawning individuals (Fig. 3). This was further supported by the individual-based assignment tests, indicating a mixture of homogeneous groups within each sample. This is probable because all larval samples were collected downstream more than 350 km away from the spawning grounds. Consequently, mixing of sutchi catfish occurs at a very early stage, possibly on the spawning ground proper, and definitely in the main river channel. Synchronized hatching on the spawning grounds (where eggs stick in clusters to submerged branches, roots, and rocks) may cause such overlap in larval peaks.

Despite the genetic heterogeneity observed in each larval peak sample, there is also evidence for a significant but weak genetic differentiation among samples. Therefore, all larvae collected on the same river branch over a period of approximately 8 weeks, may have originated from several spawning groups involving different spawning events. Previous studies on divergence within pelagic larval aggregations have provided evidence for genetically heterogeneous groups of many marine invertebrate larvae (Johnson & Black, 1984; David *et al*., 1997; Li & Hedgecock, 1998) and marine fishes (Ruzzante *et al*., 1996; Planes

Table 3. *Pangsianodon hypophthalmus*: pairwise F_{ST} (below diagonal) and R_{ST} (above diagonal) estimates between larval peak samples

	PV1	VX1	PV2	PV3
PV1	–	0.0069	0.0238*	0.0017
VX1	0.0061*	–	0.0287*†	0.0052
PV2	0.0057*	0.0128**†	–	0.0160
PV3	0.0009	0.0044	0.0013	–

Associated significance levels: *$P < 0.05$, **$P < 0.01$. †Significant after sequential Bonferroni correction for multiple comparisons.

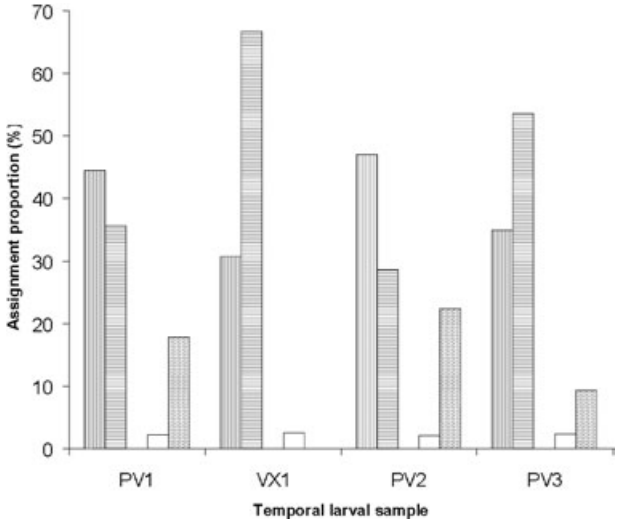

Figure 3. Estimated assignment proportions (%) of different larval peak samples to each putative spawning population of sutchi catfish. Spawning population symbols: population 1, vertical lines; population 2, horizontal lines; population 3, dark grey (not found); population 4, white; population 5, wave.

& Lenfant, 2002; Pujolar, Maes & Volckaert, 2006). Distinct larval groups separated by periods of several days within a large planktonic aggregation have also been observed in herring (*Clupea harengus*) (McPherson, Stefenson & Taggart, 2003) and capelin (*Mallotus villosus*) (Lambert, 1984). Lambert (1984) discussed the possibility that such temporally reproductive strategy might be widespread among marine species with pelagic eggs and larvae. It might have important consequences for the survival of early life stages by spreading the effort over time to take advantage of a variable environment. In sutchi catfish, the temporally structured reproductive strategy not only takes advantage of a fluctuating environment, but also, and unlike marine fish, a limited availability of spawning ground (So *et al.*, 2006). Thus, each temporally genetically heterogeneous group is on its turn influenced by biotic, hydrologic and climatic processes (Poulsen *et al.*, 2002; Van Zalinge *et al.*, 2002), which may affect the reproductive success among individual spawners and/or spawning groups (Sinclair, 1988; Ruzzante *et al.*, 1996; Li & Hedgecock, 1998; Planes & Lenfant, 2002). The availability of food resources to larvae (i.e. match/mismatch theory; Hjort, 1914; see also Cushing, 1972, 1975; Levitan & Petersen, 1995) appears to be a major contributing mechanism to survival. Hence, it may partly explain the relationship between effective population size and observed census population size, as proposed by Hedgecock (1994). Under such hypothesis, natural populations should generally show significant random genetic drift and detectable genetic heterogeneity among larval peaks because only a small proportion of adults contribute to the population (i.e. sweepstakes hypothesis). Spawning time should account for interpopulation differences in life-history patterns (a hypothesis opposed to the match/mismatch theory; Sinclair, 1988). If our interpretation is correct, then differences in life-history traits (such as spawning time) among populations might provide an explanation for the genetic composition of sutchi catfish larval peaks. The spawning waves are produced by the reproductive contribution of dominant year classes; age structure thereby determines the number of spawning waves per season in a population (Lambert, 1987).

Genetic composition of consecutive larval peaks

Parallel to the above findings, individual-based assignment tests revealed that each temporal larval sample consisted of larvae likely originating from several different spawning groups involving discrete spawning events. By using spawning populations from two different years as baseline data, we avoid an underestimation of the number of populations due to possible sampling bias in only one protracted spawning season (So, 2005). Subsequently, we were able to redefine five spawning populations, likely contributing to the larval peaks analysed here. The first (PV1), third (PV2), and fourth (PV3) temporal larval samples corresponded to the first, second, and third peak of larval drift in Cambodia, respectively, and originated from the reassigned populations 1, 2, 4, and 5, whereas the second larval sample (VX 1), collected 45 km downstream in Vietnam and belonging to the first peak of larval drift, originated from the populations 1, 2, and 4. Population 5 was not present in the VX1 sample. It is possible that it drifted from the main channel of the Mekong onto the nursery grounds (flooded floodplain) before reaching the Mekong delta (southern Vietnam). Sutchi catfish from population 3,

which is considered the smallest from the five (So, 2005), was absent in all downstream larval samples. Because we sampled larvae daily during the whole period (3 days) of each larval peak, there is good evidence that this small population does not occur in the river branch targeted. Furthermore, similar results were obtained using individual assignment tests by assuming the admixture model (i.e. allowing the genetic composition of individuals to be a mixture from different populations). Consequently, this small population may be present in any of the two other major river branches (i.e. Tonle Sap and upper stretches of the Mekong river). This is in agreement with an ongoing study (So, 2005) where this population was not found among 387 (sub)adult sutchi catfish collected on the floodplain feeding grounds in the year 2002. For a better understanding, additional larval peak samples collected at critical sites along Tonle Sap, the Mekong delta, and the upper stretches of Mekong river (upstream of Chaktomouk-Phnom Penh; Fig. 1) are required.

Furthermore, there is evidence for a temporal shift in the proportions of populations among the peaks of larval drift. In other words, each population does not appear to be in the same proportions in every temporally separate peak of larval drift in the Lower Mekong river. Temporal variation in the cohort structure among (sub)adult sutchi catfish on the feeding grounds in the Lower Mekong may be the cause (So, 2005). Floodpulse and water temperature are obvious ecological triggers for spawning of sutchi catfish and other fish species in the Mekong river (Poulsen *et al*., 2002). Spawning at the right time and place will enable offspring to enter the floodplain habitats to feed. Hence, temporal variation in the distribution of populations among sutchi catfish larvae might be due to intra-annually fluctuating conditions of the natural physical environment of the Mekong river. Alternatively, availability of food resources (phytoplankton and zooplankton; i.e. match/mismatch theory), which affects larval survival, may be another explanation for such a temporal shift.

In conclusion, the present study suggests the existence of genetic heterogeneity in the larval peaks of sutchi catfish, including an intra-annual shift in the contribution of spawning populations to larval samples. For management purposes, it is crucial to understand why two out of five groups are so underrepresented in the larval peak samples. A priority for genetic research in the Mekong basin should be an analysis of the spatial and temporal patterns of various life stages of known age. Insights into the dynamics of adaptation and the interaction between life-history (i.e. age, size, sex, growth, and survival) and genetic structure are essential for sound fisheries and conservation management.

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