Molecular Ecology (2005) **14**, 1367–1375 doi: 10.1111/j.1365-294X.2005.02538.x

Genetic structure in the coral-reef-associated Banggai cardinalfish, *Pterapogon kauderni*

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Abstract

In this study, we used 11 polymorphic microsatellite loci to show that oceanic distances as small as 2–5 km are sufficient to produce high levels of population genetic structure (multilocus F_{ST} as high as 0.22) in the Banggai cardinalfish (*Pterapogon kauderni*), a heavily exploited reef fish lacking a pelagic larval dispersal phase. Global F_{ST} among all populations, separated by a maximum distance of 203 km, was 0.18 (R_{ST} = 0.35). Moreover, two lines of evidence suggest that estimates of F_{ST} may actually underestimate the true level of genetic structure. First, within-locus F_{ST} values were consistently close to the theoretical maximum set by the average within-population heterozygosity. Second, the allele size permutation test showed that R_{ST} values were significantly larger than F_{ST} values, indicating that populations have been isolated long enough for mutation to have played a role in generating allelic variation among populations. The high level of microspatial structure observed in this marine fish indicates that life history traits such as lack of pelagic larval phase and a good homing ability do indeed play a role in shaping population genetic structure in the marine realm.

Keywords: allele size permutation test, Banggai cardinalfish, coral reef, fish, population genetic structure, *Pterapogon kauderni*

Received 14 November 2004; revision accepted 11 February 2005

Introduction

Coral reef ecosystems are ecologically diverse and a major focus of marine conservation, so they represent an excellent system in which to study marine population structure. Several recent studies have investigated population genetic structure to elucidate current and historical patterns of dispersal in various organisms associated with coral reefs (e.g. Lavery *et al*. 1996; Williams & Benzie 1998; Ayre & Hughes 2000; Uthicke & Benzie 2000; Barber *et al*. 2002; Taylor & Hellberg 2003; Hohenlohe 2004). However, they have produced no clear answer concerning how patterns of larval dispersal influence population genetic structure (for review see Palumbi 1992; Mora & Sale 2002), so additional studies will be necessary to resolve this debate.

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Of the studies concerned with population structure in coral-reef-dwelling organisms, relatively few have focused on fish. These studies have found that meaningful population genetic structure is usually detectable only in studies that involve large geographical areas. Populations occupying small geographical areas tend to be more genetically uniform (e.g. Doherty *et al*. 1995; Shulman & Bermingham 1995; Dudgeon *et al*. 2000; Nelson *et al*. 2000; Bernardi *et al*. 2001; Ovenden *et al*. 2002; Planes & Fauvelot 2002; Hickford & Schiel 2003; Van Herwerden *et al*. 2003). In general, it appears that species with longer larval duration do exhibit higher levels of gene flow (Palumbi 1992). Most studies that have investigated population connectivity in marine fish focus on species with pelagic larval dispersal. Largely ignored in this debate are species that lack larval dispersal abilities. Two questions that remain unanswered concerning the relationship between dispersal, gene flow, and genetic structure are (i) to what extent do life history

traits enable marine species to disperse? and (ii) at what spatial scales do these factors influence dispersal (Riginos & Victor 2001)?

One species that has the potential to provide useful information concerning the extent that life history traits can lead to limited gene flow in a marine fish is the Banggai cardinalfish (*Pterapogon kauderni*). One key difference between *P. kauderni* and most other marine fish is that *P. kauderni* does not exhibit a pelagic larval phase (Allen & Steene 1995; Vagelli 1999). This coral-reef fish is found exclusively in the Banggai archipelago, off central-eastern Sulawesi, Indonesia. Despite being 'rediscovered' as recently as 1994 (Allen & Steen 1995), a number of studies have investigated various aspects of *P. kauderni* ecology. This species is a male mouth-brooding fish and both adults and young exhibit a close association with branching hard corals, sea urchins, and anemones (Vagelli & Erdmann 2002; Kolm & Berglund 2003). Additionally, Banggai cardinalfish exhibit strong homing behaviour (Kolm *et al*. in press). However, Kolm *et al*. (in press) found no evidence for recognition of familiar group members, discovering instead that homing was based on the original location of their group. Moreover, the same author found no evidence for kin structure within these groups: individuals were not more related within groups than between groups. Additionally, the Banggai cardinalfish is a species of conservation concern (Kolm & Berglund 2003). The aquarium trade reportedly extracts approximately 50 000–60 000 *P. kauderni* for export each month (Vagelli & Erdmann 2002).

Our *a priori* hypotheses concerning the population genetic structure of *P. kauderni* arise from two sources: ecological studies of *P. kauderni* and a study of mitochondrial DNA (mtDNA) variation in this species (Bernardi & Vagelli 2004). First, we predicted that we would find significant population genetic structure among the populations we investigated and that a genetic break would occur between the island of Bangkulu and the other islands in the archipelago. This prediction arises from ecological observations as well as mtDNA data (Bernardi & Vagelli 2004) indicating that *P. kauderni* exhibits a genetic break between the island of Bangkulu and the other islands of the Banggai archipelago. Second, we predicted that we would find some populations to be genetically misplaced given their geographical location. This prediction is rooted in the fact both Vagelli & Erdmann (2002) and Bernardi & Vagelli (2004) identified populations believed to have been founded by artificial introductions associated with the pet trade. Finally, we predicted that we would find genetic evidence that populations of *P. kauderni* have undergone bottlenecks. Bernardi & Vagelli (2004) found some evidence for population bottlenecks, but acknowledged that their findings could have been a result of small sample sizes.

Our study had three specific goals. First, we used 11 polymorphic microsatellite loci to investigate the degree to which this species exhibits genetic structure among reefs distributed over a microgeographical scale (populations separated by 2–203 km). Second, we used microsatellite loci to determine whether there was genetic evidence for recent population bottlenecks. Third, we sought to address how structure in this species compares to genetic structure in other marine fishes. We discuss these results with respect to the question of how the level of population genetic structure found in this species should influence the future of marine reserve design.

Materials and methods

Pterapogon kauderni samples were collected from seven sites in the Banggai archipelago, located around the east coast of central Sulawesi, Indonesia (Fig. 1, Table 1). Distances, measured as the minimal distance a fish could swim between any two populations (see Table 2), ranged from 2 to 203 km. All samples consisted of nonfatal fin clips collected

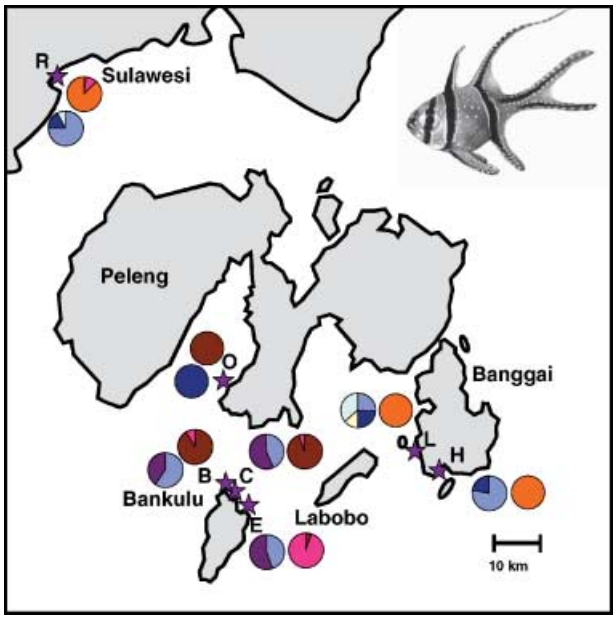

Fig. 1 Map of population localities (stars) and allele frequency pie charts for locus *Pka11* (reddish in colour and placed to the right) and locus *Pka06* (bluish in colour and placed on the left), the two microsatellite loci with the lowest expected heterozygosities (H_E). Each individual colour represents an allele at the appropriate locus and the percent of the pie chart displaying that colour represents the frequency of that allele in the population. Because of their lower levels of heterozygosity, these loci may provide a more accurate description of population differentiation than the estimates over all loci (see text). The genetic similarity of population R to populations L and H suggest that population R may be a secondary introduction as a consequence of the aquarium trade, as has been previously observed in this and other populations of this species (Vagelli & Erdmann 2002; Bernardi & Vagelli 2004).

Table 1 Population name, location, and summary statistics for seven populations, including allele numbers (*A*), sample sizes (*N*), and observed and expected heterozygosities (H_O and H_E) in seven populations of *Pterapogon kauderni*. An asterisk identifies the one population where a significant departure from Hardy–Weinberg equilibrium (after Bonferroni correction) was identified (*Pka16*, population R)

Population			B	C	E	H	L	O	R
Latitude/ Longitude			–1.770/ 123.103	–1.775/ 123.122	–1.797/ 123.139	–1.723/ 123.529	–1.689/ 123.480	–1.563/ 123.127	–0.950/ 122.800
Locus	*A*								
Pka03	17	*N*	16	32	19	15	17	31	19
		A	7	6	6	7	5	13	7
		H_O	0.813	0.781	0.684	0.800	0.647	0.968	0.842
		H_E	0.843	0.715	0.612	0.749	0.734	0.892	0.859
Pka06	17	*N*	16	36	20	13	19	36	20
		A	6	10	7	2	4	1	3
		H_O	0.500	0.722	0.600	0.308	0.737	NA	0.450
		H_E	0.583	0.774	0.755	0.369	0.741	NA	0.412
Pka07	33	*N*	14	28	19	12	13	29	18
		A	12	14	12	10	7	12	10
		H_O	0.929	0.786	0.842	0.917	0.615	0.862	0.889
		H_E	0.907	0.883	0.885	0.895	0.849	0.898	0.875
Pka09	15	*N*	13	29	19	14	19	43	20
		A	7	10	7	5	7	8	12
		H_O	0.615	0.621	0.842	0.857	0.684	0.814	0.950
		H_E	0.837	0.670	0.836	0.717	0.819	0.740	0.887
Pka11	5	*N*	17	27	19	12	17	41	20
		A	2	2	2	3	2	1	5
		H_O	0.059	0.111	0.105	0.250	0.588	NA	0.650
		H_E	0.166	0.107	0.102	0.649	0.499	NA	0.644
Pka13	23	*N*	15	36	20	15	18	43	20
		A	12	15	8	12	4	13	12
		H_O	0.867	0.833	0.650	0.867	0.778	0.884	0.900
		H_E	0.922	0.853	0.737	0.915	0.660	0.818	0.849
Pka16	24	*N*	15	32	20	10	13	42	15
		A	15	12	8	10	11	15	10
		H_O	1.000	0.656	0.750	0.800	0.769	0.881	0.533*
		H_E	0.936	0.873	0.841	0.916	0.849	0.904	0.855
Pka19	19	*N*	16	37	20	13	20	44	20
		A	11	11	9	11	12	13	12
		H_O	0.875	0.730	0.950	0.846	0.950	0.864	0.850
		H_E	0.853	0.815	0.876	0.858	0.900	0.878	0.849
Pka21	14	*N*	16	35	20	16	19	43	20
		A	8	4	9	9	6	6	11
		H_O	0.875	0.800	0.800	0.875	0.789	0.791	0.950
		H_E	0.808	0.655	0.809	0.893	0.818	0.735	0.913
Pka24	16	*N*	17	25	19	12	19	16	15
		A	6	2	3	11	6	6	9
		H_O	0.412	0.400	0.211	1.000	0.789	0.563	0.800
		H_E	0.563	0.470	0.198	0.931	0.743	0.784	0.864
Pka25	19	*N*	18	30	19	13	20	34	20
		A	13	15	5	12	7	12	9
		H_O	0.722	0.933	0.632	0.846	0.700	0.794	0.600
		H_E	0.838	0.928	0.777	0.932	0.762	0.894	0.862

between 4 and 19 November 2000 and preserved in 95% ethanol. Fish were released at the site of capture after tissue collection. All sites were similar in appearance and depth and consisted of shallow, sheltered lagoons with a mix of coral, sea grass, and bare sand bottom. The average depth of the sites was 1.8 m ± 0.5 (mean ± SD). We collected tissue samples from between 16 and 44 individuals per site via scuba diving or snorkelling. For molecular analysis, total genomic DNA was extracted following a standard phenol–chloroform technique (Sambrook *et al*. 1989)

Table 2 Table of pairwise distances (geographical and genetic) among populations and global genetic distance. See Fig. 1 for population locations. Values set in bold indicate those not significantly different from 0. At $\alpha = 0.05$, only one of the 21 genetic estimates (for each type of estimate) would be expected to appear statistically significant by chance alone

Population pair	Distance (km)	F_{ST} (95% confidence interval)	R_{ST} (95% confidence interval)	Allele size permutation test (test: $R_{ST} = F_{ST}$)
B-C	2	0.06 (0.03)	**0.01 (0.03)**	$P = 0.920$
B-E	5	0.16 (0.14)	**0.10 (0.13)**	$P = 0.280$
B-H	50	0.14 (0.11)	0.25 (0.13)	$P < 0.001$
B-L	44	0.18 (0.11)	0.35 (0.17)	$P < 0.001$
B-O	23	0.17 (0.14)	0.26 (0.12)	$P = 0.156$
B-R	143	0.13 (0.10)	0.29 (0.11)	$P < 0.001$
C-E	3	0.22 (0.15)	0.24 (0.20)	$P = 0.008$
C-H	49	0.18 (0.12)	0.36 (0.21)	$P < 0.001$
C-L	43	0.22 (0.11)	0.41 (0.12)	$P = 0.001$
C-O	23	0.19 (0.11)	0.40 (0.13)	$P < 0.001$
C-R	145	0.17 (0.10)	0.38 (0.17)	$P < 0.001$
E-H	44	0.20 (0.11)	0.45 (0.26)	$P < 0.001$
E-L	41	0.20 (0.12)	0.57 (0.39)	$P < 0.001$
E-O	28	0.27 (0.17)	0.26 (0.15)	$P = 0.491$
E-R	148	0.17 (0.09)	0.51 (0.23)	$P < 0.001$
H-L	9	0.11 (0.04)	**0.15 (0.17)**	$P = 0.096$
H-O	60	0.21 (0.16)	0.49 (0.43)	$P < 0.001$
H-R	203	0.04 0.02)	**0.06 (0.12)**	$P = 0.254$
L-O	60	0.22 (0.14)	0.60 (0.58)	$P < 0.001$
L-R	193	0.10 (0.03)	**0.18 (0.19)**	$P = 0.047$
O-R	138	0.19 (0.16)	0.51 (0.38)	$P < 0.001$
Global		0.18 (0.09)	0.35 (0.14)	$P < 0.001$

or a standard Chelex technique (Miller & Kapuscinski 1996).

For the analysis of genetic structure we used 11 microsatellite markers developed specifically for *P. kauderni* (Hoffman *et al*. 2004). Polymerase chain reaction (PCR) conditions were identical to those described in Hoffman *et al*. (2004), and PCR fragments were separated and scored on an ABI 3100 capillary electrophoresis system. We used GENEPOP version 3.3 (Raymond & Rousset 1995) to estimate expected and observed heterozygosities and to test for Hardy–Weinberg equilibrium (HWE) via exact tests [applying a sequential Bonferroni correction for multiple comparisons (number of loci × number of populations = 77); Rice 1989]. Genetic differentiation among all seven populations and between pairs of populations was estimated with F_{ST} (Weir & Cockerham 1984; calculated by GENEPOP) with 95% confidence limits determined by FSTAT version 2.9.3.2. (Goudet 1995).

Because F_{ST} can underestimate population differentiation when within-population heterozygosities are high (Hedrick 1999), we tested the relationship between F_{ST} and population differentiation using two statistical procedures. First, we tested whether our F_{ST} values were influenced by the upper bound on the value of F_{ST} ($F_{ST} = 1 - H_E$, with H_E equal to the average within-population heterozygosity; Hedrick 1999). Here, we plotted H_E vs. F_{ST} for all pairwise population comparisons. Second, we compared F_{ST} and R_{ST} values for our populations using the computer program SPAGEDI (Hardy & Vekemans 2002). We estimated global and population pairwise genetic differentiation with R_{ST} as calculated by SPAGEDI (95% confidence intervals calculated by jackknifing over loci). The comparison of F_{ST} and R_{ST} values can provide insights into the main causes of population differentiation, such as drift vs. mutation because these statistics should be similar under drift, whereas R_{ST} should be larger if stepwise mutation played a role in population differentiation (Hardy *et al*. 2003).

We tested for isolation by distance by computing the regression of $F_{ST}/(1 - F_{ST})$ on distance between population sites as determined by the minimum distance a fish could swim between populations (Rousset 1997). We assessed significance levels using a Mantel test (Mantel 1967) using the program ISOLDE in GENEPOP. Although it would be ideal to incorporate oceanic currents into our distance measures, too little is known about the specific minor currents between these islands to assess their influence with accuracy. The closest major current that influences the region is the Indonesian Throughflow. However, this current occurs northwest of the island of Sulawesi and does not influence the islands in this study.

To test for the genetic signature of recent reductions in population size (bottlenecks) in our populations, we used the statistical approach of Cornuet & Luikart (1996) as implemented by the computer program BOTTLENECK (Piry *et al*. 1999). This method uses the fact that populations that have experienced a recent reduction in population size suffer a greater reduction in number of alleles than in observed gene diversity. This is because at a polymorphic locus, rare alleles are lost first and have little effect on gene diversity. We used the Wilcoxon sign rank test to assess evidence of bottlenecks because this test does not require large numbers of loci or individuals per population. Specifically, we ran the model with 11 microsatellite loci and 1000 replications for each population. We ran the program such that all loci followed the two-phase model of mutation, which consists mostly of one-step changes with a low percentage of multistep changes (9:1), as suggested by Di Rienzo *et al*. (1994), and with 10% variance.

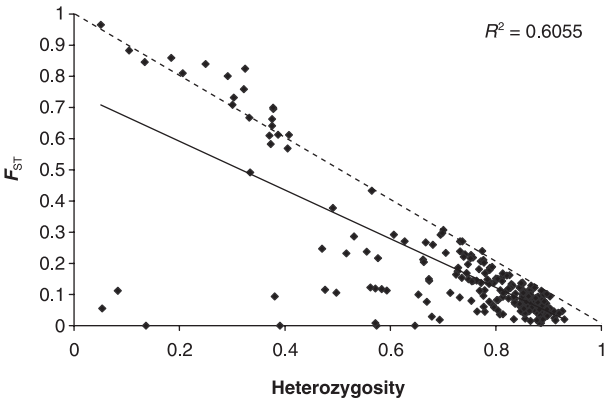

Fig. 2 Plot of F_{ST} vs. expected heterozygosity for all pairwise population comparisons. The dotted diagonal line represents the upper theoretical maximum for F_{ST} for any given value of expected heterozygosity. The solid line represents the least squares regression line for all points plotted.

Results

Microsatellite data are summarized in Table 1. Only one locus in a single population showed a deviation from HWE after of the Bonferroni correction (*Pka16* in population R). In general, these populations appear to satisfy approximately the assumptions of HWE, but there may be a low frequency null allele at *Pka16* in population R. All 11 microsatellite markers were variable across the seven populations, but the level of within-population variation was highly dependent on the marker (Table 1). Across all populations, the numbers of alleles per locus ranged from five to 33, and average expected heterozygosities (H_E) ranged from 0.361 to 0.885 (Table 1).

Our results revealed high levels of genetic subdivision over distances as small as 2 km (Fig. 1, Table 2). All F_{ST} estimates (global as well as pairwise) and all but five pairwise R_{ST} estimates were significantly greater than zero as indicated by 95% confidence intervals non-overlapping with zero (Table 2). The most striking result was that two populations (populations E and O) separated by only 28 km were nearly fixed for different alleles at locus *Pka11*, resulting in a value of F_{ST} of 0.97 (Fig. 1). A second locus (*Pka06*) displayed a similar pattern (F_{ST} = 0.70). Single-locus estimates could be artificially inflated if some of the microsatellite loci are indirectly influenced by selection. The fact that all loci reveal significant population structure, however, suggests that these results are due to limited gene flow rather than selection at loci linked to our microsatellites. Considering all populations in the study, the greatest multilocus F_{ST} was 0.27, between populations E and O (see Table 2). The greatest multilocus pairwise R_{ST} value was 0.60 between populations L and O. Genetic differentiation across all populations for all loci was estimated as 0.18 (F_{ST}) and 0.35 (R_{ST}), indicating that this species displays an unusually high level of population structure over such a small geographical scale for a marine organism. Moreover, considerable population subdivision was evident even in populations separated by as little as 2–5 km. Of the three population pairs separated by less than 5 km (Fig. 1), multilocus F_{ST} ranged from 0.06 to 0.22 and multilocus R_{ST} ranged from 0.01 to 0.24 (Table 2).

Although the multilocus F_{ST} values between populations are less striking than certain single-locus values (see previous discussion), some of our loci may exhibit values of F_{ST} that are deflated owing to high within-population variability. Because most of our microsatellite loci were highly polymorphic (Table 1), many of our observed values of F_{ST} were very close to the upper theoretical bound. Indeed, the plot of all pairwise population estimates of F_{ST} vs. H_E indicated that across a broad range of values of H_E, the corresponding F_{ST} values often are located close to the theoretical maximum (Fig. 2). Given this pattern, the amount of population structure in this species may be even higher than that indicated by the F_{ST} values. Further evidence that the F_{ST} values underestimate the actual levels of genetic structure arises from the allele size permutation test. This test indicated that across all loci for all populations R_{ST} was significantly greater than F_{ST} ($P < 0.001$) and that 15 of 21 multilocus pairwise comparisons also exhibited significantly greater R_{ST} than F_{ST} (Table 2).

The analysis of isolation by distance gave mixed results. When all populations were included in the analysis, there was no significant relationship between genetic and geographical distance (Mantel test: $P = 0.066$ with R_{ST} and $P = 0.66$ with F_{ST}). With population R removed from the analysis (see Discussion), there was indeed a positive trend of increasing genetic distance with geographical distance (Mantel test: $P = 0.021$ with R_{ST}). However, this trend is not

significant when genetic distance is measured via F_{ST} (Mantel test: $P = 0.13$). These mixed results could be due to the small number of populations used in the analysis. Further work that included more populations would lead to a clearer picture pertaining to the extent to which this species exhibits isolation by distance.

We found no evidence for a species-wide genetic signature of recent reductions in population size. A statistically significant departure from mutation–drift equilibrium was detected in only a single population (population E) and this was only marginally significant (Wilcoxon sign rank test $P = 0.042$). In all other populations, there was no evidence for a reduction in heterozygosity relative to the number of alleles present (Wilcoxon sign rank test $P > 0.10$ for all populations).

Discussion

This study found significant genetic subdivision (as measured by both F_{ST} and R_{ST}) among populations of Banggai cardinalfish. We expected to find genetic subdivision among our populations because these fish lack a pelagic larval phase. However, the spatial scale over which the genetic subdivision occurred was unexpectedly small. This study documents high levels of genetic subdivision over microgeographical distances as small as 2 to 5 kilometres.

Although we did not find evidence for recent species-wide reductions in population size, our other *a priori* predictions were supported by the data. Examination of the genetic relationships among populations indicated that the initial finding that there was a lack of a pattern of isolation by distance might partially be caused by the genetic similarity of the most western population (population R) and the two most eastern populations (populations L and H). The genetic similarity among these populations may indicate an artificial origin for population R. The seeding of artificial populations is believed to have occurred several times as a consequence of the aquarium trade (Bernardi & Vagelli 2004). Moreover, Bernardi & Vagelli (2004) suggest that our population R (their population 'Luwok 1') may well have been established in this fashion given their genetic data. Once this population is removed, the data indicate a trend toward isolation by distance when population structure is measured by R_{ST}.

How does genetic structure found in *Pterapogon kauderni* compare to other studies of genetic structure conducted within the marine realm? In the recent past, the prevailing view of genetic structure in marine organisms was that they would display very little genetic structure, even across broad geographical distances. This viewpoint was upheld by many early studies of population structure in marine organisms, which found little genetic structure over great distances. For example, Shaklee (1984) found no genetic differentiation in the damselfish (*Stegastes fasciolatus*) throughout their 2500-km range off the Hawaiian Islands, and Uthicke & Benzie (2000) found no genetic differentiation in populations of the sea cucumber *Holothuria nobilis* throughout 1300 km of their range off the Great Barrier Reef. Other studies found significant, albeit small, genetic structure over equally large (e.g. Ovenden *et al*. 2002) or smaller distances (e.g. Fauvelot & Planes 2002; Planes & Fauvelot 2002). Some studies found genetic structure over a large geographical scale (e.g. between the Pacific and Indian oceans), but little genetic variation within each region (e.g. Nelson *et al*. 2000; Bernardi *et al*. 2001; Riginos & Nachman 2001). In these studies, the lack of genetic structure has been attributed to high migration rates resulting from the excellent dispersal abilities of the pelagic larval forms of many marine fish, invertebrates and algae (Bohonak 1999).

The lack of genetic structure in marine organisms has led to research concerning the degree to which life history traits, such as dispersal ability, influence population genetic structure. Reviews of the correlation between dispersal ability and genetic structure have produced mixed results. Waples (1987) found an inverse correlation between larval duration and genetic variation. However, Waples & Rosenblatt (1987) re-analysed this data set and concluded that most species were more strongly influenced by local current patterns than dispersal abilities. In contrast, Bohonak (1999), Doherty *et al*. (1995), and Riginos & Victor (2001) all looked at a number of fish to determine whether a relationship existed between dispersal ability and genetic structure. These studies all concluded that increased dispersal ability was associated with decreased differentiation among populations.

The few studied fish species that completely lack a pelagic larval phase have shown relatively high levels of among-population genetic differentiation. Including the results of the present study, patterns of population structure have been described in three species of reef fish that lack a pelagic larval phase. Doherty *et al*. (1995) found great genetic variation ($F_{ST} = 0.79$), using allozymes, between populations of the damselfish *Acanthochromis polyacanthus* separated by about 1000 km. Genetic variation among populations of *A. polyacanthus* separated by approximately 50 km was also high ($F_{ST} = 0.17$, northern populations; $F_{ST} = 0.18$, southern populations). Planes *et al*. (2001) investigated mtDNA sequence variation among the same populations of *A. polyacanthus* studied by Doherty *et al*. (1995) plus one additional population. Across all populations, average sequence divergence was 7.6%. This amount of sequence divergence may correspond to a date of divergence of about 5 million years ago (Ma), and is greater than that found among populations within other recognized species of fish. Bernardi (2000), investigating genetic structure of the black surfperch, *Embiotoca jacksoni*, via sequencing of mtDNA, found that F_{ST} among the most distantly separated

populations (about 1500 km) was 0.72. Interestingly, Bernardi (2000) found that variation within known phylogeographical provinces was relatively low (F_{ST} = 0.05) which may explain why a previous smaller-scale allozyme study of this species detected little genetic variation (Waples 1987).

With respect to marine fish, the Banggai cardinalfish exhibits the highest degree of population subdivision at nuclear loci that has yet been documented over so small a geographical scale. The pattern of genetic subdivision in this species strongly suggests that few migrants are exchanged between populations and that populations should be free to adapt separately to local conditions. This conclusion is supported by a recent study of mtDNA sequence polymorphism in the Banggai cardinalfish, which showed significant mtDNA genetic structure among populations (Bernardi & Vagelli 2004). Only one other study has found significant population genetic structure on such a small microgeographical scale (less than 5 km) in a marine fish. Planes *et al*. (1998) investigated allozyme variation in two species of fish, *Acanthurus triostegus* and *Chaetodon ulietensis*, with populations separated by less than 1 km, and found that the populations did indeed exhibit significant genetic differentiation (highest F_{ST} was equal to 0.055). However, the populations were in near complete geographical isolation because one population occurred in a completely enclosed lagoon. Moreover, the level of genetic structure found between these two populations was equivalent to the levels of population structure observed among open-ocean populations of this species separated by distances greater than 1000 km (Planes *et al*. 1998).

In the comparison of different studies of population genetic structure, one important consideration is the type of molecular marker used. Here, we employed microsatellite markers, whereas the other studies mentioned previously either used allozymes or mtDNA sequence variation. In general, we expect mtDNA to display greater between-population levels of genetic variation than either microsatellites or allozymes, because of the four-fold smaller effective population size of mitochondrial loci relative to nuclear markers. Additionally, we would predict that F_{ST} values from studies of allozyme polymorphisms should be higher than those observed in studies using microsatellites because high levels of heterozygosity within populations (as are typically observed at microsatellite loci) place an upper bound on the value of F_{ST} (Hedrick 1999). Our data show a pattern of decreasing F_{ST} with increasing H_E (Fig. 2), suggesting that the F_{ST} values derived from the microsatellites exhibiting the highest values of H_E are reduced as a consequence of the upper ceiling that H_E place on F_{ST} (Hedrick 1999). Indeed, most of the values of F_{ST} observed in this study are very close to the upper theoretical bound. Hence, it appears likely that nuclear markers with lower average levels of polymorphism, such as allozymes, would yield even higher values of F_{ST} in this species than those reported here.

The high degree of genetic structure found in this species leads to two questions. First, what mechanisms maintain the genetic structure observed in this fish? And second, how should knowledge of this high level of structure influence marine reserve design? The answer to the first question is that genetic structure in *P. kauderni* is likely maintained by two life history properties of this species. First, *P. kauderni* does not have a pelagic larval phase. Juvenile Banggai cardinalfish therefore possess limited dispersal ability and are likely to recruit to the same reef as their parents (Vagelli & Erdmann 2002). Hence, *P. kauderni* has inherently less gene flow potential than most other marine species. Second, *P. kauderni* exhibit a keen homing ability (Kolm *et al*. in press) likely mediated by olfactory cues. The use of olfactory cues has been observed in other cardinalfish species (Atema *et al*. 2002), and such cues may enable the fish to orienteer toward their original locations after displacement by physical oceanic disturbances. Hence, these two aspects of the natural history of *P. kauderni* (i.e. natal site fidelity and homing ability) may predispose this species to exhibit high levels of population structure.

Pterapogon kauderni is heavily exploited by the aquarium trade, making it a potentially useful species in which to investigate the use of marine reserve design for reef-dwelling species. Hence, we must consider the important second question: How does the high population genetic structure found in this species influence current marine reserve design? Overall, it appears that the results of this study provide a cautionary tale for practitioners of marine conservation. Marine reserves that are overly fragmented or too small in size could have catastrophic consequences for species with strong microgeographical genetic structure (Palumbi 2003). Our results show that populations of *P. kauderni* occurring on separate reefs of the same island separated by as little as a few kilometres may not exchange enough migrants for meaningful 'spill-over' to occur between reefs. Thus, poorly constructed marine reserves would fail to conserve the biodiversity they are designed to protect.

Acknowledgements

We thank K. Mobley for discussion on the topic. We would also like to thank G. Bernardi and A. Vagelli for discussion of their data before publication. Also, we thank C. Fenie for arranging all logistics during field sampling. The study was financed by the Swedish Research Council, GH nation, and the Zoological Foundation.

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