

Evidence for Female-Biased Dispersal in the Protandrous Hermaphroditic Asian Seabass, *Lates calcarifer*

Gen Hua Yue*, Jun Hong Xia, Feng Liu, Grace Lin

Molecular Population Genetics Group, Temasek Life Sciences Laboratory, National University of Singapore, Singapore, Republic of Singapore

Abstract

Movement of individuals influences individual reproductive success, fitness, genetic diversity and relationships among individuals within populations and gene exchange among populations. Competition between males or females for mating opportunities and/or local resources predicts a female bias in taxa with monogamous mating systems and a male-biased dispersal in polygynous species. In birds and mammals, the patterns of dispersal between sexes are well explored, while dispersal patterns in protandrous hermaphroditic fish species have not been studied. We collected 549 adult individuals of Asian seabass (*Lates calcarifer*) from four locations in the South China Sea. To assess the difference in patterns of dispersal between sexes, we genotyped all individuals with 18 microsatellites. Significant genetic differentiation was detected among and within sampling locations. The parameters of population structure (F_{ST}), relatedness (r) and the mean assignment index (mAIC), in combination with data on tagging-recapture, supplied strong evidences for female-biased dispersal in the Asian seabass. This result contradicts our initial hypothesis of no sex difference in dispersal. We suggest that inbreeding avoidance of females, female mate choice under the condition of low mate competition among males, and male resource competition create a female-biased dispersal. The bigger body size of females may be a cause of the female-biased movement. Studies of dispersal using data from DNA markers and tagging-recapture in hermaphroditic fish species could enhance our understanding of patterns of dispersal in fish.

Citation: Yue GH, Xia JH, Liu F, Lin G (2012) Evidence for Female-Biased Dispersal in the Protandrous Hermaphroditic Asian Seabass, *Lates calcarifer*. PLoS ONE 7(6): e37976. doi:10.1371/journal.pone.0037976

Editor: Cédric Sueur, Institut Pluridisciplinaire Hubert Curien, France

Received: February 13, 2012; **Accepted:** April 27, 2012; **Published:** June 12, 2012

Copyright: © 2012 Yue et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This research is funded by the National Research Foundation of Singapore. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: genhua@tll.org.sg

Introduction

Dispersal is an important life history trait. It influences individual fitness, reproductive success, genetic variation and relationships among individuals within populations, gene flow among populations and the potential to colonize in new habitats [1]. The study of dispersal has been an active research area in evolutionary biology and molecular ecology [2]. In the early 1980s, studies on sex-biased dispersal in both birds and mammals set the basis for subsequent researches on theories of sex-biased dispersal [3]. Generally, mammals display male-biased dispersal [4], whereas most of the birds show the reverse pattern [5,6].

Several theories have been developed to explain the sex-biased dispersal. They relate the difference in dispersal between sexes to gender-specific differences in the advantages that philopatry conveys to males and females, or to sex-specific effects of fecundity costs. Competition for mates and breeding resources, and inbreeding avoidance can result in sex-biased dispersal [3,5]. The sex that gains more from prior ownership of a territory would be favoured by selection to move less when the fitness effects of gaining and defending reproductive resources are different between two sexes [3,5,7]. Besides resource competition, sex-biased dispersal was also attributed to mating systems [8,9]. Competition among kin and inbreeding avoidance can also produce sex-biased dispersal in mammals (Handley & Perrin 2007), birds [6,10] and some fish species [11,12], because competition among kin diminishes the inclusive fitness. Whether

avoidance of kin competition can result in sex-biased dispersal is associated with the possibility that the two sexes compete for the same resources [13]. In polygamous mating systems, the breeding success of males may be limited mainly by the number of mating partners (i.e. females). Therefore, the local competition for mating with females is possibly higher for males. In contrast, when males make no parental investment or the male contribution to parental care is minor, the competition for local resource among relatives for limited breeding resources may be greater among females [14,15]. Previous studies suggested that the sex differences in migrating patterns are associated with the balance among kin for mates and/or for resources [16,17,18]. When the breeding resources limit the female reproductive success in polygynous or promiscuous systems, or when the effect of local competition among males and females is the same in monogamous systems, dispersal is not sex-biased [19].

Sex-biased dispersal in fish has been studied only in a few species, although there are over 30,000 teleost fish species on the earth [20]. Male-biased dispersal has been reported in some fish species, such as brook trout [11] and three-spined stickleback [21]. Female-biased movement has also been described in Lake Malawi cichlids [22], dwelling Dolly Varden [23] and salmonids [24]. In salmonids, migrating individuals moved to beneficial habitats to feed and gain larger body size [24]. It is believed that female-biased dispersal may have caused the female-biased sexual size dimorphisms in some fish species [25,26,27]. The patterns and

evolutionary significance of sex-biased dispersal in protandrous hermaphroditic species remain unexplored. The genetic differentiation of most marine fish populations is usually weak [28] which is probably the consequence of unrestricted gene flow in marine environment [28,29]. Therefore, it is believed that dispersal is unbiased between sexes in these marine fish species where population differentiation is low. In fishes, direct observation of movements is very difficult, but dispersal can be inferred using polymorphic DNA markers, such as microsatellites and other DNA markers [12,21].

The Asian seabass (also called barramundi), *Lates calcarifer*, belongs to the family Centropomidae. It is distributed in coastal waters, freshwaters and estuaries through the whole of Southeast Asia to Papua New Guinea and northern Australia. It can also be found from western India to the Bay of Bengal [20]. It has a complex life history and is a protandrous hermaphrodite, which starts life as male first and later changes to female after males reached sexual mature at the age of 3 to 4 years [30]. The transition from male to female is short (i.e. a few months) and may not occur in all individuals [31]. The fecundity of *L. calcarifer* is among the highest of any teleost fish, and its life span is over 25 years. Usually, an adult female produces 0.5–40.0 million eggs [32,33]. Spawning takes place in the sea all year around in Southeast Asia, but only occurs in the sea from October to February in Australia [30,32,33]. Females spawn every three months in Southeast Asia while males reproduce all year around [34]. *Lates calcarifer* is a non-guarding species. There is no parental involvement in the development of fry and juvenile fish [30,32,33]. *Lates calcarifer* is catadromous and migrates substantially to specific spawning grounds in order to breed [32,35,36]. Previous data on tagging and recapture showed that juvenile *L. calcarifer* remained resident until reaching sexual maturity at the age of 3–4 years [37,38] and during spawning, sexually matured individuals migrated to coastal areas for breeding [38]. Recent studies indicated that adult *L. calcarifer* did not always migrate to breeding grounds to spawn, with a lifetime non-participation rate of as much as 50% and bigger individuals migrated more than smaller ones [39,40]. Because *L. calcarifer* is a broadcast breeder, there might be little interaction between males and females. Since species with external fertilization and little or no post-spawning parental investment may not exhibit sex-biased movement [5,41], we hypothesize that dispersal of *L. calcarifer* is unbiased between the two sexes.

In this study, we collected 549 Asian seabass individuals from four locations along the coast of Thailand, Malaysia, Singapore and Indonesia. To quantify genetic parameters in these four sampling locations, we used 18 polymorphic microsatellites to genotype the 549 individuals. We tested the hypotheses of unbiased dispersal in *L. calcarifer* by combining genetic parameters estimated in this study with data on tagging-recapture published by Australian scientists in the past 30 years [35,36,38,42]. To our best knowledge, this is the first study aiming to analyze the dispersal patterns and the potential reasons for sex-biased dispersal in a protandrous hermaphroditic marine fish species. The results of this study could add new data on dispersal patterns of fish species, in which the patterns of dispersal have not been extensively studied, thus enhancing our understanding of dispersal patterns of fish.

Materials and Methods

Ethics Statement

All handling of fishes was conducted in accordance with the guidelines on the care and use of animals for scientific purposes set up by the Institutional Animal Care and Use Committee (IACUC)

of the Temasek Life Sciences Laboratory, Singapore. The IACUC has specially approved this study within the project “Molecular Breeding of Asian seabass” (approval number is TLL (F)-003-09).

Sampling

We collected 549 adult (3–4 years old) *L. calcarifer* individuals from four locations in South China Sea along the coast of Thailand, Malaysia, Singapore and Indonesia (Table 1; Fig. 1). We brought all fish back to fish facility in Marine Aquaculture Centre, Singapore. We determined the age of *L. calcarifer* by counting growth rings on their scales (otoliths) according to McDougall [43]. All individuals were weighted and sexed by squeezing for sperm or eggs. We collected fin clips from each fish and stored them individually in 95% ethanol till DNA extraction.

DNA Isolation and Microsatellite Genotyping

We extracted total DNA from fin clips of each fish using a method developed by us [44]. Extracted DNA was eluted in distilled water. DNA quality and quantity were examined using electrophoresis on 1% agarose gels and Nanodrop (Thermo Scientific), respectively.

We genotyped all individuals using 18 polymorphic microsatellite markers (see details in Table 2) developed by us previously [45,46,47] due to their polymorphism, the ease of PCR amplification and scoring genotypes. One primer of each pair was labelled with a fluorescent dye (either FAM or Hex). PCR was performed in PTC-100 thermal-cyclers (MJ Research) using the following PCR program: 94°C for 3 min, followed by 36 cycles of 94°C for 30 s; 55°C for 30 s; 72°C for 30 s and a final extension at 72°C for 10 min. Twenty-five μ l reaction mixes consisted of 20 ng of DNA, 0.5 μ m of each primer, 200 μ m of each dNTP, 0.5 units of Taq-polymerase (Finnzymes) and 1 \times reaction buffer with 1.5 mm MgCl₂. PCR products were resolved on ABI3730 \times 1 automated sequencers (Applied Biosystems). GENEMAPPER v.4.1 (Applied Biosystems) was used to score fragments.

Data Analysis

Since the existence of null alleles can result in false homozygotes and generate a pattern similar to the Wahlund effect [48], we used software MICRO-CHECKER 2.2.3 [49] to infer null alleles, stuttering bands and allele dropout. We calculated allele frequencies, allele number, observed (H_O) and expected heterozygosities (H_E) for each population using software GDA [50] and allelic richness, a parameter reflecting allelic diversity independent of sample size using software FSTAT v.2.9.3 [51].

We conducted hierarchical analysis of molecular variance (AMOVA) and estimated F_{ST} among the sampling locations, based on allele frequency information [52] using the program ARLEQUIN version 3.1 [53]. We analyzed variance components among and within sampling locations. We examined the isolation by distance with the online program IBDWS [54]. The statistical significance of the correlation between the pairwise genetic distance [$F_{ST}/(1-F_{ST})$] and the geographical distance (log of distance in kilometers between populations along the coast line), was obtained by the use of Mantel tests.

We also examined the fine-scale population genetic structure for all 549 individuals collected along the coast of Thailand, Malaysia, Singapore and Indonesia using the program STRUCTURE version 2.3.3 [55]. This program uses the genotypes of each individual to find the optimal population number (K) that minimizes Hardy-Weinberg and linkage disequilibria using a Bayesian clustering algorithm and without assigning individuals to populations a priori [56]. In simulation, we used an admixture model with correlated allele frequencies. We performed 10

Table 1. Samples of Asian seabass used in this study.

| Sampling location | N | F | M | F BW (kg) TBL (cm) | M BW (kg) TBL (cm) | H_E | A_R | F_{IS} |
|-------------------|-----|-----|----|-----------------------|---------------------|-------|-------|----------|
| Malaysia | 165 | 105 | 60 | 4.82±1.28* 68.4±0.38* | 3.05±0.68 52.6±0.40 | 0.702 | 8.23 | 0.069 |
| Singapore | 104 | 53 | 51 | 4.38±0.17* 65.5±0.76* | 2.96±0.45 53.1±0.55 | 0.681 | 7.81 | 0.008 |
| Thailand | 132 | 76 | 56 | 4.05±1.06* 66.2±0.57* | 2.56±0.27 51.9±0.27 | 0.713 | 8.92 | 0.073 |
| Indonesia | 148 | 82 | 66 | 5.20±0.92* 72.9±0.48* | 3.10±0.39 62.9±0.39 | 0.697 | 8.77 | 0.027 |

N, number of sampled individuals; M, males; F, females; BW, average body weight ± SD; TBL, total body length; H_E , gene diversity; A_R , allelic richness; F_{IS} , inbreeding coefficient and *, $P < 0.05$ indicating significant difference between males and females.
doi:10.1371/journal.pone.0037976.t001

independent runs for $K = 1-20$ using 100 000 burn-in steps and 100 000 Markov Chain Monte Carlo repetitions to ensure chain stabilization as suggested in the documentation for STRUCTURE software version 2.3.3 [55]. We chose the optimal K by identifying the mean maximum estimated logarithm of the probability of the data, $\ln Pr(X/K)$, for each K , and computing the posterior probability of each K using formula given by Pritchard and Wen [55]. We also used the method of Evanno et al [57] to obtain the optimal K . To examine the proportion of males and females migrating to other locations, we also used $K = 4$ for the LOCPRIOR model to cluster individuals by providing priors for the Bayesian assignment process based on the sampling locations. The LOCPRIOR model allows structure to be detected with lower levels of divergence and is not biased towards detecting structure when it is not present [58].

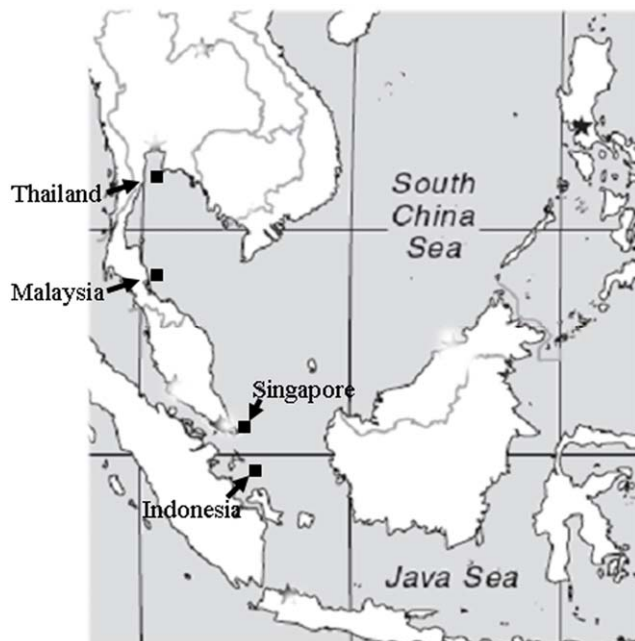
We examined the difference in dispersal between sexes using methods described by Goudet et al. [59] and software FSTAT v.2.9.3 [51]. We examined whether the genotypes at each locus deviated from Hardy–Weinberg equilibrium (HWE) within populations by calculating F_{IS} . We analyzed population differentiation using F_{ST} [60] and determined the statistical significance (P) of the F indices by 10 000 randomizations as implemented in

FSTAT v.2.9.3. To detect possible difference in dispersal between sexes, we quantified relatedness (r) [61], mean assignment index (mAIC), variance of the assignment index (vAIC), deviation from HWE (F_{IS}) and differentiation among populations (F_{ST}) for males and females separately over all four populations. The assignment index (AIC) is an estimate of the probability of a genotype belonging into a given population. Positive values of this index mean that the individual is a resident and negative values indicate that it is a migrant as AIC is centred on zero. In the case of sex-biased migration, the average AIC index (mAIC) for the sex that disperses is lower than that for the more philopatric sex because migrating individuals have lower AIC values than resident ones. Members of the dispersing sex include both residents and immigrants; therefore vAIC is larger for the dispersing sex. The β values of differences in these within-population indices were

Table 2. Details of the 18 microsatellite loci of Asian seabass used in this study.

| Locus | GenBank Accession no. | LG | A | H_O | H_E | F_{IS} | F_{ST} |
|---------|-----------------------|----|-------|-------|-------|----------|----------|
| Lca002 | AF007943 | 10 | 9 | 0.672 | 0.703 | 0.043 | 0.021 |
| BMS08 | AF404076 | – | 4 | 0.459 | 0.499 | 0.080 | 0.021 |
| Lca016 | AF406080 | – | 15 | 0.778 | 0.822 | 0.053 | 0.017 |
| Lca020 | AF404082 | 16 | 11 | 0.723 | 0.750 | 0.036 | 0.007 |
| Lca021 | AF404083 | 17 | 7 | 0.809 | 0.823 | 0.017 | 0.006 |
| Lca040 | AF404099 | 22 | 8 | 0.687 | 0.699 | 0.018 | 0.018 |
| Lca050 | AY998845 | 20 | 2 | 0.453 | 0.455 | 0.003 | 0.015 |
| Lca057 | AY998849 | 17 | 10 | 0.725 | 0.736 | 0.015 | 0.022 |
| Lca058 | AY998850 | 11 | 16 | 0.811 | 0.891 | 0.090 | 0.019 |
| Lca062 | AY998854 | 6 | 14 | 0.766 | 0.882 | 0.132 | 0.020 |
| Lca063 | AY998855 | 13 | 10 | 0.641 | 0.705 | 0.092 | 0.036 |
| Lca064 | AY998856 | 2 | 10 | 0.707 | 0.783 | 0.098 | 0.015 |
| Lca069 | AY998859 | 15 | 6 | 0.719 | 0.775 | 0.071 | 0.024 |
| Lca070 | AY998860 | 14 | 8 | 0.698 | 0.745 | 0.063 | 0.025 |
| Lca072 | AY998861 | – | 2 | 0.203 | 0.219 | 0.075 | –0.001 |
| Lca074 | AY998863 | 12 | 10 | 0.610 | 0.676 | 0.097 | 0.018 |
| Lca086 | AY998873 | 8 | 23 | 0.909 | 0.901 | –0.009 | 0.020 |
| Lca098 | AY998880 | 5 | 20 | 0.732 | 0.763 | 0.040 | 0.031 |
| Average | – | – | 10.28 | 0.712 | 0.666 | 0.065 | 0.022 |

LG, linkage group; A, number of alleles; H_O , observed heterozygosity; H_E , expected heterozygosity; F_{IS} , inbreeding coefficient; F_{ST} , differentiation in allele frequencies; and –, not mapped to linkage groups.
doi:10.1371/journal.pone.0037976.t002

**Figure 1.** Map of sampling locations of Asian seabass along the coast of Thailand, Malaysia, Singapore and Indonesia.
doi:10.1371/journal.pone.0037976.g001

determined using the randomization method with 10,000 permutations using software FSTAT v.2.9.3. Since we used microsatellites to assess the dispersal patterns, we could only detect the short-term dispersal patterns as this signal disappears after the migrating individuals mate, due to the Mendelian segregation of biparental markers [59].

To examine whether there is difference of migration distance between adult males and females, we also retrieved and analyzed tagging and recapture records released by Infofish Services (http://www.info-fish.net/reporting_recapture.html). We only used the data of tagged adult fish, which were captured at least one year after tagging to ensure that fish had enough time to migrate. We compared the average migrating distance between male and female adult individuals using t-Test as implemented in software Microsoft Excel 2003. We also analyzed the relationship between migration distance and body length using the analysis tool "Regression" in Microsoft Excel 2003.

Results

Sex, and Body Weight and Length

We collected 549 individuals in four locations along the coast of Thailand, Malaysia, Indonesia and Singapore (Table 1 and Figure 1). All fishes used in this study were at the age of 3–4 years. In all 549 individuals, the sex ratio was female-biased (F:M = 1.36). In each population, the sex ratio was also female-biased. The ratio of F:M ranged from 1.04 for the sampling location Singapore to 1.75 for the sampling location Malaysia. The average body weight and length of females were significantly ($P < 0.05$) bigger than those of males in all four populations (Table 1).

Microsatellite Polymorphism

All 549 individuals were successfully genotyped for each locus. The examination of genotypes in each locus with software MICRO-CHECKER revealed that there were no null alleles, no stuttering bands or allele dropout in all 18 loci. The average allele number/locus was 10.28. All loci conformed to HWE (Table 2).

Population Differentiation

The overall differentiation among the four populations was low ($F_{ST} = 0.022$, $P < 0.05$). The pairwise genetic differentiation was also low (F_{ST} ranged from 0.013 to 0.048; Table 3). But the genetic differentiation between pairwise populations was statistically significant ($P < 0.05$). Statistical analysis with Mantel tests revealed that there was no isolation by distance relationships among the four populations ($r = -0.16$, $P > 0.05$). The analysis of molecular variances (AMOVA) of microsatellites revealed that the variation within and among sampling locations was 97.76% ($P < 0.05$) and 2.24% ($P < 0.05$), respectively.

Table 3. Pairwise F_{ST} estimates between sampling locations of Asian seabass.

| | Malaysia | Singapore | Thailand |
|-----------|--------------|--------------|--------------|
| Malaysia | | | |
| Singapore | 0.021 | | |
| Thailand | 0.019 | 0.048 | |
| Indonesia | 0.013 | 0.019 | 0.015 |

Estimates significantly higher than zero are in bold ($P < 0.05$ in all cases).
doi:10.1371/journal.pone.0037976.t003

Using the method described by Pritchard et al. [56], the optimal K -value revealed by STRUCTURE version 2.3.3 was $K = 15$ (Figure 2), suggesting that the four sampling locations along the coast of Thailand, Malaysia, Singapore and Indonesia consist of 15 genetic clusters. Based on the ΔK method [57], the ΔK value maximized at $K = 15$ (Figure 2), confirming that the 549 individuals collected the four locations could form 15 clusters. These data indicate population differentiation among the four sampling locations and sub-structuring within sampling locations. To examine the proportion of males and females migrating to other locations, we also used $K = 4$ for the clustering analysis. The largest proportion (ranging from 31.1% for the location Indonesia to 51.0% for the location Singapore) of the individuals collected from a location was clustered into their original sampling locations (Table 4). Among the resident fish from each location, the proportion of males was much higher than that of females, while in the migrant individuals, the female proportion was much higher than the male proportion (Table 4).

Sex-biased Dispersal

We estimated the genetic parameters separately for each sex using genotypes at all 18 microsatellites. The mean assignment index (mAIC) in females (-0.89) was significantly different from the mean assignment index in males (0.88 , $P = 0.01$, Table 5). Females showed higher variance in AIC values but the difference was not significant (female $vAIC$ variance = 30.37, male $vAIC$ = 29.26, $P = 0.76$, Table 5). The population differentiation (F_{ST} , 0.036 for male vs 0.023 for female) and relatedness (r , 0.066 vs 0.044) were significantly ($P = 0.01$) higher for males than for females (Table 5). The heterozygosity deficiency (F_{IS}) was higher in males than that in females (0.046 vs. 0.035, $P = 0.036$).

We retrieved the records of tagging and recapture for 23 adult individuals released by Infofish Services. Analyzing the average migrating distance between male and female adults revealed that the females migrated more than males (75.7 ± 23.1 km for 11 males vs 357.7 ± 79.7 km for 12 females, $P < 0.01$). The migrating

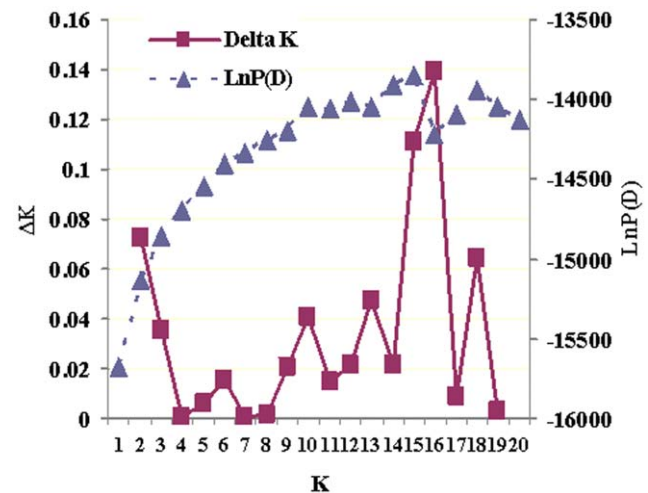


Figure 2. Results from the program STRUCTURE analysis of Asian seabass (*Lates calcarifer*, $n = 549$) from four sampling locations along the coast of Thailand, Malaysia, Singapore and Indonesia. Plot displays mean log-likelihood $\ln P(D)$ and ΔK values for 10 independent runs for each value of K for $K = 1-20$. The highest value was at $K = 15$ and $\Delta K = 15$, indicating that the four sample locations likely form 15 populations.
doi:10.1371/journal.pone.0037976.g002

Table 4. Proportion (%) of memberships of each pre-defined population of Asian seabass in each of four clusters inferred using software STRUCTURE.

| Given population | Inferred clusters | | | | | | | | Number of individuals |
|------------------|-------------------|------|------|------|------|------|------|------|-----------------------|
| | 1 | | 2 | | 3 | | 4 | | |
| | M | F | M | F | M | F | M | F | |
| Malaysia | 21.8 | 13.3 | 2.4 | 29.7 | 4.2 | 9.7 | 7.3 | 11.5 | 165 |
| Singapore | 5.8 | 11.5 | 36.5 | 14.4 | 3.8 | 7.7 | 4.8 | 15.4 | 104 |
| Thailand | 5.3 | 8.3 | 1.5 | 7.6 | 28.8 | 10.6 | 6.8 | 31.1 | 132 |
| Indonesia | 6.1 | 14.2 | 10.1 | 19.6 | 6.8 | 12.2 | 21.6 | 9.5 | 148 |

M: male and F: female.

doi:10.1371/journal.pone.0037976.t004

distance was significantly correlated to the body size ($r^2 = 0.37$, $P < 0.05$).

Discussion

Hermaphroditism is quite common in fish, being sequential in the most cases [62]. Sex reversal has evolved independently in more than 350 species from at least 23 teleost families [63] whereas our understanding of the mechanisms underlying this phenomenon is not comprehensive [63]. The Asian seabass is a protandrous hermaphroditic species. In its early life, all individuals are male but when they attain certain body size/weight or age, the majority of them become female [32,33]. In this study, we found that the sex ratio for Asian seabass at the age of 3–4 years was female-biased (F:M = 1.36) in all four sampling locations in South China Sea. At the same age, the females were heavier and longer than the males. Similar results have been seen in adult brooders of Asian seabass in breeding populations at the age of 3–4 years (Mr Tan J. personal communication). In Australia, *L. calcarifer* had a size-related sex ratio [30,32]. The smaller length classes were almost exclusively male, and the percentage of females increasing with increased total length [32]. Currently, there are several theories that can explain the sex reversal in fishes [64,65,66,67]. In most fish species, female fecundity increases dramatically with body size whereas even small mature males can produce a large number of sperm to fertilize a lot of eggs [68]. Thus, selection pressures may favour the sex change from male to female. However, female to male sex reversal is more prevalent in fishes [69]. Therefore, the evolutionary mechanisms underlying male to female sex reversal in fish merit further investigation.

Both the F_{ST} analysis and AMOVA showed that genetic differentiation of *L. calcarifer* among the four sampling locations were statistically significant, although the pairwise F_{ST} values were small. In Australia, the populations of *L. calcarifer* also showed

significant differentiation among and within locations [35,42,70,71]. The reasons for significant differentiation of *L. calcarifer* in Australia were the discontinuous distribution of appropriate habitats, an absence of extensive pre-spawning migrations and limited dispersal opportunity due to physical barriers [70,71,72]. While in Southeast Asia, along the coast of the South China Sea, there is no obvious physical barrier to prevent the migration of fish. In this study, detailed clustering analysis of all 549 individuals using a Bayesian based method [56] revealed that the 549 individuals collected in four locations likely formed 15 subpopulations. These data suggest that the significant population differentiation is related to not only geographical locations and but also the biological characters of the Asian seabass. Previous tagging and recapture studies showed that juvenile *L. calcarifer* remained resident until sexual maturity at the age of 3–4 years [37,38], and some adult individuals of *L. calcarifer* were resident while others migrated [36,37,38,39]. However, these previous studies did not examine which sex of individuals migrated more. In this study, the clustering analysis based on a Bayesian method clearly demonstrated that more females migrated than males in all four sampling locations. This result suggests that the different disposal of the two sexes may contribute to the population differentiation of *L. calcarifer* in the South China Sea.

Few studies have examined sex-biased dispersal patterns in marine fish species. Female-biased dispersal has been reported previously in a few species such as migratory salmonids [23,24,73]. To our best knowledge, our study is the first study on sex-biased dispersal in a protandrous hermaphroditic marine fish species. In this study, the differences in relatedness (r) and population divergence indices (F_{ST}) estimated using software FSTAT v.2.9.3 were significantly ($P < 0.01$) higher for males than for females in Asian seabass. Furthermore, the results from the comparisons of mean assignment index (mAIC, $P < 0.01$) indicate female-biased dispersal. Although three (i.e. F_{ST} , r and mAIC) of the four genetic parameters estimated using software FSTAT v.2.9.3 [51], provided genetic evidence for female-biased dispersal in Asian seabass, the parameter F_{IS} did not support female-biased dispersal. According to Goudet et al. [59], among the four parameters estimated by software FSTAT v.2.9.3, F_{IS} showed the lowest power in detecting sex-biased dispersal [51], which may explain why the parameter F_{IS} did not support female-biased dispersal. Furthermore, the clustering analysis based on a Bayesian method clearly demonstrated that more females migrated than males in all four sampling locations. Therefore, our molecular data provide clear indirect genetic evidence for female-biased dispersal in Asian seabass. While these results may seem to be straightforward to support female-biased dispersal, it is also possible that protandrous

Table 5. F -statistics, relatedness (r), mean assignment (mAIC) and variance assignment (vAIC) for each sex in Asian seabass.

| Sex | F_{IS} | F_{ST} | r | mAIC | vAIC |
|---------|----------|----------|-------|-------|-------|
| Males | 0.046 | 0.036 | 0.066 | 0.88 | 29.26 |
| Females | 0.035 | 0.023 | 0.044 | -0.89 | 30.37 |
| P | 0.039 | 0.010 | 0.010 | 0.010 | 0.760 |

Significance (P) was assessed using the randomisation method of Goudet et al. (2002).

doi:10.1371/journal.pone.0037976.t005

hermaphroditism in itself generated the observed results of lower differentiation among females than males. If males dispersed before sex change, and males and females dispersed equally, then migrant males would be recruited into the female pool, but there was no recruitment in the opposite direction. Hence, the results may not represent female-biased dispersal but only a feature of the peculiar phenomenon of sex-reversal. However, this possibility can be ruled out, as previous tagging and recapture studies showed that juvenile *L. calcarifer* remained resident until sexual maturity (i.e. until starting sex change) at the age of 3–4 years [37], and during the breeding season, sexually matured individuals migrated to spawning grounds for breeding [35,36,38]. We analyzed the records of long term of tagging and recapture experiments on *L. calcarifer* conducted by Australian scientists in past years. We found that females moved longer distance than males. In 2011, an escape of cultured adult Asian seabass happened in Singapore. Several months after the escape, in a place 20 Km away from the escaping places, fishermen captured large females containing electronic tags, but no males (<http://www.topix.com/forum/city/sweetwater-tn/TKL7F0RQR2GADD7U8>), which may indicate that females moved more than males. Altogether, these data provide strong evidences for female-biased dispersal in Asian seabass, which contradicts our initial hypothesis that in *L. calcarifer*, dispersal of males and females is unbiased.

Inbreeding avoidance is regarded as one of the most important causes of sex-biased dispersal in birds and mammals [3,14,74]. Theory predicts that the sex that suffers from the highest disadvantage of inbreeding should be the dispersing sex [75]. In polygynous and promiscuous mating systems, inbreeding costs are different for males and females. Females invest more on reproduction in these mating systems in comparison to males. By siring a relative's offspring, males do not lose other mating opportunities in polygynous and promiscuous systems, whereas an inbred offspring would rather replace a potentially outbred descendant in females. Inbreeding is much more costly for females than males. Therefore, females should actually be the dispersing sex in polygynous systems (Waser et al. 1986). Sex-biased dispersal is usually sufficient to minimize inbreeding [3,76,77]. In Asian seabass, for males, mating with relatives does not necessarily imply a cost as males can mate repeatedly all year around. The opposite is true for females as they typically reproduce once every three months in Southeast Asia [34]. This asymmetry of costs between the sexes could lead to the evolution of female-biased dispersal in Asian seabass. In addition, the female-biased sex ratio might make it difficult for females to find suitable males for mating to improve their reproductive success if they do not migrate to other places. On the other hand, for the males, mate competition is low in Asian seabass, as at the age of 3–4 years, the sex ratio was female-biased. Therefore, a female-biased dispersal pattern in Asian seabass may also be associated with female mate choice under the condition of low mate competition among males.

According to the resource competition theory (Greenwood 1980), the residing sex is the sex that takes most advantages by staying at its birth site. If males are responsible for defending the breeding territory significantly, then the benefits of philopatry will be greater for males than for females. In Asian seabass, males may enhance their own fitness by defending a territory that can attract large females for mating and provides resources for a large number of offspring. Asian seabass individuals are mostly solitary and may

defend territories near submerged structures that they use as hiding spots [30]. Although currently we do not know exactly which sex acquires and defends a breeding territory, there are some indications that males may defend breeding territories (Mr. Yeo, personal communication). Therefore, it is possible that the female-biased dispersal of Asian seabass is also related to the resource competition among males.

In this study, analyzing the tagging-recapture data released by Australian researchers revealed that the migrating distance was significantly related to the body size in adult *L. calcarifer*. It is generally believed that female-biased dispersal is a cause of female-biased sexual size dimorphisms in fish species [25,27,78,79], as the longer movement of females increases the feeding success, resulting in rapid growth as compared with males. For example, in sablefish females grew larger and moved further away than males [27]. However, in Asian seabass, this may not be the case. Under aquaculture conditions, enough feed was usually given to the young fishes, and at the same age (3–4 years) females usually grow bigger than males. Therefore, feeding resources could not be a determining factor promoting female-biased size dimorphisms in Asian seabass. It is highly possible that at the same age, in wild populations, the bigger size of females of Asian seabass makes them migrate faster than males, which in turn gives females more chances to find and mate suitable males in the populations where the sex ratio is female-biased. Therefore, the female-biased sexual size dimorphisms may be a cause of female-biased movements.

Conclusions

Our genetic data, in combination with data of studies on tagging and recapture published by Australian scientists in the past years [35,37,38,80], provided strong evidences for female-biased gene flow among populations of Asian seabass. We suggest that inbreeding avoidance and female mate choice under the condition of low mate competition among males, as well as male resource competition created a female-biased dispersal. The female-biased sexual size dimorphisms may be a cause of the female-biased movements. The ultimate causes behind sex reversal and sex-biased dispersal and the potential evolutionary implications of female-biased dispersal in protandrous hermaphroditic marine fish species warrant further investigation. Studies using DNA markers, in combination with data from tagging-recapture studies in the hermaphroditic fish species, could enhance our understanding of the dispersal of fish.

Acknowledgments

We thank staffs of Marine Aquaculture Centre, Agri-Food & Veterinary Authority of Singapore for help in the collecting of Asian seabass individuals and Dr Zhu ZY for assisting in the genotyping. We also thank Prof YP Zhang for his critical comments on this manuscript. The English language was kindly checked by our native English speaking colleague May Lee.

Author Contributions

Conceived and designed the experiments: GHY. Performed the experiments: GHY JHX FL GL. Analyzed the data: GHY. Contributed reagents/materials/analysis tools: GHY. Wrote the paper: GHY JHX FL GL.

References

1. Roff DA (2002) Life History Evolution. Sunderland, MA: Sinauer Associates.
2. Avise JC (2004) Molecular markers, natural history and evolution. Sunderland, MA: Sinauer.
3. Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* 28: 1140–1162.

4. Handley IJL, Perrin N (2007) Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* 16: 1559–1578.
5. Pusey A (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology & Evolution* 2: 295–299.
6. Williams DA, Hale AM (2007) Female-biased helping in a cooperatively breeding bird: Female benefits or male costs? *Ethology* 113: 534–542.
7. Johnson M, Gaines M (1990) Evolution of dispersal: theoretical models and empirical tests using birds and mammals. *Annual Review of Ecology and Systematics* 21: 449–480.
8. Austin JD, Davila JA, Lougheed SC, Boag PT (2003) Genetic evidence for female-biased dispersal in the bullfrog, *Rana catesbeiana* (Ranidae). *Molecular Ecology* 12: 3165–3172.
9. Berg EC, Eadie JM, Langen TA, Russell AF (2009) Reverse sex-biased philopatry in a cooperative bird: genetic consequences and a social cause. *Molecular Ecology* 18: 3486–3499.
10. Lebigre C, Alatalo RV, Siitari H (2010) Female-biased dispersal alone can reduce the occurrence of inbreeding in black grouse (*Tetrao tetrix*). *Molecular Ecology* 19: 1929–1939.
11. Hutchings JA, Gerber L (2002) Sex-biased dispersal in a salmonid fish. *Proceedings of the Royal Society of London Series B-Biological Sciences* 269: 2487–2493.
12. Stiver KA, Desjardins JK, Fitzpatrick JL, Neff B, Quinn JS, et al. (2007) Evidence for size and sex-specific dispersal in a cooperatively breeding cichlid fish. *Molecular Ecology* 16: 2974–2984.
13. Johns R, Quiring D, Ostaff D, Bauce E (2010) Intra-tree variation in foliage quality drives the adaptive sex-biased foraging behaviors of a specialist herbivore. *Oecologia* 163: 935–947.
14. Perrin N, Mazalov V (2000) Local competition, inbreeding, and the evolution of sex-biased dispersal. *American Naturalist* 155: 116–127.
15. Ulrich Y, Perrin N, Chapuisat M (2009) Flexible social organization and high incidence of drifting in the sweat bee, *Halictus scabiosae*. *Molecular Ecology* 18: 1791–1800.
16. De Meester N, Bonte D (2010) Information use and density-dependent emigration in an agrobiont spider. *Behavioral Ecology* 21: 992–998.
17. Wauters L, Matthysen E, Dhondt AA (1994) Survival and lifetime reproductive success in dispersing and resident red squirrels. *Behavioral Ecology and Sociobiology* 34: 197–201.
18. Long ES, Diefenbach DR, Rosenberry CS, Wallingford BD (2008) Multiple proximate and ultimate causes of natal dispersal in white-tailed deer. *Behavioral Ecology* 19: 1235–1242.
19. Clutton-Brock T, Sheldon BC (2010) Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology & Evolution* 25: 562–573.
20. Fishbase (2011) FishBase: A Global Information System on Fishes. Available: <http://www.fishbase.org/search.php>. Accessed 2012 Apr 28.
21. Cano JM, Makinen HS, Merila J (2008) Genetic evidence for male-biased dispersal in the three-spined stickleback (*Gasterosteus aculeatus*). *Molecular Ecology* 17: 3234–3242.
22. Knight M, Van Oppen M, Smith H, Rico C, Hewitt G, et al. (1999) Evidence for male-biased dispersal in Lake Malawi cichlids from microsatellites. *Molecular Ecology* 8: 1521–1527.
23. Koizumi I, Yamamoto S, Maekawa K (2006) Female biased migration of stream dwelling Dolly Varden in the Shiisorapuchi River, Hokkaido, Japan. *Journal of Fish Biology* 68: 1513–1529.
24. Tamate T, Maekawa K (2004) Female biased mortality rate and sexual size dimorphism of migratory masu salmon, *Oncorhynchus masou*. *Ecology of Freshwater Fish* 13: 96–103.
25. Andrews AH, Cailliet GM, Coale KH (1999) Age and growth of the Pacific grenadier (*Coryphaenoides acrolepis*) with age estimate validation using an improved radiometric ageing technique. *Canadian Journal of Fisheries and Aquatic Sciences* 56: pp 1339–1350.
26. Ito M, Hattori T, Narimatsu Y, Ueda Y (2008) Marine fisheries stock assessment and evaluation for stock assessment and evaluation for yellow goosefish *Lophius litulon*. *Marine Fisheries Stock Assessment and Evaluation for Japanese Waters: Japanese Waters Fisheries Agency and Fisheries Research Agency of Japan*. pp 774–788.
27. Morita SH, Morita K, Nishimura A (2011) Sex-biased dispersal and growth in sablefish (*Anoplopoma fimbria*) in the northeastern Pacific Ocean. *Environmental Biology of Fishes* DOI: 10.1007/s10641-010-9613-1 (in press).
28. Ward R, Woodwark M, Skibinski D (1994) A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology* 44: 213–232.
29. Waples R (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* 89: 438–450.
30. Russell D, Garrett R (1985) Early life history of barramundi, *Lates calcarifer* (Bloch), in north-eastern Queensland. *Marine and Freshwater Research* 36: 191–201.
31. Moore R (1979) Natural sex inversion in the giant perch (*Lates calcarifer*). *Marine and Freshwater Research* 30: 803–813.
32. Moore R (1982) Spawning and early life history of barramundi, *Lates calcarifer* (Bloch). *Australian Journal of Marine and Freshwater Research* 33: 647–661.
33. Blaber S, Milton D, Salini J (2008) The biology of barramundi (*Lates calcarifer*) in the Fly River system. *Developments in Earth and Environmental Sciences* 9: 411–426.
34. Garcia L (1992) Lunar synchronization of spawning in sea bass, *Lates calcarifer* (Bloch): effect of luteinizing hormone-releasing hormone analogue (LHRHa) treatment. *Journal of Fish Biology* 40: 359–370.
35. Moore R, Reynold L (1982) Migration patterns of barramundi, *Lates calcarifer* (Bloch), in Papua New Guinea. *Marine and Freshwater Research* 33: 671–682.
36. Russell D, Garrett R (1988) Movements of juvenile barramundi, *Lates calcarifer* (Bloch), in north-eastern Queensland. *Marine and Freshwater Research* 39: 117–123.
37. Davis T (1982) Maturity and sexuality in barramundi, *Lates calcarifer* (Bloch), in the Northern Territory and south-eastern Gulf of Carpentaria. *Marine and Freshwater Research* 33: 529–545.
38. Davis T (1986) Migration patterns in barramundi, *Lates calcarifer* (Bloch), in Van Diemen Gulf, Australia, with estimates of fishing mortality in specific areas. *Fisheries Research* 4: 243–258.
39. Milton DA, Chenery SR (2005) Movement patterns of barramundi *Lates calcarifer*, inferred from $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr/Ca ratios in otoliths, indicate non-participation in spawning. *Marine Ecology Progress Series* 301: 279–291.
40. McCulloch M, Cappo M, Aumend J, Muller W (2005) Tracing the life history of individual barramundi using laser ablation MC-ICP-MS Sr-isotopic and Sr/Ba ratios in otoliths. *Marine and Freshwater Research* 56: 637–644.
41. Croft DP, Albanese B, Arrowsmith BJ, Botham M, Webster M, et al. (2003) Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia* 137: 62–68.
42. Salini J, Shaklee J (1988) Genetic structure of barramundi (*Lates calcarifer*) stocks from northern Australia. *Marine and Freshwater Research* 39: 317–329.
43. McDougall A (2004) Assessing the use of sectioned otoliths and other methods to determine the age of the centropomid fish, barramundi (*Lates calcarifer*) (Bloch), using known-age fish. *Fisheries Research* 67: 129–141.
44. Yue GH, Orban L (2005) A simple and affordable method for high throughput DNA extraction from animal tissues for PCR. *Electrophoresis* 26: 3081–3083.
45. Wang CM, Bai ZY, He XP, Lin G, Xia JH, et al. (2011) A high-resolution linkage map for comparative genome analysis and QTL fine mapping in Asian seabass, *Lates calcarifer*. *BMC Genomics* 12: 174.
46. Yue GH, Li Y, Chao TM, Chou R, Orban L (2002) Novel microsatellites from Asian sea bass (*Lates calcarifer*) and their application to broodstock analysis. *Marine Biotechnology* 4: 503–511.
47. Zhu ZY, Wang CM, Lo LC, Lin G, Feng F, et al. (2010) A standard panel of microsatellites for Asian seabass (*Lates calcarifer*). *Animal Genetics* 41: 208–212.
48. Wahlund W (1928) Zusammensetzung von population und korrelationserscheinung vom standpunkt der vererbungslehre aus betrachtet. *Hereditas* 11: 65–106.
49. van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.
50. Lewis PO, Zaykin D (2000) Genetic Data Analysis (GDA). Available at <http://hydrodictyon.ceb.uconn.edu/people/plewis/software.php>. Accessed 2012 April 28.
51. Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (v. 2.9.3). Available: <http://www2.unil.ch/popgen/softwares/fstat.htm>. Accessed 2012 Apr 28.
52. Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics Selection Evolution* 131: 479–491.
53. Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 1: 47–50.
54. Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. *BMC Genetics* 6: 13.
55. Pritchard JK, Wen XQ, Falush D (2010) Structure 2.3.3. Available: <http://pritch.bsd.uchicago.edu/structure.html>. Accessed 2012 Apr 28.
56. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945.
57. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611–2620.
58. Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9: 1322–1332.
59. Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using biparentally inherited genetic markers. *Molecular Ecology* 11: 1103–1114.
60. Weir B, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.
61. Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43: 258–275.
62. Warner RR (1988) Sex change in fishes: hypotheses, evidence, and objections. *Environmental Biology of Fishes* 22: 81–90.
63. Avise J, Mank J (2009) Evolutionary perspectives on hermaphroditism in fishes. *Sexual Development* 3: 152–163.
64. Charnov EL (1982) The theory of sex allocation. New Jersey, USA: Princeton University Press.
65. Devlin R, Nagahama Y (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* 208: 191–364.

66. Ross RM (1990) The evolution of sex-change mechanisms in fishes. *Environmental Biology of Fishes* 29: 81–93.
67. Shapiro DY (1987) Differentiation and evolution of sex change in fishes. *Bioscience* 37: 490–497.
68. Chopelot J, Waples RS, Mariani S (2009) Sex change and the genetic structure of marine fish populations. *Fish and Fisheries* 10: 329–343.
69. Mank J, Avise J (2009) Evolutionary diversity and turn-over of sex determination in teleost fishes. *Sexual Development* 3: 60–67.
70. Chenoweth S, Hughes J, Keenan C, Lavery S (1998) Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer* (Australian barramundi). *Heredity* 80: 187–197.
71. Keenan CP (1994) Recent evolution of population structure in Australian barramundi, *Lates calcarifer* (Bloch): an example of isolation by distance in one dimension. *Marine and Freshwater Research* 45: 1123–1148.
72. Shaklee JB, Salini J, Garrett RN (1993) Electrophoretic characterization of multiple genetic stocks of barramundi perch in Queensland, Australia. *Transactions of the American Fisheries Society* 122: 685–701.
73. Jonsson B, Jonsson N (1993) Partial migration: niche shift versus sexual maturation in fishes. *Reviews in Fish Biology and Fisheries* 3: 348–365.
74. Costello CM, Creel SR, Kalinowski ST, Vu NV, Quigley HB (2008) Sex-biased natal dispersal and inbreeding avoidance in American black bears as revealed by spatial genetic analyses. *Molecular Ecology* 17: 4713–4723.
75. Perrin N, Mazalov V (1999) Dispersal and inbreeding avoidance. *American Naturalist* 154: 282–292.
76. Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society B: Biological Sciences* 264: 127–132.
77. Randall DA, Pollinger JP, Wayne RK, Tallents LA, Johnson PJ, et al. (2007) Inbreeding is reduced by female-biased dispersal and mating behavior in Ethiopian wolves. *Behavioral Ecology* 18: 579–589.
78. Kimley A (1987) The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. *Environmental Biology of Fishes* 18: 27–40.
79. Itaya K, Fujioka T (2006) The growth of pointhead flounder *Hippoglossoides pinetorum* in Ishikari Bay, Hokkaido. *Fisheries Sciences Report* 70: 89–94.
80. Bird C (1992) Trial barramundi trapping and tagging program on the Ord River. Bernard Bowen Fisheries Research Institute, Western Australian Marine Research Laboratories: Perth.