Marine Biology
August 2008, Volume 155, Number 2 : Pages 147-157
http://dx.doi.org/10.1007/s00227-008-1013-0

Genetic structure at different spatial scales in the pearl oyster (*Pinctada margaritifera cumingii*) in French Polynesian lagoons: beware of sampling strategy and genetic patchiness

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Abstract:

In order to study further the genetic structure of the pearl oyster *Pinctada margaritifera* in French Polynesia with a special consideration for the sampling scale, we analyzed or re-analyzed sets of data based on nuclear DNA markers obtained at different spatial scales. At a large scale (several 1,000 km), the remote Marquesas Islands were confirmed to be significantly differentiated from Tuamotu–Gambier and Society archipelagos, with a marked difference however for the two main islands that are different from each other. At a medium scale (several 10 to several 100 km), overall homogeneity was observed within and between these two archipelagos, with some exceptions. This could be attributed both to large-scale larval dispersal and to human-driven spat translocations due to pearl oyster cultivation. These results contrast with those observed (1) at a small scale (less than 10 km) in a lagoon heavily impacted by translocation and cultural practices, where significant genetic differentiation was detected among three laying beds, and (2) at a micro scale where we detected an important variability of the genetic composition of young spat recruited on artificial collectors. Such patterns could result from a high variance in the number of genitors at the origin of each cohort, or from pre- or post-settlement selection on linked loci. Altogether, our data support the hypothesis that under certain conditions populations of bivalves may exhibit patterns of chaotic genetic patchiness at local scale, in line with the increasing report of such patchiness in marine benthic organisms. This underlines the importance of sampling scale that should be rigorously defined depending on the questions to be answered. Nevertheless, a survey of about 80 articles dealing with population genetics of marine invertebrates showed that only 35% of those studies disclosed details about the sampling strategy (particularly the area explored). These results emphasize the need for cautious interpretation of patterns of genetic structure at medium scale when rigorous sampling strategies are not deployed.

Keywords: genetic patchiness; sampling strategy; benthic organisms; pearl oyster; bivalve; Polynesia; population genetics

Introduction

Genetic homogeneity over large geographic scales has been long expected, and sometimes observed, due to adult or larval mobility and to the absence of physical barriers to dispersal (Vermeij 1987). A large number of marine invertebrates exhibit large population sizes, external fertilization, high fecundity, an extensive pelagic larval phase, and a benthic adult stage. These characteristics lead us to an expectation of Hardy-Weinberg equilibrium as well as low genetic divergence, due to extensive gene flow during the larval stage, whereas the low mobility at the adult stage may favor local adaptation at the latest life stages.

Studies of the distribution of genetic variability of the black-lipped pearl oyster, *Pinctada margaritifera* from the Central Pacific, performed on samples collected in the 1980's, suggested a natural pattern of restriction to gene flow at both large (more then 1000km) and medium scales (200 to 400 km). For example, populations from the Society archipelagos are significantly differentiated, although separated by less than 200 km (Arnaud-Haond et al. 2003a). Previous studies, based on allozymes, even suggested the possible existence of genetically differentiated laying beds coexisting within the atoll of Takapoto (Blanc et al. 1985; Durand and Blanc 1986). However, studies performed on more recent samples showed homogenization of genetic pools of the archipelagos of Society and Tuamotu-Gambier (Arnaud-Haond et al. 2004). At the archipelago scale, this homogenization was attributable to the massive spat translocation in that area during 1990s. At the intra archipelago scale, within the Tuamotu-Gambier archipelago, where few samples were analyzed before the translocation events, it is more difficult to distinguish between the hypothesis of extensive natural gene flow at small spatial scale (several tens of kilometers) and the hypothesis of artificial gene flow linked to farming practices

(Arnaud-Haond et al. 2003b). In any event, further screening of isolated islands should be performed to identify possibly still divergent natural populations in atolls where no pearl culture is developed or no translocation has been performed, and which may represent interesting genetic resources in the perspective of future hatchery produced stock and selection programs. For *P. margaritifera*, at the intra lagoon spatial scale, as for other sessile species presenting pelagic larval stage (Roughgarden et al. 1988; Gaines and Bertness 1992), a very stochastic dynamic of spat recruitment has been reported in both space and time (Friedman et al. 1998; Friedman and Bell 1999). Whether this chaotic pattern is accompanied by spatial or temporal variance in the genetic composition of spat is still not known. Although no such data exist on *P. maxima* recruitment, some genetic data suggest the occurrence of localized heterogeneity in the genetic constitution of recruits, interpreted by the authors as resulting from large variance in the local reproductive success (Benzie and Smith-Keune 2006). Recent studies on *P. margaritifera* showed no significant difference in the genetic variability of wild samples and spat-collected samples from farms (Arnaud-Haond et al. 2003b). To what extent this observation reflects high genetic variability of locally collected spat, or high genetic variability of admixtures of distinct groups of collected spats in farms remains to be determined.

In benthic species for which migration is restricted to the larval stage, most species with lecithotrophic larvae exhibit more restricted gene flow compared with those with planctotrophic larvae, who remain longer in the plankton (Hunt 1993; Hellberg 1996; Poulin and Féral 1996; Hoskin 1997; Arndt and Smith 1998; Bonhomme and Planes 2000). However, in several invertebrate species that show substantial larval dispersal capability and large-scale genetic homogeneity, small-scale spatial and temporal genetic

patchiness has been reported (Johnson and Black 1984; Watts et al. 1990; David et al. 1997b; Johnson and Wernham 1999). The hypotheses invoked to explain such sub-structure of populations at small scale include both pre- or post-settlement selection of genotypes, as well as the different genetic origins of settling larvae (Johnson and Black 1982; Johnson and Black 1984; David et al. 1997a; David et al. 1997b) due to the stochastic recruitment in the sea (Roughgarden et al. 1988; Gaines and Bertness 1992). High variance in reproductive success, implying low effective number of genitors at the origin of a cohort, have already been reported in bivalves (Hedgecock 1994; Li and Hedgecock 1998; Boudry et al. 2002) and may favor the occurrence of genetically distinct pools of recruits at small geographic or temporal scales. A complementary explanation is the occurrence of differential selection in space and time favoring genetic differentiation of recruited cohorts. This hypothesis requires a strong genetic linkage between the markers used and some fitness component. The occurrence of post-settlement selection hypothesis was supported in *Littorina saxatilis* (Johannesson et al., 1995), and pre-settlement selection hypothesis has never been tested due to the technical difficulties in isolating and scoring very young larvae. It is therefore hard to distinguish between the hypotheses of distinct genetic origin of recruits: the synthesis of both hypotheses can be summarized as the “recruitment history” (Johnson and Black 1984; Watts et al. 1990; David et al. 1997b; Johnson and Wernham 1999). The existence of mosaic patterns at small scale, independent of the possible large scale homogeneity of population and gene flow among distant sites, underlines the importance of sampling scale for population genetics and biogeographic studies (Benzie 2000). The collection of samples representative of the populations studied is a pre-requisite to the interpretation of data in terms of gene flow, and may be influenced by the scale of sample collection compared to the area where the species collected is locally distributed. Yet, for most species studied so far, the difficulty of access to marine

128 environments and the lack of knowledge of the existence and scale of possible genetic
129 patchiness often hamper the application of a strict sampling strategy. In this context, the
130 interpretation of population genetics data in terms of gene flow may therefore often be a
131 difficult and risky exercise (Johanesson et al., 1995).
132
133 We used four anonymous nuclear markers to assess the pattern of genetic structure of the
134 Polynesian black-lipped pearl oyster populations at four spatial scales: among
135 archipelagos, among islands within archipelagos, among distinct sampling sites within an
136 island, and among artificial spat collectors within sampling sites. We addressed the
137 following questions: 1) At large and medium scale, among archipelagos and among islands
138 within archipelagos, can some genetically divergent populations still be detected in
139 isolated or non harvested atolls? 2) When sampled at micro-scale, do *P. margaritifera*
140 laying beds exhibit genetic heterogeneity? , 3) what are the possible consequences of
141 genetic patchiness for sampling strategy in population genetics and biogeographic studies?
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Material and Methods

2.1. Sample collection and DNA extraction

For large and medium-scale analysis, wild samples from fifteen geographic lagoons in French Polynesia, ranging from the western Society (Manuae) to the South-Eastern Tuamotu-Gambier (Mangareva) and the Northern Marquesas (Nuku-Hiva) were sampled by SCUBA-diving during 1999-2002 (Figure 1; Figure 2; Table 1). When possible, samples were collected in areas as large as possible, encompassing different laying beds, in order to collect a sample as representative as possible of the population studied. Details of the area encompassed by sampling are given in Table1 when available. Considering the difficulties in gathering samples from so many dispersed islands, we were able to get some samples thanks to the kindness of inhabitants, and information as to the detailed sampling area was not always possible to obtain. Eight of the fifteen samples (Manuae, Maupihaa, Arutua, Apataki, Manihi, Takaroa, Mangareva and Hiva Oa) have already been analyzed for all nuclear markers in recent studies (Arnaud-Haond et al. 2003a; Arnaud-Haond et al. 2004).

Small-scale study was performed within Takapoto lagoon (Tuamotu-Gambier): wild samples from three distinct zones were sampled by diving in May 2002 (Figure 2; Table 1), each in restricted areas of several m^2 in order to avoid mixing distinct beds. Finally, six spat samples of 42 to 50 individuals each were collected in February 2002 in three distinct collection stations (Figure 2; Table 1), representing different levels of spat density (high density and homogeneous distribution of spat on the station and collectors, mid-density and relative homogeneity, very low density and heterogeneity).

A piece of adductor muscle or gill (Raroïa) was removed from each specimen and preserved in 80% ethanol. The procedure of DNA extraction, precipitation and storage were similar to those described in Sambrook et al. (1989); we used approximately 0.5g of chopped and subsequently air-dried tissue. The nucleic acid pellet obtained after precipitation in 100% ethanol was washed with 70% ethanol, air-dried, resuspended in 100 to 200µl of deionised water and preserved at –20°C. DNA concentrations, obtained by fluorimetry, were found to be about 300ng/µl.

2.2. Polymerase Chain Reaction (PCR) and electrophoresis of anonymous nuclear loci

All samples were analyzed with four markers (Arnaud-Haond et al. 2002) developed using the DALP (Desmarais et al. 1998) and the EPIC (Palumbi 1995) methods. Although they have never been tested formally on controlled crosses and progeny, these markers are co-dominant and their previous use in natural laying beds supported the hypothesis they are Mendelian.

PCR was performed in a 20µl reaction volume with final concentrations of 300µM each dNTP, 1.8 mM $MgCl_2$, 0.4µM of each primer, about 30ng of template DNA, 1X Taq buffer and 0.75 units of Taq polymerase. In order to resolve length polymorphism, PCR products were separated through 6% denaturing polyacrylamide gels (acrylamide:bisacrylamide, 29:1, 7M Urea) using 1X Tris-Borate-EDTA buffer. The gels were then silver stained (Bassam et al. 1991). Ambiguities in genotype reading were checked by rerunning either the same or a new PCR product.

2.3. *Genetic diversity analyses, Hardy Weinberg equilibrium, linkage disequilibrium and genetic structure*

Genetic diversity within populations was estimated by unbiased (H_{nb}) and observed (H_{obs}) gene diversity (Nei 1987). We estimated the overall values for the inbreeding coefficient (F_{is}) as described by Weir and Cockerham (1984) and we used a permutation procedure (1000 permutations) to test whether a particular F_{is} value was significantly different from 0. The two-locus correlation coefficient R2 (Weir 1979) was estimated with the procedure of Black and Krafsur (1985), and its departure from zero was tested by a permutation approach.

Genetic differentiation (F_{st}) was estimated between pairs of populations with the estimator θ of Weir & Cockerham (Weir and Cockerham 1984). The significance of the θ values was tested by randomly permuting 3000 X the individuals between samples. Those calculations were performed using the GENETIX 4 package (Belkhir et al. 1996-2001).

2.4. *Variance of allelic frequencies*

In order to estimate the effective number of genitors at the origin of each spat sampled, the standardized variance in allele frequency change was estimated according to the theory of selectively neutral alleles in finite populations (Waples 1989):

$$\hat{F}_k = \frac{1}{k-1}\sum_{i=1}^{k}\frac{(x_i - y_i)^2}{(x_i + y_i)/2}$$

Where k is the number of alleles and x_i and y_i the allelic frequencies of the i^{th} of k alleles. A mean F_k across loci was weighted by the number of alleles at each locus, and N_e was estimated using the formula:

$$\hat{N}_e = \frac{t}{2\left[F_k - (1/2n_o) - (1/2n_t)\right]}$$

where t is the number of generations (we assumed one generation separated the spat from the putative pool of genitors), n_o the sample size at the generation 0 and n_t the sample size at the generation t. The confidence interval at 95% is estimated as:

$$\left[\frac{n\hat{F}}{\chi^{2}_{\alpha/2[n]}};\frac{n\hat{F}}{\chi^{2}_{1-\alpha/2[n]}}\right]$$

The estimation was made using the software *NeEstimator* (Peel et al. 2004) with all putative genetic pools of genitors: each of the three wild samples from Takapoto, the synthetic population obtained while pooling these three samples, and the synthetic population obtained while pooling the four samples from Tuamotu (Arutua, Apataki, Manihi and Takaroa).

2.5 Literature screening

A general search was performed in ISI (Web of Science) for the keywords [("population genetics" OR "genetic structure") and (benthic OR "marine invertebrate" OR "mollusks" OR "echinoderm" OR "bivalve" OR "gastropods")], which returned a list of 170 published studies. Although we were aware that this does not represent an exhaustive sampling of the existing work, we considered that this 'sampling strategy' was able to deliver a representative sample of the existing literature. Among those, 88 were found to actually deal with population genetics or biogeography of benthic organisms and 66 could be gathered and screened for details of sampling strategy. Information retained was classified as follows: A) as for the area explored 1/no information disclosed, 2/ details given about the area explored for each site and B) as for the strategy for choosing samples 3/ mention of the strategy as "random or haphazard" 4/ exhaustive sampling 5/ else when

samples collected were chosen according to a particular strategy such as the use of a transect or a grid.

Results

3.1 Level of genetic variability, Hardy Weinberg equilibrium, linkage disequilibrium

The level of unbiased gene diversity H_{nb} ranged between 0.30 (Manuae) and 0.45 (Kauehi) in natural laying beds, where the number of alleles was of 2.5 (Hao) to 5 (Hereheretue), and on collectors, the heterozygosity ranged from 0.29 to 0.40 and the number of alleles from 3.5 to 4.00 (Table 1).

Significant heterozygote deficiency values were observed in four of the six collector samples, and in five of the eighteen samples from natural laying beds (Table 1).

Linkage disequilibrium was observed to be significant between pinucl3 and pinaldo in one sample from natural population: Takapoto 3. All the remaining significant values concerned samples from spat collectors, and locus pinucl2: with pinucl3 for collector 3 and 4, with pinaldo for collectors 2 and 3, and with pinU4 for collectors 5 and 6.

3.2 Pairwise genetic differentiation

For samples from wild laying beds, pairwise genetic differentiation was analyzed at three geographic scales, among lagoons among archipelagos, among lagoons within archipelagos, and within lagoon. At the among archipelagos scale, despite the inclusion of samples from remote atolls from Tuamotu-Gambier that supposedly received no income of spat, no significant genetic differentiation was detected between the Society and Tuamotu archipelagos. As for the samples from the Marquesas archipelagos, the sample from Hiva Oa was significantly different from all other samples, except the ones from Makemo and

Hao. The sample from Nuku-Hiva, despite being more northern (but less eastern), was less differentiated from samples from the Northern Tuamotu-Gambier, but remained distinct from the southern part of the archipelago (Hereheretue, Marutea and Mangareva), and Society samples. At the within-archipelago scale, despite the inclusion of samples from remote atolls the values of F_{st} between pairs of samples for the nine wild populations from Tuamotu-Gambier were very low, and most were null (Table 4). The significant values mostly reflected the differentiation of the sample from the very isolated atoll of Hereheretue (Tuamotu-Gambier), one sample from the center of Tuamotu-Gambier (Makemo) as well as two samples from southern Tuamotu-Gambier (Marutea and Mangareva). The differentiation between the northern sample from Arutua (Northern Tuamotu-Gambier) and the samples from Takapoto3 and Marutea (Southern Tuamotu-Gambier) is also noteworthy. Finally, at the within-lagoon scale, Takapoto 1 and 3 are significantly differentiated from each other, despite being the geographically nearest samples and not showing any significant differentiation with the remote sample of Takapoto 2.

As for the collectors, more differentiation is observed, particularly for the collector 1, which is almost completely differentiated from all the other samples from laying beds or collectors from Takapoto (except collector 6), and from most samples from other Tuamotu islands. Collector 4, 3 and 6 are respectively differentiated of one (Takapoto 3), two (Takapoto 2 and 3) and all three samples from Takapoto natural population, whereas all collectors from 2 to 6 are not different one from the others, and not significantly different from any other Tuamotu samples.

3.3 Variance in allelic frequencies and estimation of the effective number of genitors.

The effective number of genitors estimated for each collector was low in any case, being estimated by considering as a putative mother population either : i) each of the three samples from Takapoto (from 3 to 93, except for one case with collector 2, for which no upper limit could be estimated if Takapoto 2 would be the origin of recruits), ii) the pool of those three samples (5 to 136), or iii) the pool of all other natural laying beds sampled in Tuamotu archipelago. However, significantly more important values can be observed while considering this last case (from 22 to ∞).

3.4 Results of the literature survey

Results of the literature survey are summarized in Figure 3. Among 66 articles screened about 65% did not report any indication as to the sampling area explored or distance among samples; among these none gave any indication of the strategy for choosing individuals (according some particular criteria like size for example), and only 8 of those 65% acknowledged a random or haphazard strategy for picking samples. Now, 35% of studies screened gave indication as to the area encompassed by sampling, or the distance among collected samples, and 90% of those documented studies also reported the strategy for picking up samples (17% reported an exhaustive collection, 65% a random choice and 9% other kind of strategy such as sampling along a transect or in a grid).

Discussion

Spatial distribution of genetic variability at large scale

At the large scale (more than 1500km), in agreement with previous studies (Arnaud-Haond et al., 2003a; Arnaud-Haond et al., 2004), the Marquesas samples, and particularly the one from Hiva Oa, display a significant level of differentiation with those from the two other archipelagos, reaching 8 to 10 % when comparing Hiva Oa with Society samples. Genetic differentiation of Marquesas populations is also evident in other species, and particularly well described for the surgeonfish *Acanthurus triostegus* (Planes et al. 1996; Planes and Fauvelot 2002). Two main phenomena are commonly invoked to explain this isolation. The first is the Marquesas countercurrent, which is regular throughout the year and which is opposed by the South Equatorial current; this might constitute a barrier preventing larval dispersal (Vermeij 1987; Planes and Fauvelot 2002). A second possibility is the influence of glaciations and co-occurring sea level drops and lagoon drainage (Paulay 1990), have led to extinction-recolonization of most inner-reef species in the lagoons; whereas the Marquesas islands might have represented refugia for some of those species. In the case of the pearl oyster, it is likely that populations persisted in Marquesas during the glacial episodes, whereas extinction and post-glacial (re) colonisation is suspected for other Polynesian archipelagos (Arnaud-Haond et al. 2003a). Although no pattern of monophyletism of Marquesas haplotypes was observed with mitochondrial DNA analysis (Arnaud-Haond et al. 2003a), the present data support previous findings that suggest a present day restriction to gene flow that may be partly attributed to the existence of current limiting exchange with other archipelagoes.

The distribution of genetic variation between the populations of the Tuamotu-Gambier archipelago supports the proposition that very little genetic differentiation exists

within this area. In part, this phenomenon probably reflects the occurrence of larval dispersal during the three-week pelagic larval stage of *P. margaritifera*. Yet, it is also partly attributable to the transfers practiced over the ten years preceding our last sampling campaign (Arnaud-Haond et al. 2004). The interpretation of the results obtained in terms of natural patterns of gene flow must then be performed with caution. Nevertheless, our data suggest the existence of only a slight restriction to gene flow at a medium scale (more than five hundred kilometers), and the occurrence of genetically differentiated stocks. Indeed, some isolated atolls do exhibit indices of genetic differentiation, for example: the atolls of Southern Tuamotu-Gambier (Marutea and Mangareva), and the isolated atoll of Hereheretue (Southern Tuamotu-Gambier). Given the scale of sampling for those populations (see Table 1), we are confident the samples are likely to be representative of the populations inhabiting those islands, and structure observed reflects restriction to gene flow rather than genetic patchiness.

It is striking that at the smaller scale, of less than 300 km (Table 4), much more differentiation (20% of the sample pairs) is observed than at medium scale (Table 2; 8% of the sample pairs). This discrepancy is however mostly driven by the genetic heterogeneity of samples gathered in very restricted areas: the ones from collectors, and one of the three Takapoto samples (Takapoto 3) that shows indices of slight differentiation with both Arutua and Takapoto 1 samples. These findings support the occurrence of recruitment patchiness at that scale, rather than long term and stable restriction to gene flow between those sampling locations.

Spatial genetic heterogeneity of recruitment

Both biotic and abiotic factors have been proposed as possible sources of the observed stochasticity as well as of the spatial and temporal patchiness of cohort recruits,

but the factors that may explain the genetic patchiness are still unknown (Johnson and Black 1984; Watts et al. 1990; McShane and Smith 1991; David et al. 1997a). The main observations that can be retained from the collector data include (1) the positive relationship between the spat density and its departure from HWE and (2) the negative relationship between spat density and genetic differentiation. All spat samples from high density collectors (C4, C5, C6) exhibit significant heterozygote deficiencies and linkage disequilibrium, but are not significantly differentiated from most other samples. On the other hand, two of the three spat samples from low-medium density collectors (C1, C3) show HWE and are responsible for most genetic differentiation observed, particularly the very low density one (C1 where all individuals have been sampled).

Recruitment is a spatially and temporally stochastic process: what is observed at one site might represent only a single recruited cohort; whereas, at the neighbour site, several distinct cohorts might have settled. When combined with the heterogeneity in the genetic composition of recruits (due to difference in the source of recruits or to pre-settlement selection), this suggests HWE and genetic originality may occur mostly in infrequent recruiting sites. In contrast recruited pools made-up of an admixture of distinct cohorts will exhibit linkage disequilibrium and departure from Hardy-Weinberg equilibrium due to Wahlund effect, but allelic frequencies more homogeneous with that of the population they come from.

The estimation of the putative number of genitors N_g at the origin of a given spat sample gives low values for all collectors, would we consider Takapoto or the whole Tuamotu Gambier as a putative mother population. Trying to interpret those values in terms of an absolute numerical estimation of N_g would be risky because these are calculated on the basis of *F*st estimates that may have a large error variance. However,

386 interesting information can be extracted from those data if one compares the different N_g
387 estimates involving different putative parents' genetic pools: a larger number is
388 systematically estimated while considering the whole Tuamotu- Gambier genetic pool as
389 putative parents' pool. This suggests that spat recruited in Takapoto represent an admixture
390 more representative of islands other than Northern Tuamotu-Gambier, and indicates that
391 the low number of parents at the origin of a given cohort is probably one of the factors
392 responsible for the heterogeneity of the genetic composition of recruits, as observed for the
393 closely related species P. maxima in Australia (Benzie and Smith-Keune 2006).

394 In conclusion, our data indicate that the spatio-temporal variability of spat
395 recruitment is accompanied by high spatio-temporal variability of the genetic composition
396 of recruits at local scale. Would this be an isolated observation, one may question the
397 generality of such finding, wondering about the possible influence of the large densities of
398 farmed stocks in Takapoto. However this fine scale "genetic patchiness", as described by
399 Johnsson et al. (1982, 1984), has been reported on a large range of non human-influenced
400 populations of marine invertebrates including gastropods (Campton et al. 1992; Johnson et
401 al. 1993; Holborn et al. 1994; Tatarenkov and Johannesson 1994), sea urchin (Watts et al.
402 1990; Edmands et al. 1996) and bivalves (Hedgecock 1994; David et al. 1997b; Benzie
403 and Smith-Keune 2006). Hedgecock proposed a "sweepstakes-chance matching
404 hypothesis", suggesting that in a heterogeneous and changing environment, young
405 succeeding in recruiting and surviving are the products of spawning by only a small
406 fraction of the adult population that spawned in the right windows of time and
407 environmental conditions. This hypothesis favours the importance of pre-settlement factors
408 at the origin of genetic mosaics in natural populations, rather than micro-environmental
409 post-settlement selection that has been demonstrated on one species of gastropods
410 (Johannesson et al. 1995). In other cases, the variation of the pattern over time as well as

411 the chaotic distribution of genetic structure in space, suggested that pre-settlement factors
412 were more likely than post-settlement micro-environmental selection (Johnson and Black
413 1982; Johnson and Black 1984; Watts et al. 1990). In our case, the lack of consistence in
414 space and the genetic differentiation of collectors located close to each other suggest that
415 the "sweepstakes-chance matching hypothesis" is more likely to explain the genetic
416 patchiness observed than a post settlement selective process.
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419 *Implication for sampling and interpreting data in population genetics and biogeographic*
420 *studies*
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422 This study illustrates a finding reported in several other benthic marine species: local
423 genetic structuring (several metres to several kilometres) sometimes reaching or exceeding
424 the differentiation reported at larger scale (hundreds to thousands kilometres). We have
425 highlighted that this apparent inconsistency is strongly related to the sampling design of
426 the study. The detection of genetic patchiness was only achieved by sampling in very
427 restricted areas compared to the ones that were explored (when such information was
428 available) when assessing large scale patterns and processes.
429 Chaotic genetic patchiness is now increasingly reported in marine invertebrates
430 (Jolly et al. 2003; Juinio-Menez et al. 2003; Casu et al. 2005; Virgilio and Abbiati 2006;
431 Virgilio et al. 2006; Andrade and Solferini 2007) and on fishes (Doherty et al. 1995;
432 Exadactylos et al. 1998; Planes et al. 2002; McPherson et al. 2003; Selkoe et al. 2006;
433 Burford and Larson 2007; Gonzalez-Wanguemert et al. 2007). Besides the evolutionary
434 and biological causes and implications of chaotic genetic patchiness, this phenomenon
435 becomes an issue when it comes to collecting a sample 'representative of the population

studied'. All marine biologists face the problem of access to samples that is increasingly difficult when studying a large-scale metapopulation system. It is seldom realistic to expect an extremely sophisticated, hierarchically designed, and standardized sampling scheme, particularly when working on sub-tidal and deep-sea organisms. Most of us have to be content with a "statistically satisfying" number of samples collected, sometimes including samples kindly provided by other researchers or local people, without the minimum information as to the habitat, area covered, density of populations, etc. As a result, only 33% of 68 studies dealing with population genetics of marine benthic organisms gave indication as to the area and strategy of sampling in each population, and 57% did not give any indication of either the area explored, nor the choice of samples collected. Technical and logistical difficulties may not allow much improvement in the sampling we, as researchers, will have access to. However, the occurrence of genetic patchiness should warn us against conclusions of genetic composition or limitations to gene flow based on blind samples that depending on some characteristics of the sampling sites (such as area explored or density) may reflect transient and localized genetic patchiness rather than significant and long-term restriction to gene flow among localities analyzed.

Acknowledgements

We would like to thank all the people who helped us to collect samples: Terii Seaman and Patrick Jilcot from the Pearl-Culture Service, Peva Levy, Claude Soyez, Marcel Matarere, Destremeau Poroi, Herle Gorager and Johan Hamblin from Ifremer and Philippe Shoun. We wish to address a very special thank to Gaby Hauamani, without whom none of the work on spat would have been possible, and Vincent Prasil for useful discussions and information about spat translocations. We thank Helen Boudry for help with the English in this manuscript, and three anonymous referees for their useful comments and suggestions. This work was supported in part by the "Contrat de développement n°2 2000-2003 entre l'Etat et le Territoire de Polynésie Française" and the BRG (Bureau des Ressources Génétiques)

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640 **Table 1 :** Sample size (N), geographic location in the lagoon (see Fig 2), number of

641 alleles, expected (H_{nb}) and observed (H_{obs}) heterozygosity, and heterozygote deficiency

642 (F_{is}) * significant after a permutation test (1000 permutations).

643

archipelagos	sample	Samples	area	N	Alleles Nb	H_{nb}	H_{obs}	F_{is}
Society	Manuae	MA	-	23	3.00	0.304	0.268	0.118
	Maupihaa	MP	-	40	4.25	0.364	0.266	0.273*
Tuamotu-Gambier	Arutua	AR	-	28	3.25	0.376	0.314	0.167*
	Manihi	MH	-	22	3.25	0.377	0.263	0.307*
	Apataki	AI	1 km^2	28	3.75	0.359	0.372	0.092
	Hereheretue	HERE	>10 km^2	27	5.00	0.397	0.369	0.071
	Takaroa	TA	-	30	3.50	0.339	0.303	0.108
	Raroia	RA	-	15	3.25	0.406	0.293	0.285*
	Kauehi	KA	-	19	3.75	0.451	0.3691	0.188
	Makemo	MK	-	28	3.25	0.410	0.366	0.109
	Hao	HAO	0.06 km^2	12	2.50	0.355	0.275	0.234
	Marutea	MRT	0.4 km^2	29	3.00	0.323	0.294	0.094
	Mangareva	MG	4 km^2	40	3.75	0.368	0.323	0.125*
Marquesas	Nuku Hiva	NH	>10 km^2	42	3.00	0.396	0.353	0.111
	Hiva Oa	HO	>10 km^2	31	2.75	0.389	0.378	0.029
Takapoto beds	Takapoto 1	Tkp1	1	37	3.75	0.342	0.212	0.383*
	Takapoto 2	Tkp2	2	54	3.75	0.346	0.300	0.138
	Takapoto 3	Tkp3	3	29	4.25	0.366	0.315	0.142
Takapoto collectors	Collecteur 1	C1	C	37	4.00	0.294	0.252	0.144
	Collecteur 2	C2	B	52	3.75	0.365	0.242	0.338*
	Collecteur 3	C3	B	41	3.50	0.382	0.374	0.021
	Collecteur 4	C4	A	50	3.50	0.360	0.240	0.283*
	Collecteur 5	C5	A	50	4.00	0.392	0.282	0.148*
	Collecteur 6	C6	A	50	4.00	0.404	0.344	0.336*

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Table 2: F_{ST} values for pairwise comparison among samples from wild populations from *P. margaritifera*, based on four anonymous nuclear DNA markers. Abbreviations for samples are as detailed in Table 1. Significant values after the 1000 permutation test are in bold.

Archipelagos	Tuamotu-Gambier											Society		Marquesas	
	AR	MH	AI	KA	TA	HERE	RA	MK	HAO	MRT	MG	MA	MP	NH	HO
AR	-	0.000	0.004	0.002	0.011	0.010	-0.005	0.001	-0.016	**0.031**	0.014	0.027	**0.024**	0.003	**0.049**
MH		-	-0.013	-0.016	-0.009	-0.014	-0.016	0.015	-0.018	0.015	0.014	-0.014	-0.004	0.011	**0.080**
AI			-	0.006	0.001	0.009	-0.012	0.013	-0.024	0.002	0.002	-0.008	-0.001	**0.025**	**0.085**
KA				-	0.006	-0.014	-0.013	0.012	-0.011	**0.040**	**0.031**	0.007	0.007	**0.020**	**0.080**
TA					-	0.009	-0.005	0.004	-0.014	0.005	0.015	-0.007	-0.006	0.011	**0.056**
HERE						-	-0.006	**0.029**	0.006	**0.048**	**0.042**	0.001	0.011	**0.032**	**0.105**
RA							-	-0.005	-0.029	-0.000	-0.005	0.005	-0.009	0.017	**0.069**
MK								-	-0.021	0.013	0.006	**0.030**	0.020	0.027	0.017
HAO									-	-0.015	-0.012	-0.008	0.007	-0.000	0.041
MRT										-	0.000	0.004	0.011	0.029	**0.067**
MG											-	0.023	0.023	0.016	**0.046**
MA												-	-0.007	**0.036**	**0.102**
MP													-	**0.022**	**0.078**
NH														-	0.014
HO															-

Table 3 : Significant correlations (R) between allelic frequencies at the different loci, per loci pair in all the spat and wild samples. *P<0,05, **P<0,01. No significant value was observed for the other natural populations.

Pair	Tkp3	C1	C2	C3	C4	C5	C6	Total
Pin2-pin3	-	-	-	**0.43****	0.09*	-	-	-
Pin2-Pinaldo	-	-	**0.19****	**0.29****	-	-	0.13**	-
Pin2-PinU4	-	-	**0.18****	-	-	0.20*	**0.18****	-
Pin3-Pinaldo	**0.26****	-	-	-	-	-	-	-
Pin3-PinU4	-	-	-	-	-	-	-	-
Pinaldo-PinU4	-	-	-	-	-	-	-	-

Table 4 : Detail of pairwise F_{ST} estimates of the genetic differentiation. Significant values after the permutation test (1000 permutations) are indicated in bold.

F ST	Ma	Ap	Tkr	Tkp 1	Tkp 2	Tkp 3	C 1	C 2	C 3	C 4	C 5	C 6
Arutua	0,000	0,004	0,012	0,009	0,019	**0,034**	**0,049**	0,009	-0,007	0,013	0,009	0,018
Manihi	-	-0,013	-0,010	-0,003	0,007	0,009	**0,028**	-0,012	0,000	-0,022	-0,002	-0,009
Apataki		-	-0,003	0,002	-0,005	0,003	0,012	-0,006	0,003	-0,002	-0,003	0,008
Takaroa			-	0,023	0,013	0,016	0,008	-0,001	0,001	0,001	0,003	-0,007
Takapoto 1				-	0,011	**0,029**	**0,054**	0,005	0,018	0,010	0,015	**0,033**
Takapoto 2					-	-0,005	**0,032**	0,006	**0,019**	0,015	0,000	**0,029**
Takapoto 3						-	**0,034**	0,014	**0,032**	**0,020**	0,009	**0,027**
Collecteur1							-	**0,023**	**0,023**	**0,039**	**0,026**	0,015
Collecteur2								-	0,007	-0,001	0,004	0,009
Collecteur 3									-	0,013	0,004	0,004
Collecteur 4										-	0,008	0,003
Collecteur 5											-	0,009
Collecteur 6												-

Table 5 : Estimation of the average (N_e), maximum (max, P<0.975) and minimum (min, P<0.025) number of genitors of the cohorts sampled in the different collectors $N_{e\,\mathrm{min}}^{\mathrm{max}}$, using as a putative parental group the samples from Takapoto 1,2,3, the average of these three samples, and the average of all samples from samples from Tuamotu (Apataki, Manihi, Arutua and Takaroa).

sample	N_e	Tkp 1	Tkp 2	Tkp 3	Takapoto	Tuamotu
Collecteur 1	37	16_{5}^{93}	13_{4}^{47}	11_{4}^{43}	18_{6}^{59}	57_{13}^{∞}
Collecteur 2	52	14_{5}^{59}	12_{4}^{36}	10_{4}^{33}	18_{7}^{48}	41_{12}^{382}
Collecteur 3	41	14_{4}^{79}	9_{3}^{23}	8_{3}^{21}	12_{5}^{31}	132_{17}^{∞}
Collecteur 4	50	15_{43}^{69}	12_{4}^{43}	11_{3}^{40}	20_{7}^{65}	79_{16}^{∞}
Collecteur 5	50	14_{4}^{49}	35_{6}^{∞}	15_{5}^{75}	30_{10}^{136}	37_{12}^{270}
Collecteur 6	50	11_{4}^{36}	9_{3}^{24}	9_{3}^{28}	13_{5}^{34}	46_{22}^{14381}

Figure 1: Localization of the Polynesian islands where samples were collected, on the three principals archipelagoes from French Polynesia: Society, Tuamotu-Gambier and Marquesas.

Figure 2: Map of the Takapoto lagoon, indicating the spat collection stations (with high and homogeneous density: A, and low and heterogeneous density : B and C), where samples were collected (respectively 3, 2, and 1 spat collector sampled). Sampling zones of wild beds are also indicated (1, 2 and 3).

Figure 3: Results of the literature survey for sampling details and strategy.

Appendix : list of articles screened for sampling strategy

Figure 1

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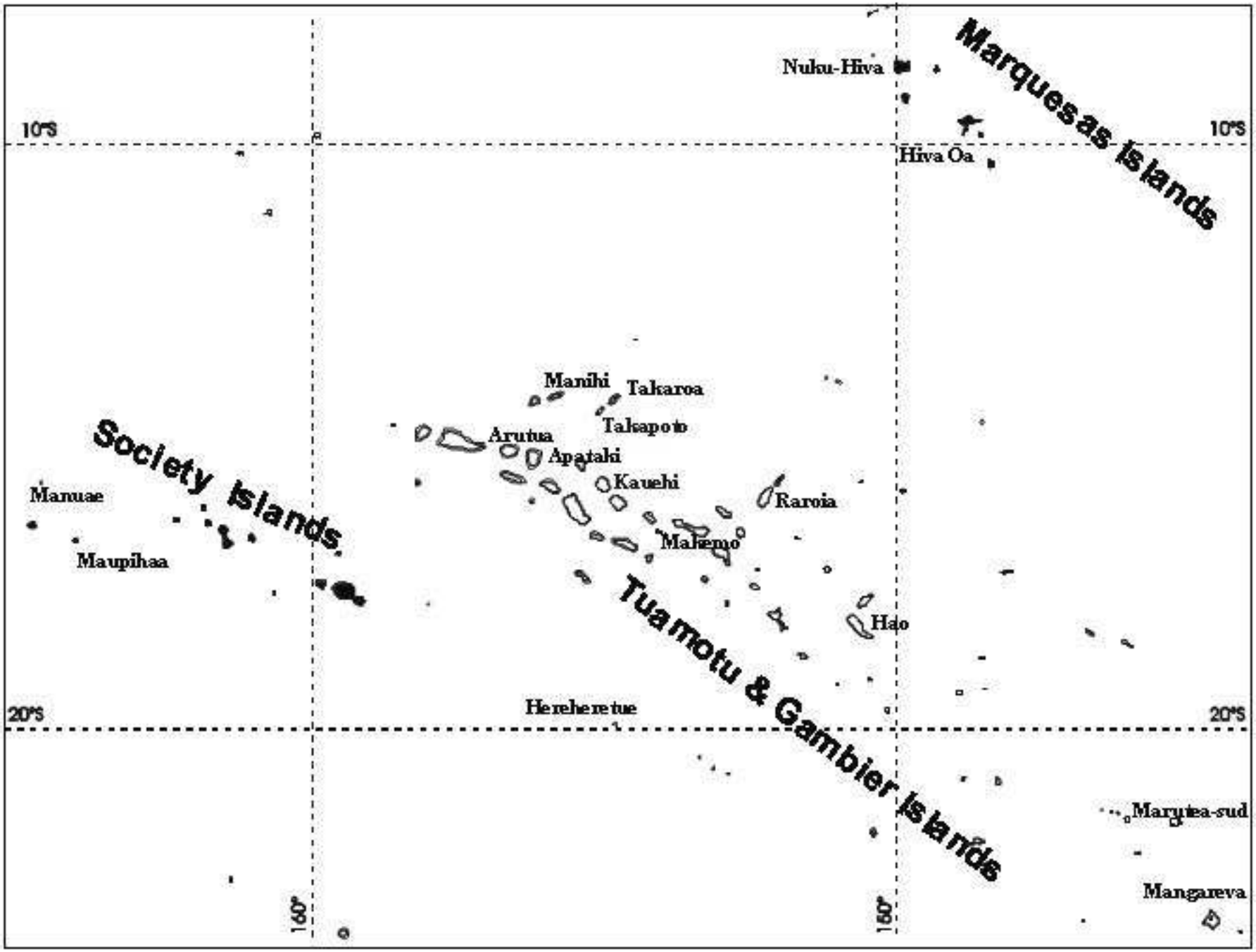

Figure 2

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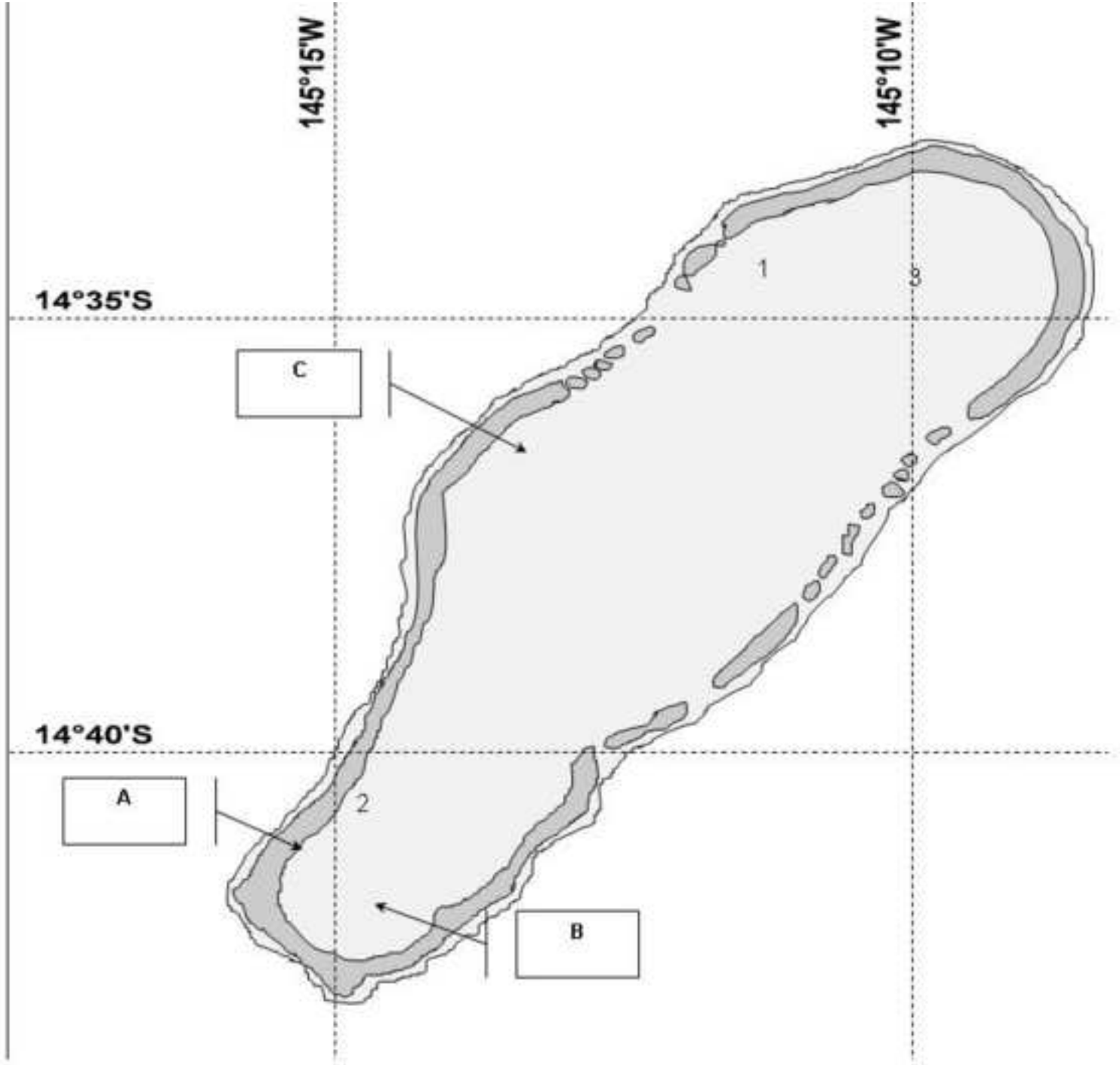

Figure 3

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