Genetic analysis of threatened Australian grayling *Prototroctes maraena* suggests recruitment to coastal rivers from an unstructured marine larval source population

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Population genetic variation of Australian grayling *Prototroctes maraena* was examined to determine whether the dispersal strategy of this amphidromous species favours retention of larvae and juveniles in close proximity to their natal river, or mixing of populations via marine dispersal. Variation in microsatellite and mitochondrial DNA markers was unstructured and differentiation was indistinguishable from zero across four coastal rivers spanning approximately one-quarter of the continental range of the species. This result indicates that the marine larval and juvenile phase probably facilitates extensive gene flow among coastal rivers and agrees with a previous analysis of otolith chemistry that suggested larvae probably move into the sea rather than remain in estuaries. It appears likely that the dispersal strategy of *P. maraena* would enable recolonization of rivers that experience localized extinction provided that connectivity between freshwater habitats and the sea is sufficient to permit migration and that enough source populations remain intact to support viability of the wider population.

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INTRODUCTION

Diadromy is a life-history character of aquatic organisms that entails bidirectional migration between freshwater and marine environments during the life cycle of an individual. Variation in the direction and developmental stage at which migration occurs gives rise to several recognized sub-categories of diadromy including amphidromy, where reproduction and development both occur in fresh water, but individuals spend a period of their juvenile phase in the sea (McDowall, 2007, 2008). In evolutionary terms, diadromous migrations are thought to increase fitness by allowing species to take advantage of variation in habitat productivity between freshwater and marine habitats during particular stages of the life history (Gross *et al.*, 1988), and by providing a dispersal mechanism that facilitates habitat recolonization and replenishment of local populations (McDowall, 2008, 2010).

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1 The opportunity for connectivity *via* the sea leads to the expectation that diadromy
2 promotes broad geographical ranges and inhibits the development of genetic struc-
3 turing, even among geographically isolated populations (McDowall, 2001a). Genetic
4 data are generally consistent with this pattern because both marine and anadromous
5 fishes exhibit high levels of genetic variation and low population structuring relative
6 to freshwater species (Ward *et al.*, 1994; DeWoody & Avise, 2000). Behavioural traits
7 of some diadromous fishes tend to counteract the potential homogenizing influence
8 of marine dispersal. In particular, philopatry, or the tendency for individuals to repro-
9 duce at the same site as their parents, is common among anadromous fishes in the
10 form of 'homing' to the natal stream (McDowall, 2001b; Hendry *et al.*, 2004). Hom-
11 ing behaviour may contribute to remarkably fine-scale structuring of populations.
12 For example, despite their close proximity (<1 km) in Little Togiak Lake, Alaska,
13 U.S.A., spawning groups of sockeye salmon *Oncorhynchus nerka* (Walbaum) are
14 sufficiently differentiated that straying individuals can be genetically distinguished
15 (Lin *et al.*, 2008).

16 Homing is not known in amphidromous species and seems unlikely to be a pro-
17 fitable strategy given that juvenile stages would be required to undertake the precise
18 transition from the sea back to the natal freshwater spawning area (McDowall, 2010).
19 Rather, amphidromy is regarded primarily as a dispersal strategy: for example, the
20 colonization of ephemeral freshwater habitats, such as those of tropical islands and
21 archipelagos where freshwater supply is unreliable leading to frequent localized
22 extinction (McDowall, 2007, 2010; Lord *et al.*, 2010). Island-scale genetic structure
23 is absent for most amphidromous species examined to date, indicating that larval
24 movement through the sea promotes gene flow (Ward *et al.*, 1994; Berrebi *et al.*,
25 2005; Yamamoto *et al.*, 2007; Cook *et al.*, 2009). Lack of genetic structure may even
26 extend over thousands of kilometres in some cases (Hoareau *et al.*, 2007; Crandall
27 *et al.*, 2010). Conversely, migratory behaviour can be lost by some populations of an
28 amphidromous species after colonization of a freshwater system (Michel *et al.*, 2008),
29 or populations can exhibit facultative amphidromy where some individuals migrate
30 to the sea while others remain in fresh water (McDowall, 2001a; Crook *et al.*, 2008).
31 While amphidromy is often seen as an adaptation for dispersal and colonization in
32 unstable environments, it is possible that a reduction in dispersal might result from
33 selection on the behaviour of amphidromous fish larvae living in continental river sys-
34 tems that have stable and predictable river flows. Stable connectivity between marine
35 and freshwater habitats could favour retention of the marine larval population close to
36 the river mouth or in the estuary, thereby allowing larvae and juveniles to take advan-
37 tage of increased marine productivity while reducing the risk of dispersal into ocean
38 currents. The prediction that juvenile amphidromous fishes might behave in this way
39 under certain conditions is given the term 'dispersal-limitation strategy'. The return
40 of larval recruits to their source catchment in this scenario could lead to genetic struc-
41 turing of populations similar to that observed in salmonids, as opposed to the absence
42 of detectable structure observed for island populations of amphidromous fishes.

43 The Australian grayling *Prototroctes maraena* Günther (Retropinnidae) is a small
44 to medium-sized (up to 300 mm total length, L_T) amphidromous fish, once com-
45 mon in coastal rivers of south-eastern Australia (Berra, 1982). Its extinct congener,
46 *Prototroctes oxyrhynchus* Günther was last seen alive in New Zealand in the 1920s
47 (McDowall, 1996). Due to declines in abundance throughout most of its range includ-
48 ing Victoria, Tasmania and southern New South Wales, *P. maraena* is listed as

Vulnerable under the Australian Government Environment Protection and Biodiversity Conservation Act 1999. The life history of *P. maraena* involves spawning in fresh water from late summer to winter followed by larval transport to the marine environment where development occurs for *c.* 6 months before the return migration to fresh water in spring (Berra, 1982). Thereafter, adults remain in fresh water where some individuals may survive up to 5 years, although most spawn only once and die after 2 years (Berra, 1982).

A recent analysis of Sr:Ca and Ba:Ca ratios in otoliths of *P. maraena* from several rivers in Victoria showed that amphidromy may be an obligate strategy for this species (Crook *et al.*, 2006). An outstanding question arising from this work is whether populations in coastal rivers of Victoria constitute a single stock that is maintained by among-catchment dispersal during the obligatory marine juvenile phase. Alternatively, if a dispersal-limitation strategy is employed as outlined above, then stock structure may exist between catchments with important conservation implications for the species. In particular, a relatively short life span means that localized extinction may be inevitable if recruitment were disrupted for several consecutive years by impeding connectivity between freshwater spawning habitat and the sea. After re-establishment of connectivity, recolonization would be relatively slow if dispersal between catchments was limited.

Several anthropogenic factors are thought to impact upon *P. maraena* populations, including instream barriers such as dams and weirs, sedimentation of habitat due to erosion of degraded catchments, loss of critical flows due to water abstraction and the presence of introduced piscivorous fishes (Wager & Jackson, 1993; Backhouse *et al.*, 2008). Additionally, many of the river systems within the range of the species have sand bar estuary entrances that periodically close over during periods of low river discharge, potentially limiting larval access to the sea and recolonization by juveniles back into the rivers upstream. Future climate change predictions suggest that south-eastern Australia is likely to experience major declines in river discharge (Jones & Durack, 2005), thus increasing the potential impacts of estuary closures on *P. maraena* recruitment into the future. Knowledge of how threatening processes interact with the species' dispersal strategy would be informative for understanding the reasons for the species' decline and the management interventions most likely to improve its status. Effective conservation management of *P. maraena* therefore requires knowledge of the degree of population structure that exists across coastal catchments. The aim of this study was to estimate the level of population genetic structure among Victorian coastal river populations of *P. maraena* using microsatellite and mitochondrial DNA (mtDNA) markers and to integrate this knowledge into a life-history model for the species that provides a sound basis for its management and conservation.

MATERIALS AND METHODS

FISH COLLECTION AND STUDY SITES

Samples of 152 *P. maraena* were collected using a bank-mounted electrofishing unit from the freshwater reaches of four rivers in coastal Victoria (Thomson River, December 2006, *n* = 30, 120–200 mm total length, *L*_T; Tarwin River, December 2006, *n* = 22, no *L*_T data; Bunyip River, December 2006, *n* = 46, 53–157 mm *L*_T; Barwon River, May 1997 to June 1998,

FIG. 1. Map of study area showing locations of the Thomson, Tarwin, Bunyip and Barwon Rivers. Grey shading on inset map shows the approximate geographic range of *Prototroctes maraena*.

$n = 54$, 76–241 mm L_T) (Fig. 1). This sampling area covers *c.* 400 km of coastline and is at the centre of the geographic range of this species. A small (2 mm²) section of the pelvic fin was excised and stored in 100% ethanol and the fish was quickly returned to its point of capture.

MOLECULAR METHODS

Genomic DNA was extracted from fin tissue using a standard phenol–chloroform extraction method with ethanol precipitation (Sambrook *et al.*, 1989). A 950 bp fragment of mitochondrial DNA spanning ATP synthase subunit 8 and ATP synthase subunit 6 genes was amplified by polymerase chain reaction (PCR) using primers ATP8.2L8331 and COIII2H9236 (S. McCafferty, unpubl. data; <http://striweb.si.edu/bermingham/research/primers/>) and the protocol described by Woods *et al.* (2010). The PCR product was enzymatically purified then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems; www.appliedbiosystems.com). Sequencing was performed on a 3130xl Genetic Analyser (Applied Biosystems) at Griffith University DNA sequencing facility.

Sixteen microsatellite loci developed for Australian smelt *Retropinna semoni* (Weber) (Retropinnidae) were screened on a sub-set of *P. maraena* samples. Five published loci (Hillyer *et al.*, 2006) and 11 unpublished primer sets (R. Woods, unpubl. data) were tested. Four loci were polymorphic and a 20 mer oligonucleotide tail was added to the 5'-end of each forward primer to facilitate fluorescent labelling (Real *et al.*, 2009) (Table I). PCR conditions were: 1× reaction buffer, 1.5 mM MgCl₂, 0.1 μM tailed forward primer, 0.4 μM reverse primer, 0.4 μM fluorescent-labelled tag primer, 0.2 μM dNTPs and 0.2 U *Taq* polymerase. Cycling conditions for PCR included 35 cycles of denaturation (95° C for 30 s), annealing (58° C for 30 s) and extension (72° C for 30 s), followed by a final extension of 40 min at 72° C. Fragment analysis was conducted on a 3130 Genetic Analyser (Applied Biosystems) and genotypes were scored using GeneMapper v4.0 software (Applied Biosystems). A second round of amplification and genotyping of all samples suggested a low incidence of genotyping error (<3.8%). Individuals with mismatched genotypes between the two runs were subjected to a third round of amplification and genotyping to confirm heterozygotes.

DATA ANALYSIS

Microsatellite genotype frequencies were checked for the presence of null alleles, large allele dropout and stuttering artefacts using Micro-Checker v2.2.3 (Van Oosterhout *et al.*, 2004). Tests for linkage disequilibrium (LD) and departures of genotypic proportions expected under Hardy–Weinberg Equilibrium (HWE) were calculated with exact tests for each locus

TABLE I. Primers used for PCR amplification of microsatellite loci in *Prototroctes maraena*

Locus	Sequence 5'–3'	5' tail*	Repeat motif	Allele size range (bp)	Source
sm-G7	GAACATCCTTCCAGTCAAGAAC	1	(GCT) ₅ (GTT) ₁₃	250–298	R. Woods, unpub. data
sm-E8	CGGATTGGCAGCAGTATC CGGAAGTGGAGGTTTCAGCA	1	(CAG) ₆ (CAA) ₁₆ (CAG) ₅	116–171	R. Woods, unpub. data
sm-H2	GACGAGTTTCATCACGGGAAA TGAGAAAGGTAGCCTGTCTCG	3	(GTT) ₉	249–284	R. Woods, unpub. data
sm-77	CCATATAAAGACGATCAGGGTCC AGGAGAAAGTTGCCTCACGGTG AGAAAGGAATGACAGCTCATTC	4	(CT) ₁₀ TT(CT) ₂	171–197	Hillyer <i>et al.</i> (2006)

*See Real *et al.* (2009).

and population combination using default settings in GENEPOP v4.0 (Rousset, 2008). Microsatellite allele frequencies, heterozygosity at each locus and allelic richness (A_R) for each locus and population combination were estimated using MSA v4.05 (Dieringer & Schlötterer, 2003). Diversity statistics and neutrality tests (Tajima's D and Fu's F_s) for the mtDNA data set were calculated using DNAsp v5.0 (Librado & Rozas, 2009) and the genealogical relationship of the haplotypes was estimated using statistical parsimony in TCS v1.21 (Clement *et al.*, 2000). Geographic structuring of genetic variation was assessed in ARLEQUIN v3.1 (Excoffier *et al.*, 2005) by estimation of the fixation index F_{ST} using an analysis of molecular variance framework. Estimates of F_{ST} were obtained for each locus separately and as a weighted average over the four microsatellite loci. Statistical significance of F_{ST} estimates was determined by 1000 permutations of individuals among populations.

The power to detect specified levels of population structure given the sampling effort was assessed using Powsim v4.0 (Ryman & Palm, 2006). F_{ST} values specified in this analysis were calculated from Wright's (1943) infinite island-model expectation using effective population size (N_e) estimated using Migrate v3.1.6 (Beerli, 2006) and a range of migration rates (m ; $m = 0.2, 0.1, 0.05, 0.01, 0.005, 0.0025, 0.001$ and 0.0005).

$$F_{STmsat} = (1 - m)^2 [2N_e - (2N_e - 1)(1 - m)^2]^{-1} \quad (1)$$

$$F_{STmtDNA} = (1 - m)^2 [0.5N_e - (0.5N_e - 1)(1 - m)^2]^{-1} \quad (2)$$

Equation (1) was used to determine F_{ST} values for microsatellite data and equation (2) was used to determine corresponding values for mtDNA data (Larsson *et al.*, 2009). The theoretical threshold migration rate corresponding to demographic independence of populations is $m = 0.1$ (Hastings, 1993; Palsbøll *et al.*, 2007). Power was estimated as the proportion of significant outcomes (*i.e.* rejecting the null hypothesis of no allele frequency difference among populations using a χ^2 contingency test at $P < 0.05$), based on 1000 simulations for each m value, incorporating observed allele frequencies and sample sizes for each locus.

An N_e estimate required for calculation of F_{ST} values used in the power analysis was obtained using Migrate v3.1.6 (Beerli, 2006). A single estimate of N_e was obtained for the microsatellite data set by assigning the four river samples to a single population using the 'population relabel' command. Microsatellite mutation was modelled using the Brownian motion approximation of the stepwise mutation model and the Bayesian implementation of Migrate involved an MCMC search of 50 000 burnin steps followed by 1 000 000 steps with parameters recorded every 50 steps; a static heating scheme of four chains with temperatures (1.0, 1.25, 3.0 and 1000.0); uniform prior on theta (minimum: 0.0, maximum: 30.0, delta: 3.0); uniform prior on migration (minimum: 0.0, maximum: 100.0, delta: 10.0). The analysis was repeated seven times to assess the behaviour of the MCMC runs and resulting parameter estimates. Each run used a different random number seed, random starting tree and parameter values starting from F_{ST} -based estimates. The posterior distribution of the parameter value was then compared across runs to ensure overlap of 95% CI and the coefficient of variation (c.v.) of the modal estimate was calculated. Following recommendations in the Migrate manual, the analysis was run long enough to achieve an effective sample size of > 1000 for the genealogy likelihood. Effective sample size for the theta parameter was > 5000 in all runs. After observing similar posterior distributions for the theta parameter across replicate runs and a low c.v. (0.15), the parameter estimate was reported from the run achieving the highest effective sample size. The population-scaled mutation rate (theta) was converted to N_e using the standard assumption that microsatellite mutation rate (μ) equals 5.0×10^{-4} per site per generation (Estoup & Angers, 1998) and by substituting these values into the equation $N_e = \Theta (4\mu)^{-1}$.

The potential for the existence of rare immigrant individuals originating from genetically differentiated source populations not sampled in the present study was tested using individual assignment of microsatellite genotypes. The individual assignment approach estimates the probability that each individual belongs to a single population defined by simulation of multilocus genotypes conditioned on allele frequencies and gametic disequilibrium present in the observed data minus the focal individual. The observed data in this case consist of the microsatellite genotypes of all sampled individuals disregarding sampling units (*i.e.* four

1 rivers). The assignment probability calculation of Rannala & Mountain (1997) and simulation
 2 method of Paetkau *et al.* (2004) were implemented in GENECLASS v2.0 (Piry *et al.*, 2004).
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6 RESULTS

7 GENETIC DIVERSITY

8 Null alleles, large allele dropout and stuttering artefacts were not detected in the
 9 microsatellite data set. No cases of significant deviation from HWE were observed
 10 at any microsatellite locus in any population nor was there evidence for LD between
 11 loci (Table II). From a total of 119 genotyped individuals, 45 alleles were identified
 12 and the number of alleles per locus ranged from six to 18 (Table II). Allelic richness
 13
 14

15 TABLE II. Microsatellite diversity in four river samples of *Prototroctes maraena*. *P*-value
 16 corresponds to exact test for Hardy–Weinberg equilibrium
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18 Locus	Barwon River	Bunyip River	Tarwin River	Thomson River
19 <i>sm-G7</i>				
20 <i>n</i>	51	21	22	23
21 <i>A</i>	13	12	11	13
22 <i>A_R</i>	8.924	10.584	9.797	10.607
23 <i>H_E</i>	0.835	0.854	0.874	0.853
24 <i>H_O</i>	0.765	0.810	0.864	0.870
25 <i>P</i>	>0.05	>0.05	>0.05	>0.05
26 <i>sm-E8</i>				
27 <i>n</i>	45	20	17	15
28 <i>A</i>	13	10	9	9
29 <i>A_R</i>	9.400	8.872	8.636	9.000
30 <i>H_E</i>	0.867	0.829	0.847	0.853
31 <i>H_O</i>	0.933	0.900	0.941	1.000
32 <i>P</i>	>0.05	>0.05	>0.05	>0.05
33 <i>sm-H2</i>				
34 <i>n</i>	47	18	21	20
35 <i>A</i>	6	6	6	5
36 <i>A_R</i>	4.382	5.476	5.047	4.430
37 <i>H_E</i>	0.370	0.432	0.344	0.318
38 <i>H_O</i>	0.383	0.500	0.381	0.300
39 <i>P</i>	>0.05	>0.05	>0.05	>0.05
40 <i>sm-77</i>				
41 <i>n</i>	52	22	22	23
42 <i>A</i>	5	5	4	4
43 <i>A_R</i>	4.221	4.336	3.682	3.641
44 <i>H_E</i>	0.626	0.476	0.603	0.457
45 <i>H_O</i>	0.615	0.409	0.455	0.304
46 <i>P</i>	>0.05	>0.05	>0.05	>0.05

47 *n*, sample size; *A*, number of alleles ; *A_R*, allelic richness; *H_E*, expected heterozygosity; *H_O*, observed
 48 heterozygosity (*H_O*).

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FIG. 2. Mitochondrial DNA haplotype network. Numbers refer to haplotype codes in Table IV. Each pie chart represents a haplotype partitioned into frequency of occurrence in the four rivers sampled: Barwon River (■), Bunyip River (■), Tarwin River (□) and Thomson River (□). The size of each haplotype corresponds to the frequency of the haplotype within the entire sample as indicated in the open circles.

values were similar across the four populations for each locus (Table II). The edited alignment of 97 *P. maraena* mtDNA sequences was 740 bp in length with 19 variable positions and 12 haplotypes (Fig. 2). Tests for deviation from neutrality (Tajima's *D*, Fu's *F_s*) were close to zero and non-significant in each population and overall (Table III). The number of haplotypes sampled in each population ranged from seven to nine and both haplotype and nucleotide diversities were similar in each population (Table III). The estimated genealogical relationship among the 12 haplotypes is shown in Fig. 2. The most internal haplotype in the network (Hap 1; Fig. 2) comprised 52% of the total sample and had the highest frequency of occurrence in every population (Table IV).

GEOGRAPHIC POPULATION STRUCTURE

Geographic partitioning of genetic variation into the four coastal river samples was either extremely low or zero for microsatellites and mtDNA (Table V). The

TABLE III. Mitochondrial DNA diversity in four river samples of *Prototroctes maraena*. Values in parentheses represent 1 s.d.

	Barwon River	Bunyip River	Tarwin River	Thomson River	Overall
<i>n</i>	22	28	22	25	97
<i>H</i>	7	7	8	9	12
<i>h</i>	0.727 (0.088)	0.611 (0.095)	0.740 (0.092)	0.793 (0.075)	0.7092 (0.046)
<i>π</i>	0.004 (0.002)	0.003 (0.002)	0.004 (0.002)	0.004 (0.002)	0.004 (0.002)
Tajima's <i>D</i>	−0.605	−0.926	−1.211	−0.956	−0.779
Fu's <i>F_s</i>	0.177	0.028	−0.694	−1.513	−0.813

n, sample size; *H*, number of haplotypes; *h*, haplotype diversity; *π*; nucleotide diversity.

TABLE IV. Mitochondrial DNA haplotype codes corresponding to haplotype network (Fig. 2). GenBank accession numbers and the frequency of each haplotype sampled in four river populations of *Prototroctes maraena* are given

Haplotype code	GenBank accession number	Barwon River	Bunyip River	Tarwin River	Thomson River	Total
1	HQ285982	11	17	11	11	50
2	HQ285983	2	1	2	0	5
3	HQ285984	4	1	3	2	10
4	HQ285985	1	1	1	3	6
5	HQ285986	1	1	0	0	2
6	HQ285987	2	0	2	2	6
7	HQ285988	1	5	1	2	9
8	HQ285989	0	2	1	1	4
9	HQ285990	0	0	0	2	2
10	HQ285991	0	0	0	1	1
11	HQ285992	0	0	0	1	1
12	HQ285993	0	0	1	0	1
Total		22	28	22	25	97

global estimate of F_{ST} for microsatellite loci was negative and the estimate for each independent locus was negative with the exception of sm-77 which returned a non-significant estimate of 0.00652 (Table V). All pair-wise F_{ST} tests were non-significant and the estimates were either negative or low ($F_{ST} < 0.003$). Negative estimates of F_{ST} are indicative of an absence of population structure and should be interpreted as equal to zero (Meirmans, 2006). In effect, the level of genetic variation detected within each river population of *P. maraena* was very close to, or indistinguishable from, the total amount of variation sampled. Based on these results, no detectable geographic structuring of *P. maraena* populations exists among the coastal rivers of Victoria sampled in this study.

STATISTICAL POWER ANALYSIS

As the level of population structure obtained from the present sampling design was not significantly different from zero, a retrospective assessment of power was conducted to determine what minimal level of structure (*i.e.* F_{ST}) could be confidently

TABLE V. Estimates of genetic structure among the four river samples for each locus and the average over four microsatellite loci

Locus	F_{ST}	P
sm-G7	-0.00010	>0.05
sm-E8	-0.00084	>0.05
sm-H2	-0.00987	>0.05
sm-77	0.00652	>0.05
Microsatellite average	-0.00026	>0.05
mtDNA	-0.01418	>0.05

distinguished from the random expectation using the sample sizes, number of loci, allelic variation and marker inheritance of this study. The modal estimate of theta found by Migrate v3.1.6 was 7.838 (95% CL 4.125, 8.925) yielding an N_e estimate of 3919 (95% CL 2063, 4463). Substitution of this value and specified m into equation (1) produced a range of F_{ST} values appropriate to diploid microsatellite loci under Wright's infinite-island model; substitution into equation (2) produced the corresponding range of F_{ST} values for a haploid, maternally transmitted locus (Larsson *et al.*, 2009). Simulations conducted using Powsim v4.0 showed that migration would need to be $m \leq 0.005$ before a high probability of rejecting the null hypothesis of no population structure when its false was achieved (Fig. 3). In the present case, for the infinite-island model at equilibrium, a migration rate of $m = 0.005$ corresponds to an F_{ST} value of *c.* 0.013 for nuclear loci and *c.* 0.048 for mtDNA. A threshold migration rate expected to produce demographic independence ($m = 0.1$) corresponds to F_{ST} values of *c.* 0.00054 and *c.* 0.0022 for nuclear and mtDNA loci, respectively. Population structure at this rate of migration would be undetectable using the sampling effort employed here, as power at this level of differentiation merely approaches the type I error rate of 0.05 (Fig. 3).

CRYPTIC POPULATION STRUCTURE

The potential to detect rare immigrants originating from a genetically distinct but unsampled source population was tested using an individual assignment approach. A population exclusion test conducted in GENECLASS v2.0 found that 100% of the 119 observed likelihood scores were within the 99% CI of the likelihood score distribution simulated using the method of Paetkau *et al.* (2004). In other words, no sampled individual could be excluded as a member of a single population whose allele frequencies were defined by pooling all of the 119 multilocus genotypes sampled across the four rivers.

FIG. 3. Power test for microsatellite (—□—) and mtDNA (---○---) loci. Migration rates (m) of 0.2, 0.1, 0.05, 0.01, 0.005, 0.0025, 0.001 and 0.0005 were used to obtain corresponding F_{ST} values for each data set and simulations were conducted using observed allele frequencies and sample sizes for each locus.

DISCUSSION

Genetic variation of *P. maraena* sampled from four coastal rivers spanning approximately one-quarter of the species' continental range was unstructured. Based on these data, the hypothesis that a single stock exists in coastal Victoria cannot be rejected. It is likely that gene flow among catchments mediated by dispersal of larvae and juveniles during the marine phase of the life cycle is responsible for this pattern. Consequently there is no evidence to support a dispersal-limitation strategy that might develop if larvae and juveniles remained in close proximity to the natal river to minimize oceanic dispersal. This result agrees with a recent study of otolith chemistry which showed that larval and juvenile *P. maraena* probably enter a relatively homogenous chemical environment such as the sea, rather than remaining in estuaries with distinct chemical signatures (Crook *et al.*, 2006). Previous studies of amphidromous fishes inhabiting islands have also found low levels of population structure that are often indistinguishable from zero (Berrebi *et al.*, 2005; Hoareau *et al.*, 2007; Yamamoto *et al.*, 2007).

Power analyses demonstrated that migration rate would need to be relatively low ($m \leq 0.005$) for this study to detect significant differentiation. Therefore, it cannot be ruled out that a lower level of population structure does truly exist which is inaccessible to the present analysis. Increasing power to detect lower levels of structure could be achieved by increasing sample sizes and the number of assayed nuclear loci (Ryman *et al.*, 2006). It is questionable, however, as to whether any useful biological information relevant to management could be gained from more precise estimation of very low F_{ST} values (Waples, 1998). On the other hand, the ability to discriminate between source and sink populations *via* estimation of asymmetrical migration rates could provide useful guidance for targeting management efforts such as habitat rehabilitation. This goal may require both an increase in statistical power of the genetic analysis as well as complementary information on individual movement from environmental sources such as otolith microchemistry (Selkoe *et al.*, 2008; Woods *et al.*, 2010). All of the available microsatellite loci developed for a related retropinnid species were tested, so increasing the number of loci in future will require development of new microsatellite markers for this species. It should also be noted that the low abundance of this threatened species makes it difficult to achieve large sample sizes and limits the scope for research that requires destructive sampling (*e.g.* otolith analysis).

Although the number of microsatellite loci used here was limited to four, these provided greater resolution for detecting low levels of differentiation than the mtDNA locus under the conditions of this study. This result is expected whenever differentiation is low because of high migration and effective size (Larsson *et al.*, 2009). These conditions are likely to characterize many amphidromous species, so a more refined understanding of population genetic structure relevant to amphidromous life histories will best be achieved using multiple nuclear markers rather than mtDNA. The mean $\pm$ s.d. F_{ST} estimate for anadromous fishes exhibiting homing behaviour is 0.06 ± 0.036 (Bradbury & Bentzen, 2007). Values of this magnitude would be detected with high confidence in the present sampling scheme, so these results indicate that homing is not a behavioural strategy used by *P. maraena*.

The present study adds to the current life-history model for *P. maraena* by showing that at least moderately high dispersal among coastal rivers occurs during the

marine larval and juvenile phases. The distribution of *P. maraena* extends beyond the limits of the present study into eastern South Australia, southern New South Wales and around the coast of Tasmania: it is possible that future studies may identify stock structure in these areas, although no genetically differentiated rare immigrants that might provide a clue to the existence of additional stocks were identified. The dispersive capacity of *P. maraena* means that recolonization of freshwater habitats may be expected to occur naturally after localized extinctions, provided that connectivity to the sea is maintained and sufficient source populations remain viable throughout the range of the species. This suggests that rehabilitation of degraded coastal rivers has the potential to greatly benefit *P. maraena* by encouraging the re-establishment of source populations in regions where the species has declined. McDowall (1999) suggested that the extinction of *P. oxyrhynchus* might have been due to populations in highly productive habitats ('sources') losing recruits to unproductive habitats ('sinks'), with a reduction in the ratio of source to sink populations due to anthropogenic effects gradually forcing the species onto a trajectory towards extinction. Given these findings, such a hypothesis has great relevance to *P. maraena* and demonstrates the importance of protecting the few rivers known to support breeding populations of the species. Such populations are the source of recruits to other river systems and are critical to the long-term viability of *P. maraena* across its range.

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