Marine Genomics 1 (2009) 129–134

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

# Fine scale dispersal in Banggai Cardinalfish, *Pterapogon kauderni*, a coral reef species lacking a pelagic larval phase

Alejandro Vagelli [a], Martha Burford [b], Giacomo Bernardi [b,*]

[a] New Jersey Academy for Aquatic Sciences, 1 Riverside Drive, Camden, New Jersey 08103, USA
[b] Department of Ecology and Evolutionary Biology, University of California Santa Cruz, 100 Shaffer Road, Santa Cruz, California, 95060, USA

ARTICLE INFO

*Article history:*
Received 8 August 2008
Accepted 25 January 2009

*Keywords:*
Pterapogon kauderni
Banggai Cardinalfish
Marine dispersal
Microsatellites

ABSTRACT

Dispersal in marine species results from complex interactions between biotic and abiotic factors. Importantly, the pelagic larval phase of most marine species adds a significant degree of complexity. Therefore, a growing body of work is focusing on those rare species that lack a pelagic larval phase (usually brooding species). For such species, large-scale gene flow has been shown to be very low, thus following the expectation of a relationship between realized dispersal and pelagic larval duration. Yet, little is known about the dispersal of those species at very small geographic scales. In this study, we focused on the Banggai Cardinalfish, *Pterapogon kauderni*, a mouthbrooding species that lacks a pelagic larval phase. Based on previously identified microsatellites, we scored 12 populations around the southern island of Bangkulu, in the Banggai Archipelago, Indonesia. While only 60 km in perimeter, we found that this island harbors very distinct populations of *P. kauderni*. Indeed, assignment tests self-assigned 10 out of those 12 populations. These results mirror the very high level of self-assignment at the level of the entire archipelago, where, out of 13 populations, 70% of the individuals were reassigned to their source population. Therefore, our data show consistency between small and large-scale dispersal. In addition, in light of the recent expansion in the harvesting of this species for the pet trade, our data have important conservation implications.



## 1. Introduction

In marine species, understanding the relationship between connectivity and geographic distance is problematic because of the complexity of the dynamics of dispersal at different spatial scales. Since most marine fishes typically exhibit a planktonic larval stage that may last days to months and a non-planktonic post larval stage, several studies have focused on understanding the relationship between the pelagic larval duration (PLD) and realized dispersal. Genetic studies that used gene flow as a proxy for dispersal have produced inconsistent results (e.g. Waples, 1987; Doherty et al., 1995; Shulman and Bermingham, 1995; Riginos and Victor, 2001). In addition to the variability of the life cycle between different species, the role of spatial scales is also difficult to predict (Bernardi et al., 2001). Small-scale phenomena (centimeters, meters, or few kilometers) do not appear to be good predictors for large-scale (hundreds or thousands of kilometers) genetic structure (Hellberg, 1995, 1996; Moberg and Burton, 2001).

In order to remove the complicating factor of the bipartite life cycle (adult and larval stages), some studies focused on species that lack a dispersing planktonic larval phase (or period) (Hellberg, 1995; Doherty et al., 1995; Bernardi, 2000, 2005; Planes et al., 2001; Bernardi and Vagelli, 2004; Hoffman et al., 2005). In those few species for which data are available, results at large-spatial scales were mostly consistent with expectations, geographic distance and genetic divergence were correlated. However, little is known about the dispersal of these species at finer spatial scales.

In this study, we have focused on the small-scale dispersal of a coral reef fish, the Banggai Cardinalfish, *Pterapogon kauderni*. In addition to the absence of a pelagic larval stage, juvenile and adult lifestyle characteristics of *P. kauderni* make the species extremely philopatric. *P. kauderni* settles within the parental habitat, shows a strong attachment to particular microhabitats confined to very shallow waters, and exhibits low motility and dispersing capabilities as adults. Overall, these characteristics have limited the species to a very small geographic range, the Banggai Archipelago, off central Sulawesi, Indonesia (Vagelli, 2004; Vagelli and Erdmann, 2002; Bernardi and Vagelli, 2004).

Out of the about 55 islands that comprise the Banggai Archipelago, *P. kauderni* inhabits 31, covering an area of approximately 6000 km$^2$. The greatest distance that separates two populations is 153 km (Patikaman, Peleng Is.–Kano Is.). The most isolated population is located in Tempau Is., which is separated from the population inhabiting Masoni Is. by 16 km, and by 30 km from the population found in Bokan Is. The second most isolated Island is Bangkulu, with its north population separated by about 13.6 km from the South

* Corresponding author. Department of Ecology and Evolutionary Biology, University of California Santa Cruz, 100 Shaffer Road, Santa Cruz, California, 95060, USA. Tel.: +1 831 459 5124; fax: +1 831 459 3383.
*E-mail address:* bernardi@biology.ucsc.edu (G. Bernardi).

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doi:10.1016/j.margen.2009.01.001

Peleng population and by about 13.5 km from the Labobo Kenecil population. Then, the population inhabiting Masoni Is. is separated by about 11 km from the population located in Limbo, and by 16 km from that inhabiting Tempau Is.

In addition to the Banggai Archipelago, *P. kauderni* is found in Luwuk, Central Sulawesi. This population is restricted within the harbor, which is about 1.2 km in length by 0.5 km and opens to the ocean through a passage of about 150 m. Besides from being restricted to a very small area, this population is exposed to high levels of pollution, including regular fuel spills, fresh water and sewage discharges. Although both north and south coastlines have suitable habitats with adequate substrates, and environmental conditions far better than within the Luwuk harbor, no population was found from Luwuk to Botok (68 km to the north, including Lamala Bay), and from the harbor to Luk (46.5 km to the south). The Luwuk population is therefore isolated from the populations inhabiting the Banggai Archipelago. The closest population is localized in Patikaman (south-central Peleng) at about 120 km, and separated by the Peleng Strait with strong currents and depths up to about 920 m (Vagelli, 2005). Because of its isolation, and the fact that Luwuk harbor was used by fish collectors as a distribution center during the first years of the *P. kauderni* trade, the Luwuk population has been proposed to be the result of an artificial introduction, yet no direct evidence is available.

In contrast, a clear record of artificial introduction of *P. kauderni* individuals in the Lembeh Strait (North Sulawesi), about 400 km north to the species' natural range is available. A discernable population started around the year 2000 (Erdmann and Vagelli, 2001; Vagelli, 2005) and is growing. Similarly, a small population inhabits the Tumback Bay (North Sulawesi, about 90 km South of the Lembeh Strait) where a fish trader is located, and where "undesirable" specimens have been released into the bay for several years. Most recently, evidence of a new introduction in Bali has also emerged (Gilimanuk Bay), where one the largest export center of *P. kauderni* is located.

Prior genetic work uncovered very low levels of gene flow at the scale of the entire Archipelago (Bernardi and Vagelli, 2004; Hoffman et al., 2005). Mitochondrial DNA analysis partitioned samples in two major clades, a southern clade that included samples collected on the southern part of the island of Bangkulu (an island at the southern edge of the species distribution) and a northern clade that included samples collected on the northern part of Bangkulu, as well as all the remaining samples. The island of Bangkulu, therefore, is a key location to study fine scale structuring in this species. Mitochondrial sequences, while effective at the scale of the Archipelago, provided minimal resolution of the genetic structure at smaller spatial scales (Bernardi and Vagelli, 2004), in contrast, the recent development of 11 microsatellite loci for *P. kauderni* (Hoffman et al., 2004) allowed for finer genetic resolution (Hoffman et al., 2005). In this latter study, two out of the 11 original microsatellites (*Pka6* and *Pka11*) provided most of the power for resolving the genetic structure of *P. kauderni* populations.

The goal of the present study was to understand the fine scale population structuring of *P. kauderni* at the island of Bangkulu, and its relationship with the overall population structure of the species. We surveyed extensively the island of Bangkulu, off Central Sulawesi, and analyzed samples from twelve localities using the *Pka6* and *Pka11* microsatellites. In addition, we also analyzed 15 localities that encompassed the full natural range of the species and one introduced population.

## 2. Materials and methods

### 2.1. Sample collections and surveys

We used two sets of samples in this study. The first set of samples corresponded to 122 individuals that we previously collected in 2001–2002 and used for the study of Bernardi and Vagelli (2004) (Table 1, Fig. 1). Of those original samples, 23 individuals were collected from 4 sites on the island of Bangkulu (sites 2, 4, 5 and 6). For the present study,

**Table 1**
Population name and summary statistics for *Pterapogon kauderni* populations, including sample size ($N$), number of distinct alleles (A), observed and expected heterozygosities ($H_O$ and $H_E$).

| Population | | BAK02 | BAK04 | BAK05 | BAK06 | BAK07 | BAK08 | BAK09 | BAK10 | BAK11 | BAK1G | BAK12 | BAK20 | BAK21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | | | | | | | | | | | | | | |
| Pka06 | N | 8 | 5 | 22 | 17 | 36 | 41 | 25 | 20 | 12 | 18 | 20 | 41 | 24 |
| | A | 9 | 5 | 7 | 11 | 7 | 9 | 8 | 6 | 5 | 4 | 7 | 14 | 7 |
| | $H_O$ | 1.000 | 0.600 | 0.636 | 1.000 | 0.806 | 0.854 | 0.720 | 0.650 | 0.750 | 0.389 | 0.600* | 0.805 | 0.667 |
| | $H_E$ | 0.908 | 0.889 | 0.722 | 0.923 | 0.758 | 0.856 | 0.780 | 0.728 | 0.656 | 0.660 | 0.817 | 0.798 | 0.757 |
| Pka11 | N | 9 | 5 | 13 | 15 | 26 | 41 | 24 | 20 | 11 | 18 | 16 | 43 | 22 |
| | A | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 |
| | $H_O$ | NA | 0.000 | NA | 0.800 | 0.039 | NA | NA | NA | NA | NA | NA | 0.488 | 0.364 |
| | $H_E$ | NA | 0.644 | NA | 0.515 | 0.180 | NA | NA | NA | NA | NA | NA | 0.450 | 0.498 |
| Population | | BAN01 | BAN02 | BOK01 | LAB01 | LEM01 | LIM01 | LOI01 | LUW01 | MASE1 | MASO1 | MEL01 | PEL01 | PEL02 | PEL04 | SEK01 | TAL01 | TEM01 |
| Locus | | | | | | | | | | | | | | | | | | |
| Pka06 | N | 5 | 5 | 12 | 5 | 9 | 5 | 8 | 17 | 5 | 4 | 3 | 5 | 5 | 2 | 14 | 8 | 5 |
| | A | 3 | 3 | 5 | 7 | 4 | 2 | 3 | 7 | 2 | 4 | 3 | 5 | 5 | 3 | 9 | 3 | 3 |
| | $H_O$ | 0.600 | 0.600 | 0.833 | 1.000 | 0.778 | 0.800 | 0.500 | 0.706 | 0.200 | 0.750 | 0.750 | 0.800 | 1.000 | 1.000 | 0.571 | 0.635 | 0.800 |
| | $H_E$ | 0.511 | 0.644 | 0.667 | 0.911 | 0.752 | 0.533 | 0.658 | 0.768 | 0.378 | 0.857 | 0.607 | 0.867 | 0.867 | 0.833 | 0.675 | 0.775 | 0.778 |
| Pka11 | N | 5 | 5 | 11 | 4 | 9 | 4 | 8 | 17 | 3 | 4 | 3 | 5 | 5 | 2 | 12 | 8 | 5 |
| | A | 3 | 2 | 3 | 2 | 3 | 2 | 4 | 4 | 3 | 1 | 2 | 1 | 3 | 2 | 2 | 3 | 1 |
| | $H_O$ | 0.400 | 0.400 | 0.091* | 0.750 | 0.778 | 0.250 | 0.500 | 0.647 | 0.667 | NA | 0.500 | NA | 0.400 | 0.500 | 0.250 | 0.500 | NA |
| | $H_E$ | 0.600 | 0.356 | 0.714 | 0.679 | 0.647 | 0.679 | 0.735 | 0.706 | 0.800 | NA | 0.429 | NA | 0.644 | 0.500 | 0.533 | 0.575 | NA |

The top panel corresponds to populations from the island of Bangkulu (BAK), the bottom panel corresponds to populations from the entire range of the species. Asterisks identify significant departures from Hardy–Weinberg expectation (after sequential Bonferroni corrections). Population abbreviations are described in Table 3.

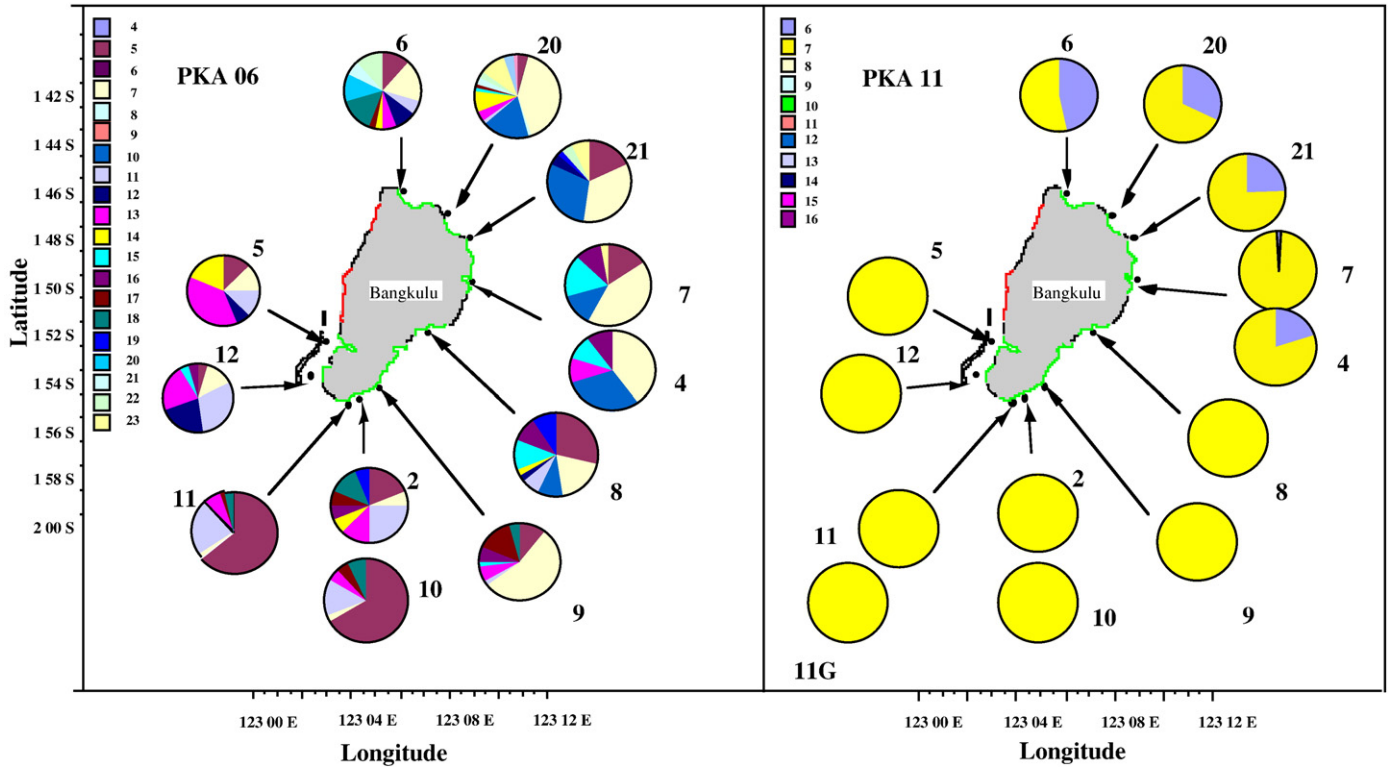


**Fig. 1.** Map of population localities around the island of Bangkulu, Banggai Archipelago, Indonesia. Allele frequency pie charts for locus Pka6 (left panel) and Pka 11 (right panel). Each individual color represents an allele at the appropriate locus and the percent of the pie chart displaying that colour represents the frequency of that allele in the population. A coastline highlighted in green on the map of the island indicates the presence of *Pterapogon kauderni*, absence of the specie is indicated in red, and the regions that were not surveyed are in black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

we did additional extensive surveys and collections at the island of Bangkulu in 2004. Wherever possible, we entered the water and scored for presence or absence of *P. kauderni*. In addition, when possible, fish were collected and these samples constitute the second set of samples. This second set comprised 273 individuals collected at 11 sites (Table 1, Fig. 2). Of those 11 sites, 3 corresponded to sites sampled for the Bernardi and Vagelli (2004) study (sites 2, 5 and 6) and at these three sites we collected additional fishes (no additional samples were obtained from site 4 of Bernardi and Vagelli, 2004). We also sampled an additional 8 new sites (Table 1, Fig. 2). We collected samples with a hand net, and the individuals were fin clipped and then returned to their habitat. We stored fin clips in 95% ethanol at ambient temperature. Considering the threatened status of this species (Allen, 2000), we tried to limit the number of sampled individuals, and we took measure not to disturb the habitat. We also collected individuals as far apart as possible to minimize possibilities of genetically related individuals.

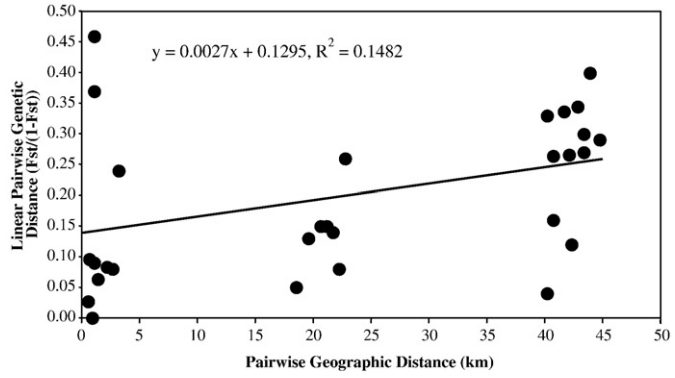


**Fig. 2.** Isolation by distance scattergram for *Pterapogon kauderni* along the east coast of Bangkulu Island, Indonesia. All pairwise distances (following contour lines) were calculated for populations from Bak6 to Bak 11 (see Fig. 1) and plotted against linear genetic distances ($F_{ST}/1-F_{ST}$).

### 2.2. DNA extractions, molecular markers and PCRs

We extracted DNA from fin clips as previously described (Bernardi and Vagelli, 2004). Eleven microsatellite primer pairs designed for *P. kauderni* have previously been described in the literature (Hoffman et al., 2004). A subsequent population study showed that out of the eleven microsatellite loci, two loci gave the strongest and most interesting results to uncover population structure, *Pka06* and *Pka11* (Hoffman et al., 2005). These two loci showed to be in Hardy–Weinberg equilibrium, neither exhibiting evidence of selection, or the presence of null alleles (Hoffman et al., 2005). Therefore we decided to use these two microsatellite markers for our analysis. Amplification protocols followed the literature (Hoffman et al., 2004, 2005) and we used an ABI 3100 automated sequencer to perform the microsatellite analyses.

### 2.3. Microsatellite analyses

We analyzed within-sample deviations from Hardy–Weinberg (HW) expectations using an exact test of HW proportions for multiple alleles (Guo and Thompson, 1992) as implemented in GENEPOP version 3.2 (Raymon and Roussset, 1995). To further examine the conformance of the data to HW expectations, we used Arlequin (Schneider et al., 2000) to estimate expected and observed heterozygosities ($H_E$ and $H_O$). Finally, to analyze the independence of the microsatellite loci, we used an exact test for linkage equilibrium in GENEPOP.

### 2.4. Population structure

We assessed population structure using both classical $F_{ST}$ calculations as well as an Analysis of Molecular Variance (AMOVA). We estimated genetic differentiation among all populations and between pairs of populations with $F_{ST}$ (Weir and Cockerham, 1984; calculated by Arlequin) using 95% confidence limits. In addition, we performed an Analysis of Molecular Variance (AMOVA) using the software package Arlequin (Schneider et al., 2000). We first analyzed the complete dataset in order to uncover any differences between the island of Bangkulu and the remainder of the samples. This allowed for comparison between the current microsatellite dataset and the mitochondrial DNA dataset of Bernardi and Vagelli (2004). Then we conducted a further analysis using the second dataset that only included samples from the island of Bangkulu. Finally, in order to test for isolation by distance in the samples from Bangkulu, we conducted a MANTEL test using linear pairwise $F_{ST}$ values and pairwise distance (km) among adult samples as implemented in GENEPOP (10,000 permutations). We conducted two MANTEL tests, one with just the *Pka 06* locus and one with both loci (*Pka 06* and *Pka 11*) and applied them to the whole island of Bangkulu, and then to the east coast and to the west coast, independently (populations Bak 6 to Bak11). Sample sizes were not large enough in the remainder of the Archipelago to perform such a test at that scale.

### 2.5. Assignment tests

Assignment tests are a robust method for testing structure in a population (Paetkau et al., 1995). Assignment tests incorporate multiple loci and therefore minimize the effect of rare alleles or low frequency alleles, common in microsatellite data, from driving the pattern of structure observed using other microsatellite analyses. The assignment test calculates a reference frequency for each location while excluding the individual in question, draws an individual from the total (all locations combined) and calculates the likelihood that this individual genotype belongs to any reference location, and then assigns it to the reference location with which it has the greatest affinity (Paetkau et al., 1995; Buonaccorsi et al., 2002). The program tests for significance using permutation resampling to test whether the frequency of self-assignment to a given location was greater than that expected in a panmictic population. We used a correction factor based on Titterington et al. (1981) when there were frequencies of zero in the data. We calculated assignment probabilities using the Doh program (Brzustowski, 2002).

## 3. Results

### 3.1. Surveys

For this study, we thoroughly surveyed the island of Bangkulu (Fig. 1). While the eastern shore of the island supports a continuous habitat suitable for *P. kauderni* (green outline, Fig. 1), the northwestern shore of the island is devoid of the species (red outline, Fig. 1), mostly due to the presence of steep slopes and lack of coral reefs and other suitable shallow habitat.

### 3.2. Microsatellite analyses

Table 1 provides a summary of the main characteristics of the microsatellite analyses. As was shown before (Hoffman et al., 2004, 2005), the two microsatellite loci *Pka06* and *Pka11* behave in an expected Mendellian fashion. Earlier surveys uncovered 17 and 5 alleles respectively (Hoffman et al., 2005), while our surveys, which were geographically more extensive, uncovered a few additional loci (for a total of 22 and 11 alleles, respectively) (Table 1). Only two samples were out of Hardy–Weinberg proportions for one locus, Bak12 for *Pka06* and Bok01 for *Pka11* (Table 1). In cases were there were enough data to make comparisons, there was no violations of linkage disequilibrium once corrections were made for multiple comparisons (sequential Bonferroni). The results of the frequency of the alleles showed a unimodal distribution (not shown) with dominant alleles for both loci, which is typical of most microsatellites.

### 3.3. Population structure

Earlier studies demonstrated that gene flow among populations was very limited (Bernardi and Vagelli, 2004; Hoffman et al., 2005). The present study confirmed these results, with high $F_{ST}$ values corresponding to low numbers of migrants per generation between populations ($Nm$). This is true both at the level of the Archipelago (average $F_{ST} = 0.258$, $Nm = 1.5$) (not shown), as well as within the island of Bangkulu (average $F_{ST} = 0.15$, $Nm = 2.92$) (Table 2). The results of the MANTEL test did not indicate significant isolation by distance when analyzing the whole island or the west coast alone (a region where suitable habitat is rare). In contrast, significant isolation by distance was observed from the northernmost location on Bangkulu Island (Bak 6) along the east coast to the most southern location (Bak 11) using only one locus or both loci ($P = 0.039$ and $P = 0.019$, respectively) (Fig. 2).

### 3.4. Analysis of molecular variance

Results based on mitochondrial DNA showed a genetic break that separates the southern region of the island of Bangkulu, from a second group that includes the northern part of the island of Bangkulu and the rest of the Banggai Archipelago (Bernardi and Vagelli, 2004). In order to uncover finer genetic structure, and expand on the isolation by distance results described above, we performed an Analysis of Molecular Variance (AMOVA) using the entire dataset, and also a dataset that was restricted to the Bangkulu populations. Both datasets were subjected to sequential partitions, starting at the northernmost

**Table 2**
Gene flow among *Pterapogon kauderni* populations from the island of Bangkulu represented by *Nm* (above the diagonal) and $F_{ST}$ (below the diagonal).

| | BAK2 | BAK4 | BAK5 | BAK6 | BAK7 | BAK8 | BAK9 | BAK10 | BAK11 | BAK11G | BAK12 | BAK20 | BAK21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BAK2 | – | 3.8* | 5.2* | inf | 3.8* | 28.6 | 2.7* | 3.1* | 10.7 | 1.1* | 74.5 | 3.9* | 3.5* |
| BAK4 | 0.11* | – | 2.5* | 7.8* | inf* | 9.8 | 9.3 | 0.9* | 1.3* | 0.4* | 4.1 | inf | inf |
| BAK5 | 0.08* | 0.16* | – | 3.7* | 1.9* | 2.7* | 1.6* | 1.2* | 1.9* | 0.7* | 6.1* | 2.1* | 1.7* |
| BAK6 | 0.00 | 0.06* | 0.11* | – | 5.8* | 9.5* | 5.1* | 2.3* | 3.5* | 1.1* | 9.4* | 8.6* | 5.9* |
| BAK7 | 0.11* | 0.00 | 0.20* | 0.07* | – | 10.2 | 11.9* | 1.4* | 1.8* | 0.9* | 3.3* | 16.8* | 11.5* |
| BAK8 | 0.01 | 0.04 | 0.15* | 0.04* | 0.04* | – | 3.6* | 3.6* | 5.9* | 1.9* | 6.7* | 6.7* | 7.8* |
| BAK9 | 0.15* | 0.05 | 0.22* | 0.08* | 0.04* | 0.11* | – | 1.0* | 1.3* | 0.6* | 2.8* | 8.3* | 4.8* |
| BAK10 | 0.13* | 0.34* | 0.28* | 0.17* | 0.25* | 0.12* | 0.31* | – | 27.6* | inf* | 1.6 | 1.2 | 1.4 |
| BAK11 | 0.04 | 0.26* | 0.20* | 0.12* | 0.21* | 0.07* | 0.27* | 0.01* | – | 4.0* | 3.8* | 1.6* | 1.8* |
| BAK11G | 0.29* | 0.50* | 0.38* | 0.29* | 0.34* | 0.20* | 0.43* | 0.00* | 0.10* | – | 0.9* | 0.8* | 0.9* |
| BAK12 | 0.00 | 0.10 | 0.07* | 0.05* | 0.12* | 0.06* | 0.14* | 0.23 | 0.11* | 0.35* | – | 3.6* | 2.9* |
| BAK20 | 0.11* | 0.00 | 0.19* | 0.05* | 0.02* | 0.06* | 0.05* | 0.28 | 0.23* | 0.38* | 0.12* | – | inf |
| BAK21 | 0.12* | 0.00 | 0.22* | 0.07* | 0.04* | 0.05* | 0.09* | 0.25 | 0.21* | 0.35* | 0.14* | 0.00 | – |

Significant p values (significance level = 0.05) are indicated by*. *Nm* and $F_{ST}$ were calculated using ARLEQUIN version 2.000 (Schneider et al., 2000).

**Table 3**
Assigment test among *Pterapogon kauderni* populations from the island of Bangkulu.

| | Bak02 | Bak04 | Bak05 | Bak06 | Bak07 | Bak08 | Bak09 | Bak10 | Bak11 | Bak12 | Bak20 | Bak21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bak02 | 0.11 | 0.00 | 0.11 | 0.00 | 0.11 | 0.11 | 0.11 | 0.00 | 0.11 | **0.22** | 0.00 | 0.00 |
| Bak04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | **0.40** | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 |
| Bak05 | 0.04 | 0.04 | **0.54** | 0.00 | 0.07 | 0.00 | 0.00 | 0.00 | 0.04 | **0.18** | 0.00 | 0.00 |
| Bak06 | 0.00 | 0.00 | 0.06 | **0.71** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.18** | 0.00 |
| Bak07 | 0.00 | 0.08 | 0.00 | 0.00 | **0.51** | **0.10** | **0.10** | 0.03 | 0.00 | 0.00 | 0.08 | 0.03 |
| Bak08 | 0.00 | 0.12 | 0.00 | 0.00 | **0.12** | **0.39** | 0.02 | 0.10 | 0.05 | **0.12** | 0.02 | 0.02 |
| Bak09 | 0.07 | 0.00 | 0.04 | 0.00 | **0.19** | 0.07 | **0.59** | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 |
| Bak10 | 0.00 | 0.00 | 0.05 | 0.00 | 0.05 | 0.00 | 0.00 | **0.60** | **0.25** | 0.05 | 0.00 | 0.00 |
| Bak11 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.25** | **0.50** | **0.17** | 0.00 | 0.00 |
| Bak12 | 0.00 | 0.00 | **0.12** | 0.00 | 0.08 | 0.04 | 0.08 | 0.00 | **0.16** | **0.44** | 0.00 | 0.00 |
| Bak20 | 0.02 | 0.09 | 0.02 | 0.11 | 0.04 | 0.04 | **0.09** | 0.00 | 0.00 | 0.00 | **0.30** | **0.22** |
| Bak21 | 0.00 | **0.20** | 0.00 | 0.08 | 0.08 | 0.04 | **0.16** | 0.04 | 0.00 | 0.00 | **0.12** | **0.24** |

Bolded figures correspond to statistically significant values.

population (BAK6), which was compared to the remaining Bangkulu populations, by adding one population at a time, moving southward. Sequential additions were done separately on the west and east coasts. Along the east coast of Bangkulu, both datasets exhibited a strong genetic break between population 9 and population 2 (Fig. 1). Indeed, sequential partitions that separated populations north of that break accounted for a between-groups variance that ranged between 3.96% to 5.91% of the total variance, while the partition at the 9-2 break accounted for 8.43% of the total variance (9.72% of the total variance when only considering the Bangkulu samples). Along the east coast, a similar break was observed at the junction of populations 12 and 11 for both datasets (Fig. 1). Thus, the southernmost portion of the island of Bangkulu seems to be genetically distinct, and defined by populations 2, 10, and 11 (Fig. 1). Grouping the southernmost populations (2, 10, and 11) against the remainder of the populations explained 14.61% of the total variance (15.67% of the total variance when only Bangkulu samples were considered).

### 3.5. Assignment tests

Due to small sample sizes at the level of the Archipelago, our analyses focused on the island of Bangkulu, the main topic of our study. Yet, because dispersal is so restricted in *P. kauderni*, even with small sample sizes, some interesting results were obtained at the level of the Archipelago. Details can be obtained in the electronic supplementary materials. Briefly, at the level of the Archipelago, 67% of the individuals re-assigned to their original population, which is remarkable considering the limited sample sizes that were available in this analysis.

Within the island of Bangkulu, 10 out of 12 populations showed a statistically significant self-assignment (Table 3). For those 10 populations, re-assignment to their own population was found on average in 48.2% of the cases (range 24% for population 21, to 71% for population 06). In addition, some individuals were significantly assigned to other populations. For example, individuals from population 5 were significantly assigned to 2 populations, population 5 (54% of the individuals) and population 12 (18% of the individuals) (Table 3). In this case, populations 5 and 12 are adjacent to each other and are less than 1 km apart (Fig. 2). In all cases where assignment was significant and was in more populations than the source population, the newly assigned populations were either immediately adjacent to the source, or very close (less than 10 km) (Table 3).

## 4. Discussion

### 4.1. Population structure in the Banggai Archipelago

*P. kauderni* exhibit a very limited dispersal potential, which is likely due to a lack of pelagic larval stage and an adult philopatric behavior. These characteristics predict strong population structure, and indeed, this is what has been previously observed based on mitochondrial DNA (Bernardi and Vagelli, 2004), and microsatellite markers over a restricted geographic sampling (Hoffman et al., 2005). In this study, microsatellite analyses over the entire range of the species resulted in low overall levels of gene flow and assignment tests that consistently reassigned individuals to their source population. These results were consistent with the prediction of extreme philopatry and highly structured populations. This study, however, was not intended to be a general survey of the species, thus additional data will be necessary to properly characterize the population genetics of *P. kauderni*.

### 4.2. Introduced populations

*P. kauderni* has been accidentally or voluntarily introduced in northern Sulawesi, at Lembeh, and Tumback, in eastern Bali, at Gilimanuk Bay, and possibly elsewhere. The Luwuk population has also been suggested to be an introduced population (Bernardi and Vagelli, 2004; Hoffman et al., 2005). Assignment tests (see supplementary materials for details) show that 40% of Lembeh individuals reassign to Lembeh (probably due to a unique mix of geographically unrelated genotypes) as well as a low reassignment to the islands of Banggai (20%), Bokan (10%), where the introduced Lembeh individuals may have originally come from. The additional 30% individuals reassigned to the Luwuk population. Luwuk individuals are mostly reassigned to Luwuk itself (53% of the individuals), and to a lower degree to Lembeh (12%). Since we know that Lembeh is an introduced population (Erdmann and Vagelli, 2001), and that Lembeh and Luwuk populations partially reassign to each other, our data indicate that Luwuk may indeed correspond to an introduced population, unless Lembeh individuals originated from Luwuk, which is unlikely since no collecting is known to have ever taken place in Luwuk harbor. More samples are needed to determine with certainty if this is the case.

### 4.3. Population structure at Bangkulu Island

The goal of this study was to determine the level of population structure at very small-scale levels in *P. kauderni*. Our data suggest that *P. kauderni* exhibit strong population structure at the scale of the entire Archipelago, as indicated by the high level of self-reassigned individuals. Yet, the precise nature of this structure needs to be evaluated using larger sample sizes. At the finer scale of the island of Bangkulu, very strong structure was also observed. Classical population structure measurements ($F_{ST}$, $Nm$) were indicative of very low dispersal at a scale of a few kilometers at Bangkulu, with less than 5 migrants per generation between most populations (Table 2). In addition, the positive result of an isolation by distance analysis (Fig. 2), and the high level of self-reassignment, or of reassignment to adjacent populations that are only separated by a few hundred meters or a few kilometers at the most, is also consistent with an extreme degree of philopatry at a very small scale level.

### 4.4. Ecology and population structure at Bangkulu Island

The island of Bangkulu, while relatively small (60 km of perimeter), presents a series of microhabitats that are not always suitable for *P. kauderni*'s ecological requirements. The east coast of the island presents a nearly continuous suitable habitat, which is occupied by discrete (importantly, not continuous) populations of *P. kauderni*. In contrast, most of the west coast of the island presents cliffs and sharp drop offs that are not suitable for *P. kauderni* and, as expected, the species is absent from these areas (Fig. 1, green and red areas respectively). Considering the mitochondrial DNA genetic break observed between the southern region of Bangkulu and all other populations (Bernardi and Vagelli, 2004), we assumed that the physical barrier present on the west coast of the island could have played a role in isolating the south of the island from the rest of the range. The results from the current study confirm the findings of Bernardi and Vagelli (2004) by showing a genetic break between the south of Bangkulu and the other populations (AMOVA, $\Phi ct = 15.7$). However, the genetic break that is described here did not correspond to the predicted region on the west coast of the island (there is no evidence of sharp genetic break between population 6 and populations 5 and 12). Instead, the genetic break is found in the southernmost region of the island, between populations 5, 12 and population 11 to the west, and between population 9 and population 2 to the east. Since all populations have high degrees of genetic isolation (as shown by low gene flow levels and high reassignment values), it is likely that once populations are established by infrequent dispersal events, they start drifting with little secondary contact. Our results suggest that the southern part of Bangkulu may be difficult to colonize, thus having allowed this region to diverge in almost complete isolation for a very long time.

The combined evidence of a genetic break and isolation by distance are not contradictory. As mentioned above, it is likely that when dispersal occurs in *P. kauderni*, it is at the same time extremely rare, and over very short distances. This situation results in a pattern consistent with isolation by distance, yet, when in some points dispersal is particularly low, it also results in genetic breaks.

## 5. Conclusion

In marine systems, population structure at a given geographic scale is, in general, a poor metric to predict population structure at a different scale. This counter-intuitive phenomenon is usually explained by the chaotic behavior of the system. The presence of a very large fraction of the population in the pelagic larval phase, where oceanography, behavior, and mass mortality are difficult to predict, significantly contributes to the complexity of the system. Unequal contribution of parental populations to the next generation (Hedgecock effect), differential selection, chaotic patchiness, and other models, have all been evoked to reconcile the empirical data with the theoretical predictions. In *P. kauderni*, where the complex pelagic larval phase is removed, the structure found at the level of the Banggai Archipelago was also reflected by structure at very small scale. In that respect, these data add to the growing body of evidence gathered from the very few marine fishes which lack a pelagic larval phase, such as the black surfperch, *Embiotoca jacksoni* (Bernardi, 2000, 2005), and the spiny damselfish, *Acanthochromis polyacanthus* (Doherty et al., 1995, Planes et al., 2001). These species could therefore be the ideal platform to begin to understand the dynamics of dispersal in marine fishes, before tackling more complex systems.

The relative easy care of *P. kauderni,* combined with its appeal, has sparked a strong interest for the species in the pet trade. Its unregulated harvest for the international aquarium trade put such a strong pressure on the species in the wild that it has been proposed to be include under Appendix II of CITES at the CoP 14, and it has been favorably assessed to be listed in the IUCN Red List of threatened species. Human impact is already evident in the introduced population in the Lembeh strait, more than 400 km from its natural range. It is likely that other releases happened within and outside the Banggai Archipelago. The most recent release in Bali, is indicative of an ongoing trend. Tampering with such a threatened species (Allen, 2000) is truly unfortunate, as its extreme philopatry has created pockets of genetic uniqueness that are extremely vulnerable to unnatural gene flow. Management of this species needs, therefore, to take into consideration each locality as a separate genetic entity.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.margen.2009.01.001.

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