Origin, genetic diversity and population history of a marine population (Chanidae: *Chanos chanos*) in an enclosed lagoon in French Polynesia

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Abstract
Geomorphological features of atolls in the Tuamatu Archipelago (French Polynesia) created during the glaciation periods of the Pleistocene enable unique studies of evolution. Atolls not far from one another may be classic open atolls with water exchange between the ocean and the lagoon, or they may have an enclosed lagoon, without a direct ocean connection since at least the last glaciation (20,000 years ago). Niau’s atoll has an enclosed lagoon that hosts a milkfish (*Chanos chanos*) population whose origin is enigmatic. The milkfish’s co-occurrence with a tilapia species suggests a human introduction. However, there is no such record, and the people of Niau consider the milkfish population origin to be natural. The fish is used as staple food, it also plays a major role in several of their cultural traditions. We compared the genetic diversity and population history of the Niau’s milkfish to the one of a nearby open atoll, Kauehi, using a mitochondrial marker. Niau's milkfish exhibit a lower genetic diversity compared to the Kauhei population, suggesting that the population experienced a genetic bottleneck. However, the two populations are not differentiated, consistent with the hypothesis that Niau's milkfish population origin is human derived from the surrounding ocean population. Its smaller effective size suggests that this population has been self-sustaining for many generations. Ancient Polynesians were known for their transport of many species throughout the Pacific; this study would be the first case suggesting the transplantation of a marine fish. In addition, our results suggest that Niau’s milkfish population is large enough to limit inbreeding depression and seems to have been effectively managed over multiple generations by the local Polynesian communities.

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**Introduction**

Geomorphological features can create unique opportunities to understand processes that drive the evolution of species. This has been the case for geographically isolated African lakes where the water level changes interrupted or connected peripheral water bodies and as a consequence contributed to the generation of an enormous diversity of cichlid fishes (Genner et al. 2007). In the same vein, landlocked marine lakes in Palau led to the isolation of some jellyfish (genus *Mastigias*) populations leading to incipient speciation, morphological evolution and even behavioral adaptation (Dawson and Hamner 2003). The most well known case of this is the split between the Atlantic and Pacific Oceans following the rise of the Isthmus of Panama (3 million years ago) resulting in numerous studies of the early characteristics of allopatric speciation in marine sister groups (Lessios 2008, Rocha and Bowen 2008). The sea urchin *Diadema* is one of the most instructive models, showing how complete physical isolation can lead in a short time to a complete speciation event (Lessios 1981).

The small atoll of Niau, in the Tuamotu Archipelago of French Polynesia is similar to the cases described above in that its geomorphology provides a potential living laboratory for the study of evolution. The strip of land surrounding Niau lagoon is raised about 7.5 m from the current level of the ocean and the marine environment is characterized by a totally enclosed lagoon of around 30 km² with a low salinity (32g/l) due to resurgences of ocean water through the mass of the calcareous and karstic reef edifice (Figure 1). The average water temperature is about 30°C, and the bottom of the lagoon is completely filled with "kopara", made up mainly of cyanobacteria, marine algae, bacteria and detritus, which can reach several meters in thickness (Rougerie et al. 1997). The lagoon is shallow, with a maximum depth of 6 m and a mean depth of 2 m (Tröndle and Salvat 2010). The closure of this system dates back to 1-2 million years ago following a
swelling of the lithosphere in response to overload from rising volcanoes (Pirazzoli and Montaggioni 1988). Several studies (Planes et al. 1998; Bernardi et al. 2008) have shown that changing sea level during the Pleistocene in this area created physical barriers affecting the historical population demography of many resident marine species. The Pleistocene was characterized by frequent transitions between glacial and interglacial phases with the sea level variations reaching positive and negative peaks (Woodruff 2003). During the last peak of the Pleistocene glaciations (21,000 calendar years before present) the level of the Indo-Pacific Ocean was estimated at 120 m below present levels and all of the lagoons made by Polynesian reefs were dry (Bard et al. 1996; Dickinson 2004). This represented a severe bottleneck event for the populations of lagoonal ecosystems (Fauvelet et al. 2003). After the last peak of the Pleistocene glaciations the sea level started to rise, but not enough to submerge the Niau coral rim and reestablish a direct connection with the ocean. We can therefore date the formation and the closure of this lagoon to at least 20,000 years. Presently, only two fish species can be found in the lagoon: the milkfish *Chanos chanos* (Forsskål, 1775) and the Mozambique tilapia *Oreochromis mossambicus*; a freshwater fish that tolerates salt water (Peters, 1852) (Tröndle and Salvat 2010).

The presence of the tilapia (*Oreochromis mossambicus*) is not natural and its introduction in the 1970s to control mosquito density of the island has been documented (G. Remoissenet, *pers. com.*). However, there is no record of the introduction of *Chanos chanos* in Niau, and it could be viewed as a relic of geological closure of the lagoon due its habitat flexibility. *Chanos chanos* is a migratory, euryhaline species that is naturally distributed in the tropical and subtropical Indo-Pacific region (Bagarinao 1994) and is an important tropical marine fish for aquaculture. Its co-occurrence with a species of tilapia and the existence of attempts to build milkfish farms in two other atolls of the Tuamotu Archipelago (Keith 2002), suggest that the presence of milkfish may
be due to human introduction. However, the fishery department of French Polynesia has no record of any milkfish introduction program for Niau lagoon. Niau belongs to the UNESCO Biosphere reserve of the "Commune de Fakarava" and the transport of species has been highly restricted since 2006. The people of Niau consider the presence of this species as natural and have unique traditions associated with the milkfish. One such tradition involves catching and eating what is known as "pati opara", or milkfish whose stomachs are greatly distended as a result of grazing on algal floc (kopara). The stomach (opara) is highly prized for eating, and is considered the ‘caviar’ of Niau, by local people (Pickering et al 2012). This species is used as a staple food for Niau’s population and it was recently estimated that in the space of one year, 36.5 tonnes of Chanos chanos were fished in Niau lagoon (Pickering et al. 2012).

In this study, we investigated the genetic diversity and the demographic history of the closed population of Chanos chanos on Niau atoll, and compared it with another population from an open atoll, Kauehi, also within the Tuamotus. Kauehi differs from Niau by the presence of a deep pass, as well as shallow channels (called ‘hoa’) that facilitate exchange with the ocean, potentially facilitating gene flow. Cytochrome b analyses allowed us to compare the two populations and to specifically look for a genetic bottleneck signal, which we expected to find in the Niau population as a result of Niau’s isolation. We also gathered demographic history from Niau’s population of Chanos chanos to evaluate its likely origin.

**MATERIALS AND METHODS**

*Sampling, DNA extraction, PCR and sequencing*

A total of 88 samples of Chanos chanos were collected from the two lagoons in 2011 with the help of local fishermen; 53 samples from Niau, and 35 from Kauehi (Figure 1). A piece of caudal
fin tissue was collected from each fish for sequencing. DNA was extracted using the QIAGEN®
DX Universal Tissue Sample DNA Extraction protocol. Cytochrome b amplification was
performed using universal primers: GLUDGL, CB3H (Palumbi et al. 1991), GLUFISH (Sevilla
et al. 2007), and CytbH15573 (Meyer 1993). Thermal cycling of polymerase chain reactions
(PCR) consisted of an initial denaturing step at 94°C for 3 minutes, then 35 cycles of
amplification (25 sec of denaturation at 94°C, 25 sec of annealing at 52°C and 25 sec of
extension at 72°C), and a final extension of 3 min at 73°C. Forward and reverse sequences of
each sample were edited and aligned using Geneious Pro v5.1 (Drumond et al. 2010). All
sequences have been deposited in GenBank (Accession numbers MF592173 - MF592260).

<<Fig. 1 near here>>

Genetic diversity analyses
Haplotype diversity (h) and nucleotide diversity (π) were estimated with Nei's algorithms (1987)
implemented in the statistical software package ARLEQUIN 3.5 (Excoffier et al. 2010).
Genealogical relationships at the haplotype level were inferred using the median-joining network
as implemented in Network v. 4.611 (Bandelt et al. 1999). We examined levels of gene flow
among sampling sites by computing the pairwise FST value and its P-value in ARLEQUIN.
Demographic analyses were used to infer the evolutionary dynamic of the system, such as
whether the populations under study are in contraction, expansion or stable through time.
Demographic history of our populations was assessed using both neutrality test and Extended
Bayesian Skyline Plots. The signals of a demographic change are deviations from a constant
population size or selective neutrality, estimated here with summary statistics Fs (Fu 1997) for
each of the two sampling locations. Tests for significance were assessed using 10,000
simulations implemented in ARLEQUIN. Negative values of Fs show evidence of an excess of
low-frequency haplotypes, a signature characteristic of either selection or recent demographic expansion while significant positive $F_s$ values indicate a genetic subdivision, a contraction of the population, or diversifying selection.

Changes in female effective population size ($N_{ef}$) through time were estimated using the Extended Bayesian Skyline Plot (EBSP) implemented in BEAST v.1.7 (Drummond et al. 2005; Drummond and Rambaut 2007; Heled and Drummond 2008). EBSP is a non-parametric model that does not specify any prior hypothesis on tempo or mode of change for the Ne. Sequence divergence estimates for cytochrome b in reef fish range from 1% to 2% per million years (Bermingham et al. 1997; Bowen et al. 2001; Lessios 2008; Reece et al. 2010). We set a strict clock with a uniform prior distribution for the clock rate with an upper and lower mutation rate ranging from $0.5 \times 10^{-8}$ to $1 \times 10^{-8}$ per site per year. We used the HKY + G model of mutation and ran 10 million MCMC iterations with a thinning interval of 1,000. We checked convergence by visually inspecting the trace and computing the Effective Sample Size (ESS) for each parameter in two independent runs using the program TRACER 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). ESSs were always higher than 200. EBSP were plotted using R version 2.10.0 (R Development Core Team 2010) and ggplot2 package (Wickham 2009) setting the burn-in to 10%. The EBSP plot thus displays $N_{ef} \times$ generation time ($g$) together with the 95% credible interval (CI) on the y-axis and time in years on the x-axis. Moreover, we compute the posterior distribution of the number of demographic changes occurring along the gene genealogies, allowing us to test whether potential changes in the reconstruction of the demography of species were significant. We rejected a constant population size model if the lower bound of the 95% high posterior density (HPD) of this distribution was higher than zero. Finally, we computed a coalescent analysis with a constant size as tree prior (Kingman 1982).
implemented in BEAST when a constant population size model was not rejected to better estimate $N_{ef}$ parameter. While generation time is unknown for *Chanos chanos*, we conditionally used the equation $g = (\alpha + \omega) / 2$, where $\alpha$ is the age at first reproduction and $\omega$ is the age at last reproduction (Pianka 1978). We used a provisional generation time of 9 years based on existing life-history information (Bagarinao 1994) to retrieve female effective size $N_{ef}$.

**RESULTS AND DISCUSSION**

Sequencing of the cytochrome b control region provided an informative sequence of 769 bp for the 88 samples split into 9 different haplotypes. A summary of cytochrome b variation for each site is provided in Table 1. Genetic diversity indices show that in spite of having a greater number of samples (53 individuals for Niau vs. 35 for Kauehi), the Niau population had a lower haplotype diversity (62% vs. 68%, Figure 2a), and a lower nucleotide diversity (0.16% vs. 0.23%, Figure 2b) than Kauehi.

<<Table1 near here>>

<<Fig. 2 near here>>

The haplotype network (Figure 2c) supported by a non-significant Fst value (-0.00307, p-value = 0.42) shows homogeneity between the two populations from the different lagoons (i.e. atolls). The network shows the presence of two main haplotypes shared by 80% of all individuals together with 6 unique haplotypes from Kauehi and 2 unique haplotypes from Niau while only 35 (vs. 53) individuals were screened in Kauehi; yet the proportion of unique haplotype is not significantly different (P=0.07). The absence of a local segregation is highlighted by the presence of
some haplotypes embedded in a network separated by only a few mutations from central haplotypes.

The genetic neutrality tests (Fu’s Fs values) for both populations of *Chanos chanos* were not significant, indicating no significant departure from a constant population size. Albeit not significant, Fu's Fs values are opposed for the two populations, negative for Kauehi and positive for Niau. The EBSP analyses could not reject a constant population size model; it is illustrated by the constant effective size through time for both populations displayed graphically (Figure 3) that corroborated neutrality test results.

<<Fig. 3 near here>>

The estimate for the effective size of Niau's milkfish population is almost 3 times lower than the one for Kauehi. The coalescent analysis and the constant population size yielded a tree prior female effective size of 11,344 for Niau and 31,873 for Kauehi (median N_{ef} x g = 1.021 x 10^5 and 2.869 x 10^5 respectively). Using a more powerful approach (likelihood), we thus further confirmed the haplotype and nucleotide diversity trends. The smaller effective population size of Niau's milkfish population could suggest that this population has been self-sustaining for several generations in the smaller and enclosed lagoon of Niau. From a conservation point of view, Lynch and Lande (1998) estimated that a population was at risk of extinction when its effective population size was less than 500 - 1,000. Our results for the effective female population size (mtDNA is maternally inherited only) of the two populations range from 11,344 (CI: 2,513-25,904) for Niau to 31,873 (CI: 11,116-64,514) for Kauehi, suggesting that diversity in both locations is sustained by large populations.
The stability through time and the lack of differentiation between the two populations do not support the hypothesis that the populations of Niau and Kauehi are isolated from each other. Instead, samples from both locations appear to be part of a single large population, despite the fact that the Niau population occurs within a closed lagoon that has been isolated for at least the last 20,000 years. There are different scenarios that could lead to the lack of differentiation and the distinct genetic diversity found between the two samples. The most likely is to consider that the milkfish population in Niau is not a relictual population trapped in Niau's lagoon after the last major sea level change but was introduced by local Polynesian communities. There is no present day record of such introduction(s). In fact, if its origin is human derived, the smaller effective size for Niau population suggests that Niau's milkfish population has been self-sustaining for many generations and that the introduction(s) was (were) not done recently, potentially facilitated by the presence of this species around Niau, outside of the lagoon. Transporting and transplanting marine species has always been a part of Polynesian culture and ancient Polynesians did carry many species throughout the Pacific. While we have an abundance of evidence that Polynesians transplanted pearl oysters, troca shells, marbled turban, and probably other marine species (Gillett 1989; Arnaud-Haond et al. 2003), this would be the first case for the transplantation of a marine fish.

Another hypothesis would be related to the possibility that occasional storms could have breached the atoll wall, allowing a natural introduction of *Chanos chanos* in Niau's atoll since its closure. There are no records of such event in Niau, and we would have expected to retrieve a sign of population bottleneck in such case. It is worth noting that sea level has fluctuated over the
past thousands years, and 20,000 years ago actually represents a time when sea level was at its lowest, making this scenario less parsimonious.

The mitochondrial cytochrome b was chosen to investigate the presence of historical patterns and population differences between Niau and Kauehi atolls as this marker is widely used in phylogeographic studies because of its high evolutionary rates, small effective population size (Moore 1995), and lack of recombination (Avise 2000). Nevertheless, this study is based on a single locus and more loci could be needed to better describe demographic events that could have been missed due to a lack of statistical power and to improve demographic estimates such as the effective population size as they could reduce the coalescent variance (Felsenstein 2006, Brito and Edwards 2009, Wakeley 2009).

Results from this study suggest that the presence of *Chanos chanos* in the Niau's atoll is not linked to the closure of Niau 20,000 years ago but instead is the result of ancient Polynesians transplanting the fish into Niau’s waters. If these results were confirmed, this would be the first case documenting the transplant of a marine fish by Polynesians. The results also show the milkfish population in Niau, while smaller than the one from an open lagoon, is large enough to maintain diversity and to limit inbreeding depression, at least at the mitochondrial level. These findings suggest that the milkfish resource is preserved and managed appropriately by the local community.
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LITERATURE CITED


http://www.geneious.com


Gillett, R. 1989. Tilapia in the Pacific Islands: Are there lessons to be learned. FAO/UNDP Fishery Support Programme, Suva


**TABLE 1** Diversity indices from 88 sequences of Cytotchrome b region. Number of individuals (N), number of haplotypes (k), number of polymorphic sites (S), haplotype diversity (h), nucleotide diversity (\(\pi\)), Fu’s \(F_s\) value with corresponding P-value in parentheses.

<table>
<thead>
<tr>
<th>Sites</th>
<th>N</th>
<th>k</th>
<th>S</th>
<th>h</th>
<th>(\pi)</th>
<th>Fu’s (F_s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niau</td>
<td>53</td>
<td>5</td>
<td>6</td>
<td>0.6161 +/- 0.0566</td>
<td>0.001593 +/- 0.001142</td>
<td>0.52 (0.60)</td>
</tr>
<tr>
<td>Kauhei</td>
<td>35</td>
<td>7</td>
<td>14</td>
<td>0.6773 +/- 0.0667</td>
<td>0.002304 +/- 0.001519</td>
<td>-0.59 (0.42)</td>
</tr>
</tbody>
</table>
FIGURE 1. Sampled sites in French Polynesia, with satellite images of Niau and Kauehi atolls.
FIGURE 2. Genetic diversity and haplotype network of Niau and Kauehi populations. (a) Haplotype diversity, (b) nucleotide diversity and (c) median-joining network. Each haplotype is represented by a circle with its size proportional to the number of individuals bearing the haplotype over the whole data set.
FIGURE 3. Extended Bayesian Skyline Plots representing the median value of the $N_e \times g$ through time (plain lines) in years for the two populations of *Chanos chanos* and their respective 95% credible interval (CI) (dotted lines).