African Journal of Marine Science 2024, 46(3): 205–216
Printed in South Africa — All rights reserved

AFRICAN JOURNAL OF
MARINE SCIENCE
ISSN 1814-232X EISSN 1814-2338
https://doi.org/10.2989/1814232X.2024.2384454

Genetic consequences in the southern African endemic seabream *Polysteganus undulosus* (Sparidae) after eight decades of overfishing

CJ Oosthuizen[1,2]*, BQ Mann[3], P Bloomer[2] and TB Hoareau[2,4]

[1] *Resolve Evolve, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa*
[2] *Molecular Ecology and Evolution Programme, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa*
[3] *Oceanographic Research Institute (ORI), Durban, South Africa*
[4] *Present address: Reneco International Wildlife Consultants LLC, Abu Dhabi, United Arab Emirates*
* *Corresponding author, e-mail*: *carel.oosthuizen@up.ac.za*

The Critically Endangered seventy-four seabream *Polysteganus undulosus*, a slow-growing sparid that forms spawning aggregations off South Africa, faced heavy exploitation from 1910 until a fishery moratorium was put in place in 1998. Utilising temporal samples from 1962/1963 (mid-collapse) and 2005/2006 (post-collapse), we assessed genetic diversity at six microsatellite loci. Amplification success for archived samples was low (43%), necessitating a rarefaction approach, revealing a 40% decrease in allelic diversity. Significant genetic differences between recent and archived samples confirmed the impact of overfishing. Simulation studies indicated that missing genotypes did not affect these tests, validating the genetic differences. Using a coalescent-based approach, a 10-fold decrease in effective population size (N_e) was estimated over this 43-year period (N_e = 43.88 to 4.76). Simulations provided corrected N_e of 36.86, accounting for missing genotypes that were responsible for inflated values. Assuming 25% of pristine levels in the 1960s, pristine N_e values ranged from 147 to 176 in 1910, suggesting a 96.8–98.3% genetic decline over 88 years of exploitation. This study emphasises severe genetic consequences of overfishing on *P. undulosus* diversity and effective population size. The results provide vital genetic baseline data for recovery assessment and conservation efforts, as well as an analytical framework to evaluate the stock decline using partial genotyping data gathered from degraded archived samples.

Keywords: catch data, conservation, effective population size, genetic differentiation, microsatellites, missing data, rarefaction, temporal samples

Introduction

Overexploitation of marine resources is a long-standing issue (Jorgensen et al. 2007; Safina and Klinger 2008). Severe reductions of targeted fish populations caused by fishery-related activities need to be studied to determine the impact of these reductions on the genetic diversity of the species. Examples of reduced genetic diversity that was completely or partly fishery-induced have been observed in Maui's dolphin *Cephalorhynchus hectori maui* (Pichler and Baker 2000; Baker et al. 2013), New Zealand snapper *Pagrus auratus* (Hauser et al. 2002), brook trout *Salvelinus fontinalis* (Jones et al. 2001) and North Sea cod *Gadus morhua* (Hutchinson et al. 2003). In the case of the North Sea cod (Hutchinson et al. 2003), genetic diversity was determined from two temporally separated samples, from 1954 and 1998. A dramatic decrease in the genetic diversity was detected using nuclear DNA microsatellite markers for the period 1954–1970. Recovery of genetic diversity was observed for the period 1970–1998, and was ascribed to the arrival of new alleles into the population through migration.

The effect of overfishing in most cases is a decline in spawning stock biomass, resulting in an observed decrease in the age of maturity, since the larger individuals are constantly removed creating a situation where there is selection for earlier maturation (Beacham 1983; Kenchington 2003). As discussed by Furlan et al. (2012), drastically lowering genetic diversity within a species also decreases fitness on an individual basis by lowering sperm quality, reducing reproductive output, increasing juvenile mortality, as well as increasing susceptibility to diseases and parasites. The result of these changes, brought about by low or no genetic diversity, is a dramatic increase in the risk of extinction (Kenchington 2003; Furlan et al. 2012).

The seventy-four seabream *Polysteganus undulosus* has undergone a substantial decline over the past century owing to intense human exploitation (Garratt 1996), resulting in its classification as Critically Endangered on the IUCN Red List (Mann et al. 2014). Historical records and stock assessments of this southern African endemic provide compelling evidence of this decline. Catches of over 1 000 tonnes (t) per annum were recorded in the commercial linefishery in the early 1900s, and by the late 1990s catches had declined to <10 t per annum (Mann and Fennessy 2013). The first stock assessment was retrospectively conducted in 1996 (Chale-Matsua et al. 2001) based on biological and catch data collected during 1962/1963 (Ahrens 1964). This study showed

that the spawner biomass per recruit (SB/R) had already declined to 25% of pristine levels in the early 1960s, and by 1996 the SB/R was estimated to be <5% (Mann 2007).

Regulatory measures for *P. undulosus* were first implemented in 1985, including a minimum size limit of 25 cm total length (TL), a daily bag limit of five fish per person per day, and a 3-month closed season (from 1 September to 30 November). In hindsight this was a case of too little, too late (Mann 2007). The regulations for *P. undulosus* were revised in 1992 and, as no recovery in catches was apparent, the minimum size limit was increased to 40 cm TL (similar to the size at first maturity reported by Ahrens [1964]), the bag limit was decreased to two fish per person per day and the 3-month closed season was maintained (Mann 2007). However, based on the continued collapse of catches and the results of the retrospective stock assessment conducted in 1996 (Chale-Matsua et al. 2001), the fishery was finally closed with the implementation of a moratorium in 1998 (Mann 2007).

Polysteganus undulosus is associated with pinnacles and steep ledges on rocky reefs, in depths of 50–180 m, and while its historical distribution ranged from Maputo Bay, Mozambique, to False Bay, South Africa (Ahrens 1964; Garratt 1996), individuals are now seldom found north of St Lucia or south of Cape Agulhas since the dramatic stock collapse in the 1960s (Mann 2007) (Figure 1). Based on this evidence, and the fact that catch per unit effort (CPUE) had declined by more than 90% throughout its distribution (Penney et al. 1999; Griffiths 2000), a moratorium was placed on the capture of *P. undulosus* in 1998 (regulations promulgated in terms of the South African Marine Living Resources Act [Act No 18 of 1998]).

Several ecological and biological traits make *P. undulosus* highly vulnerable to exploitation. The most important of these is its predictable spawning aggregation behaviour on reefs, such as the Illovo Banks in the greater Durban area which were heavily targeted by local boat-based line fishermen throughout the 20th century (Garratt 1996; Penney et al. 1999; Griffiths 2000; Chale-Matsua et al. 2001; Mann 2007). These conspicuous aggregations occurred historically from August to October, reaching their peak in September following a northward migration to the warmer waters off KwaZulu-Natal (Ahrens 1964). Fish species that form such spatially and temporally distinct spawning aggregations have been well documented in the literature and are known to be particularly vulnerable to exploitation (Sadovy and Liu 2004; Golbuu and Friedlander 2011). The slow growth and late sexual maturity (at age 9 years and 650 mm total length [TL]) exhibited by *P. undulosus* (Chale-Matsua et al. 2001; Mann 2007), may also be factors that limit the population's ability to tolerate heavy exploitation and to recover quickly from exploitation. However, its long-life expectancy of over 20 years (Chale-Matsua et al. 2001; Mann 2007) and high female fecundity (1–3 million eggs), as well as southward dispersal of their eggs and larvae that utilise reefs along the southern and eastern Cape coasts as nursery areas, are elements that may provide some degree of resilience by replenishing the population over time (Ahrens 1964; Garratt 1996; Chale-Matsua et al. 2001; Mann 2007; Connell 2012).

The combination of population genetics and temporal sampling plays a crucial role in assessing the genetic decline caused by fishery-related activities (Hutchinson et al. 2003; Kinziger et al. 2015; Silva and Gardner 2015; Bryant et al. 2016). Examples from previous studies, such as those on New Zealand snapper (Hauser et al. 2002) and North Sea cod (Hutchinson et al. 2003), demonstrate how reduced genetic diversity can be partially or entirely attributed to fishing pressure. Temporally separated samples from 1954 and 1998 for North Sea cod (Hutchinson et al. 2003) revealed a significant decrease in genetic diversity (i.e. change in allele diversity, allele frequencies and heterozygosity) between 1954 and 1970, followed by a recovery attributed to migration introducing new alleles to the population. Overfishing often leads to declines in spawning stock biomass (Beacham 1983; Kenchington 2003; Furlan et al. 2012). It also results in a severe reduction in genetic diversity, impacting fitness by reducing sperm quality and reproductive output, increasing juvenile mortality as well as the susceptibility to diseases and parasites, thereby increasing the overall risk of extinction (Furlan et al. 2012). The result of these changes brought about by lowered genetic diversity is a reduction in adaptive potential and resilience and a dramatic increase in the risk of extinction (Kenchington 2003; Furlan et al. 2012; Laikre et al. 2021).

Large populations in general are expected to be at mutation-drift equilibrium, thus carrying large numbers of alleles when compared with small/reduced populations (Garza and Williamson 2001; Hutchinson et al. 2003). Genetic studies, utilising microsatellites, provide valuable insights into recent changes in genetic diversity, allowing us to better understand population dynamics and the effects of overexploitation on marine fish known to have large population sizes (Hutchinson et al. 2003; Kinziger et al. 2015; Silva and Gardner 2015; Bryant et al. 2016). Estimated effective population size (N_e) represents the number of breeding individuals of an idealised population with the same rate of genetic drift and the same amount of inbreeding as the population under consideration (Luikart et al. 2010; Hare et al. 2011). When studied over time, N_e can provide valuable insights into changes in genetic diversity crucial for effective management (Luikart et al. 2010; Hare et al. 2011; Wang et al. 2016; Marandel et al. 2019; Laikre et al. 2020; Hoban et al. 2021; Frankham 2021). Therefore, N_e is an increasingly important measure that can lay the foundation for conservation and management decisions (Marandel et al. 2019; Hoban et al. 2020; Laikre et al. 2021).

In this study, we compared historical levels of genetic diversity of *P. undulosus* during stock collapse (mid-collapse: 1962/1963) to more-recent samples collected more than 40 years later (post-collapse: 2005/2006). This was undertaken to determine whether the genetic diversity of the species had declined as a direct consequence of the high rate of exploitation that led to the collapse of this once highly abundant marine species. To do this, we used microsatellite DNA markers to calculate genetic diversity parameters and estimate N_e for both the 1962/1963 and 2005/2006 samples. Because of the challenges experienced with DNA extraction and microsatellite amplification from the archived samples (1962/1963), we used a simulation study to investigate the

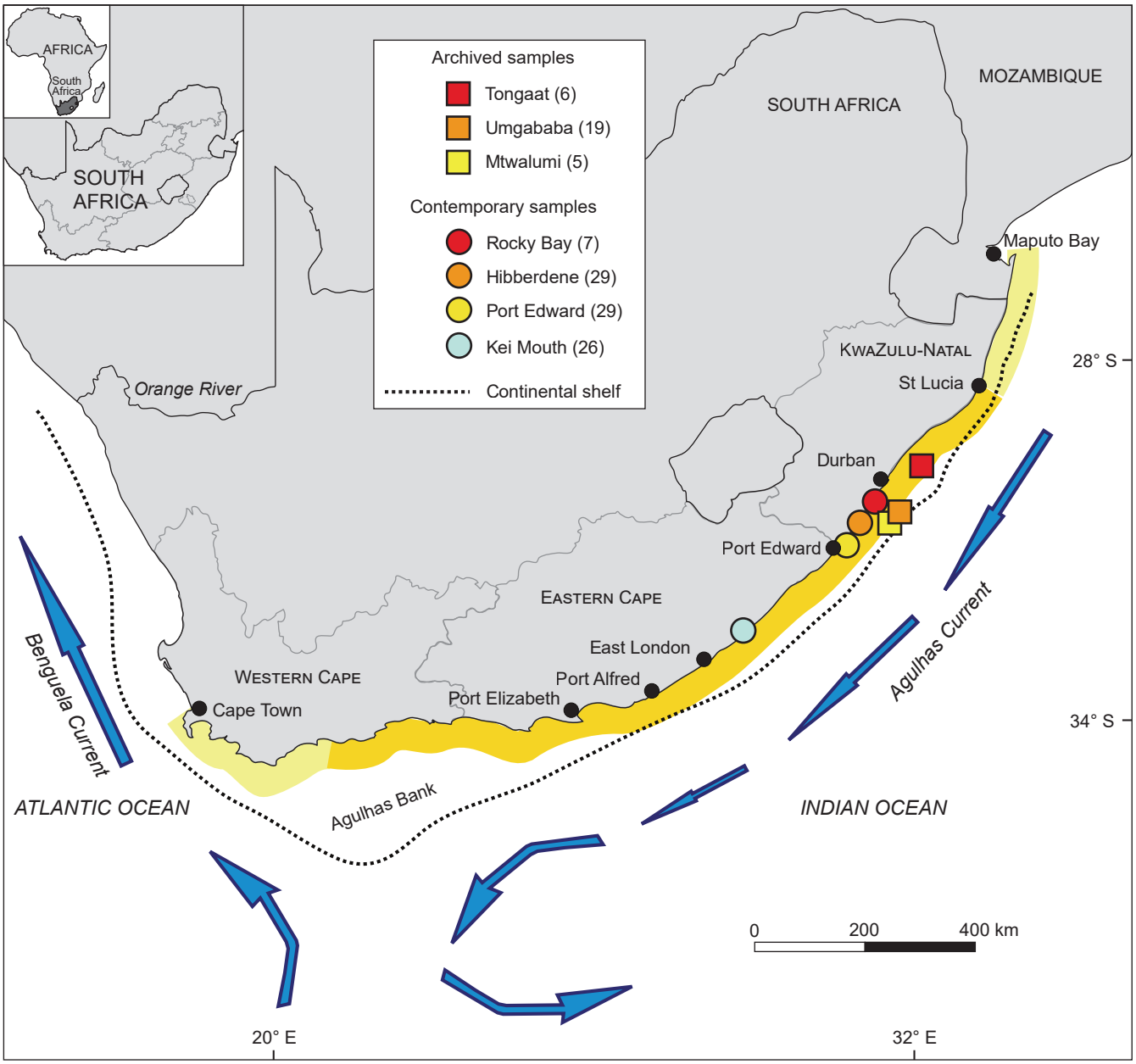

Figure 1: Map of South Africa indicating the sample locations, the distribution range of *Polysteganus undulosus* before (pale and dark yellow areas) and after (dark yellow area only) the fishery collapse (from Mann [2007]), and the prevailing ocean currents. Archived samples are from 1962/1963, and contemporary samples are from 2005/2006

effect that missing data have on the estimation of N_e. Since we expected that N_e would decline between the two time-points, we estimated N_e for samples from each time-period. Since population decline reduces genetic diversity, we expect the genetic diversity of post-moratorium samples to be significantly lower relative to pre-moratorium samples.

Materials and methods

Sampling

Archived scales of *P. undulosus* (n = 30) were collected as part of a biological study conducted in 1962/1963 for the purpose of age determination (Ahrens 1964). These samples represent the stock during the collapse phase, hereafter referred to as the mid-collapse/archived samples. At the time of this earlier study, well before genetic techniques were developed, a minimum of six scales were collected per fish, placed in a 2% solution of sodium hydroxide for 24 h, and then washed and placed in a 2% solution of sodium sulphite for several minutes before they were stored dry in individual paper envelopes (Ahrens 1964). This wash step was performed with the main aim of cleaning the scales for long-term storage, without knowing at that stage that future studies could be performed on the

genetic material obtained from dried and stored scales. The fish sampled by Ahrens (1964) came from Tongaat (n = 6), Umgababa (n = 19) and Mtwalumi (n = 5), located along the coast of KwaZulu-Natal Province, South Africa (Figure 1).

Forty years later, tissue samples of *P. undulosus* were collected for genetic analysis, as part of a stock assessment project conducted between 2005 and 2006 (Mann 2007). These samples (n = 91) represent data from the post-collapse/contemporary era. They were collected along the KwaZulu-Natal coast at Rocky Bay (n = 7), Hibberdene (n = 29) and Port Edward (n = 29), and from one location on the coast of the Eastern Cape Province at Kei Mouth (n = 26) (Figure 1). Small portions of muscle tissue (5 × 5 mm) were taken from the body wall of the abdomen after gutting and stored in 90% ethanol.

Molecular techniques

DNA was extracted for a panel of 30 archived individuals from the washed and stored scales using the forensicGEM™ DNA extraction tissue kit (BIOCOMbiotech). Other genomic DNA extraction methods that were performed without any success included DNeasy blood and tissue extraction kit (Qiagen), Wizard® genomic DNA purification kit (Promega), phenol/chloroform DNA extraction (Sambrook et al. 1989) and DNA extraction using Chelex (Estoup et al. 1996). Each of these DNA extraction techniques requires the fragmentation of the scale sample and has its own advantages and limitations, and the main differences lie in the principles behind their methodologies, as described below.

ForensicGEM™ DNA Extraction – Tissue (BIOCOM biotech) — This kit is specifically designed for forensic applications, particularly for extracting DNA from what can be considered challenging samples, such as degraded tissues or those with inhibitors. It typically utilises a combination of chemical and enzymatic methods to lyse cells and release DNA while removing contaminants. The main advantage is its robustness in extracting DNA from difficult samples, often encountered in forensic settings.

DNeasy Blood and Tissue Kit (Qiagen) — This kit is widely used for extracting DNA from various sample types, including blood and tissue. It utilises a silica-based membrane technology where DNA binds to the silica membrane in the presence of high salt conditions, while contaminants are washed away. This method is known for its simplicity, speed and ability to produce high-quality DNA suitable for downstream applications like PCR and sequencing.

Wizard® Genomic DNA Purification Kit (Promega) — Similar to the DNeasy kit, this kit is designed for the purification of genomic DNA from various sample types. It typically involves cell lysis followed by DNA binding to a solid support matrix and subsequent washing steps to remove impurities. The purified DNA is then eluted in a suitable buffer. The main difference may lie in the proprietary formulations and the specific protocols provided with the kit.

Phenol-chloroform DNA extraction — This traditional method involves organic extraction of DNA using phenol and chloroform. It relies on the differential solubility of DNA in aqueous and organic phases. After cell lysis, phenol is used to denature proteins and lipids, and chloroform is used to separate DNA from other cellular components (Sambrook et al. 1989). While effective, this method is labour-intensive, requires the use of hazardous chemicals, and is more prone to contamination compared with modern kits.

DNA extraction using ‘Chelex 100’ — Chelex resin binds divalent metal ions, which are necessary for many enzymatic reactions that degrade DNA. This method involves the incubation of the sample with Chelex resin, followed by boiling to release DNA while inactivating nucleases. Chelex-based extraction is simple, rapid, and suitable for small-scale DNA extractions, but it may not yield DNA of sufficient purity for some downstream applications (Estoup et al. 1996).

For the contemporary samples, total genomic DNA was extracted for a panel of 91 individuals using the DNeasy blood and tissue extraction kit (Qiagen) according to the manufacturer’s protocol. Care was taken to avoid contamination of these two groups of samples (i.e. the archived and contemporary samples). DNA extractions for these two groups were performed on different days; different sterilised pipette sets were used each time, and comprehensive cleaning of the workspace with a 10% bleach solution was performed before and after working with each group of samples.

Six species-specific polymorphic microsatellite loci (Appendix 1) were isolated as part of this project (GenBank accession numbers KY353254–KY353259) using the same methods as described in Reid et al. (2012). Six additional microsatellites were also included (Appendix 1) (Agata et al. 2011; Reid et al. 2012). The forward primers for these 12 primer pairs were fluorescently labelled with dyes for filter set G5: 6-FAM, VIC, NED and PET (Applied Biosystems). Fragment amplification was performed in a GeneAmp® PCR System 9700 (Applied Biosystems) using the QuantiTect multiplex PCR kit (Qiagen), according to the manufacturer’s instruction, using three multiplex combinations as indicated in Appendix 1. PCR amplifications of microsatellites were performed in 10-µl reaction volumes containing 0.2 pmol of each primer, 5 ul of the QuantiTect multiplex PCR kit (Qiagen) and 1 ul of extracted DNA. The final reaction volume was made up with water. PCR amplifications success was tested by using 2% agarose gel electrophoresis. Allele sizes were determined by comparing fragments to the LIZ™ 500 size standard (Applied Biosystems) using GeneMarker 1.5 software (SoftGenetics, USA) after fragment analysis on an ABI3100 automated sequencer.

Data analysis and simulations

Microsatellite scoring data were analysed using Micro-Checker 2.2.3 (van Oosterhout et al. 2004) for the detection of the presence of null alleles, large allele dropout and other scoring errors for the contemporary samples (2005/2006), but not for the archived samples with <60% missing data. To estimate these parameters, Micro-Checker relies on the statistical distribution of allele frequencies, the relative proportion of homozygotes and missing data. Unreliable amplifications like those observed on the archived samples disrupt these parameters, making the software’s assumptions highly biased and its results unreliable. This analysis was therefore not performed on the archived samples. The number of alleles per locus and the number of private alleles for each group of

samples (i.e. the 1962/1963 and 2005/2006 samples) were determined using GenAlEx 6.5 (Peakall and Smouse 2012). The observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated for both temporal samples, and linkage disequilibrium between loci was calculated for the contemporary sample using GenAlEx 6.5 (Peakall and Smouse 2012). A comparison of the genetic diversity parameters was performed between the archived samples (mid-collapse, 1962/1963) and the contemporary samples (post-collapse, 2005/2006) by using six microsatellite loci that successfully amplified in the archived samples. This comparison was achieved using a rarefaction method implemented in GENCLONE 1.0 (Arnaud-Haond and Belkhir 2007).

Rarefaction approach for comparing temporal samples and the evaluation of allelic diversity

To assess and compare the genetic diversity of *P. undulosus* between 1962/1963 and 2005/2006 while accounting for both missing data and difference in sample sizes, a rarefaction approach available in the program GENCLONE was employed. The mid-collapse samples consisted of 30 individuals, while the post-collapse samples comprised 87 individuals (that successfully amplified). This method involved the resampling of individuals multiple times, initially starting with two individuals and progressively increasing the group size until it reached the maximum number of individuals in each group (i.e. all 30 mid-collapse samples, and all 87 post-collapse samples). This rarefaction-based analysis provided a framework for evaluating and contrasting the genetic diversity between the mid-collapse (1963/1964) archived samples and the post-collapse (2005/2006) temporal samples, despite the difference in sample size and missing data. By extrapolating the number of alleles expected in each pseudo-sample, a standardised comparison of genetic diversity was obtained, providing meaningful comparisons between the samples.

Genetic differentiation between current and archived samples

We estimated F_{ST} between all pairs of samples collected from the different locations and across time to estimate the genetic differences between them. To do so, we used the unbiased Weir and Cockerham estimates available in the program Genetix 4.05 (Belkhir et al. 2004). The significance of these values was estimated using a permutation method based on 10 000 resamplings. We also applied a factorial correspondence analysis available in Genetix 4.05 to evaluate whether subtle genetic differences could be observed between locations among the post-collapse samples.

Decrease in effective population size (N_e) estimates over time

We used the isolation-with-migration model (IMa 2.0: Hey 2010) to estimate the effective population size N_e for both the mid-collapse and post-collapse samples. This program is based on coalescent simulations of gene genealogies that use metropolis-coupling Markov-chain Monte-Carlo methods to estimate the posterior probabilities of various parameters, including the mutational parameters of the ancestral and daughter populations (θ, θ_1 and θ_2), the divergence time (t), and the migration parameters ($m_{1,2}$ and $m_{2,1}$, denoting migration from population 2 to 1, and from 1 to 2, respectively). We chose the single-step mutation model which was the model of molecular evolution available in IMa2 for fitting the microsatellite data. Removing the parameters θ_2, t, $m_{1,2}$ and $m_{2,1}$ significantly improved the chain-mixing and the speed of the analyses compared with when all six parameters are estimated. This choice is relevant here as we have considered that the species is composed of a unique panmictic population, which is a fair assumption considering that *P. undulosus* aggregate in a single known spawning area during the reproductive season. The downstream tests of genetic structure among locations supported this assumption (see Results and Discussion).

We performed pre-simulation runs of the IMA model that helped set the upper bounds of the θ parameter prior to optimising the sampling of its posterior distribution. This provided us with an adequate burn-in period for each run, ranging from 2 to 25 million, and that allowed reaching stationarity distribution indicated by the absence of detectable trend lines. This is observed with the absence of autocorrelations, good effective sample sizes (ESS of >200) and clear marginal peak locations (no difference between first half, second half and the total datasets). With these priors set, we ran several independent runs for each sample set obtained either from the real datasets (mid-collapse versus post-collapse samples) or using simulations. Following the burn-in period, the parameter value θ was recorded every 1 000th generation. Next, the posterior probability modes were used as estimates of the parameters, and the highest posterior density at 90% (HPD90) as credibility intervals. We did not derive N_e estimates from θ considering that both are correlated following the equation $\theta = 4N_em$, and because we were interested in the relative difference of the estimates.

Effect of missing data on N_e for the archived samples

Because genetic content (number and quality of samples as well as the number of markers) may potentially have a drastic effect on the resolution of Bayesian estimates of N_e (Marandel et al. 2020), we developed a simulation study to evaluate this potential bias. To do so, we simulated genetic data with different levels of missing genotypic data using DIYABC 2.0 (Cornuet et al. 2014). The details of the coalescent simulations include the sampling of 87 samples at t_0 with N_e = 500, followed by a change in N_{e1} = 2 500 from t_1 = 20, and another change of N_{e2} = 10 000 from t_2 = 66, with an ancestral sampling of the population at t_2, with mutation rates of 10^{-5} for all microsatellites, and simulating eight microsatellites using the HKY mutation model. We then used the samples at t_0 to simulate missing genotypes by randomly removing a fraction of genotypes corresponding to the following proportions of missing genotypes: 0.00, 0.05, 0.10, 0.20, 0.40, 0.60, 0.80 and 0.90. All the simulated datasets had the same characteristics as the empirical data in terms of number and variability of the loci considered.

Comparison of N_e estimates to historical population levels and catch data

To illustrate the relationship between the level of exploitation and the N_e estimates, we analysed the catch

data for *P. undulosus* from 1900 (unexploited/pristine) up to when the fishery moratorium came into effect in 1998 (Mann 2007). Catch data served as an indicator of abundance within the vulnerable population and the corresponding trends in N_e across the two temporal samples. To provide a deeper historical perspective on the N_e estimates, we retrocalculated N_e for the pristine population in 1900 using a combination of catch data and N_e estimates from the 1960s (25% of pristine population) and 2005/2006 (5% of the pristine population: Mann 2007). By applying a rule of three to these known values, including the original population size and N_e in 1960, we derived the N_e estimate for the pristine population in 1900 (Mann 2007).

Results

Difficulty in amplifying markers and reliability of data from archived samples

DNA from a total of 30 samples was obtained for the 1962/1963 archived scale samples. The extraction of the contemporary samples from 2005/2006 was used for testing the utility of 12 microsatellite loci, 6 specifically designed for the species, and 6 others originating from cross-species amplification (Appendix 1). All the archived samples (n = 30) were genotyped and only a fraction of these samples (varying between 6 and 23 samples per locus) (Table 1) provided reliable genotypes. Only 6 of the 12 microsatellites successfully amplified in the archived samples. In addition to this, a significant proportion of the samples failed in providing genotypes for these 6 microsatellite loci, resulting in 56.7% of missing data. Genotyping for the same 6 loci (Table 1) in the contemporary samples (from 2005/2006) resulted in 27.83% missing genotypes due to low amplification success at only two loci, PBt011 and PU126 (Table 1). Three of these 6 loci were designed in *P. undulosus* (PU065, PU126 and PU165), whereas the other 3 loci were microsatellites developed for other sparid species (CL011, PA535 and PBt001: Reid et al. 2012) and were cross-amplified. Micro-Checker showed no evidence of scoring error caused by stutter, and no large allele dropout was detected for any of the loci. Low null allele frequencies were detected in all the loci, except for locus PU126 where no alleles were detected. Allele frequencies were adjusted for null alleles for all further analyses in the contemporary sample (2005/2006). Significant linkage disequilibrium was detected only between PU065 and PU165 (p = 0.007). The diversity of the microsatellite markers was moderate and ranged between 10 and 25 alleles per locus for all the samples combined.

Rarefaction approach for comparing temporal samples and the evaluation of allelic diversity

The results of the rarefaction analysis revealed a substantial decrease in allelic diversity over time (Figure 2). Specifically, when considering a standardised sample size of 30 individuals, the allelic diversity decreased by 40%, from 58 alleles in the archived sample (1962/1963) to 35 alleles in the contemporary sample (2005/2006).

Genetic differentiation between current and archived samples

When comparing the archived (1962/1963) and recent (2005/2006) samples, significant genetic differentiation (p-values of <0.001) was observed, with F_{ST} values ranging between 0.196 and 0.238 (Table 2), indicating drastic alterations of the genetic make-up of the population over this period. However, the 2005/2006 samples originating from different locations during the post-collapse era exhibited no detectable spatial genetic structure, as shown in Table 2 and Appendix 2.

To evaluate whether missing data could explain the significant level of genetic differentiation, we used a simulation study that mimicked samples with an increasing fraction of missing genotypes in order to compare this to the samples with no missing genotypes. No genetic differentiation arose from this comparison, even for cases where the fraction of missing genotypes was >50% (Table 2), which is close to the fraction of missing genotypes characterising our 1962/1963 dataset.

Decrease in effective population size (N_e) estimates over time

Using IMa2 to approximate the effective population sizes (N_e) of *P. undulosus* and provide insights into the changes in N_e over time, our analyses reached stationarity with a burn-in period ranging from 2 to 25 million steps, as illustrated by: (i) absence of detectable trend lines; (ii)

Table 1: Six polymorphic microsatellite loci used for comparing genetic variation between archived samples (1962/1963) and contemporary samples (2005/2006) of *Polysteganus undulosus* from the South African coast. Values in bold font represent loci not in Hardy–Weinberg equilibrium (p < 0.05). A_P = number of alleles specific to each sample group; H_E = expected heterozygosity; H_O = observed heterozygosity; N = number of samples successfully amplified for each locus; N_A = number of alleles detected for each locus in each sample group; N_S = number of alleles shared between the two groups; N_T = total number of all alleles for each locus

	Archived samples 1962/1963					Contemporary samples 2005/2006						
Locus	N	N_A	A_P	H_O	H_E	N	N_A	A_P	H_O	H_E	N_S	N_T
CL011	6	5	2	0.00	**0.78**	84	6	3	0.42	0.41	3	11
PA535	10	10	3	0.20	**0.88**	86	15	8	0.86	0.89	7	25
PBt011	11	11	9	0.36	**0.88**	9	2	0	0.00	**0.20**	2	13
PU065	23	16	14	0.65	**0.90**	87	3	1	0.36	0.31	2	19
PU126	17	6	4	0.29	**0.74**	37	4	3	0.81	**0.60**	2	10
PU165	11	9	2	0.73	**0.84**	87	9	2	0.64	0.71	7	18
Total		57	34				38	17				

absence of autocorrelations; (iii) good effective sample sizes (ESS of ~200); and (iv) clear marginal peak locations. The estimated N_e for the 1962/1963 sample group was 43.88 (95% confidence interval: 28.13–81.83). In contrast, N_e for the 2005/2006 sample group was significantly lower at 4.76 (95% confidence interval: 3.01–6.96). There was no overlap in confidence intervals for these two estimates, confirming the drastic difference in N_e between the two periods.

Missing data alone cannot explain the inflated N_e for the archived samples

The results of the simulation study reveal that, as the amount of missing data increases, the N_e estimates become inflated (Figure 3). However, this inflation effect was substantial only when the amount of missing data exceeded 60%. Below this threshold, the bias in N_e estimates remained below 20% of the true value. The relative difference of N_e to the true value increased exponentially

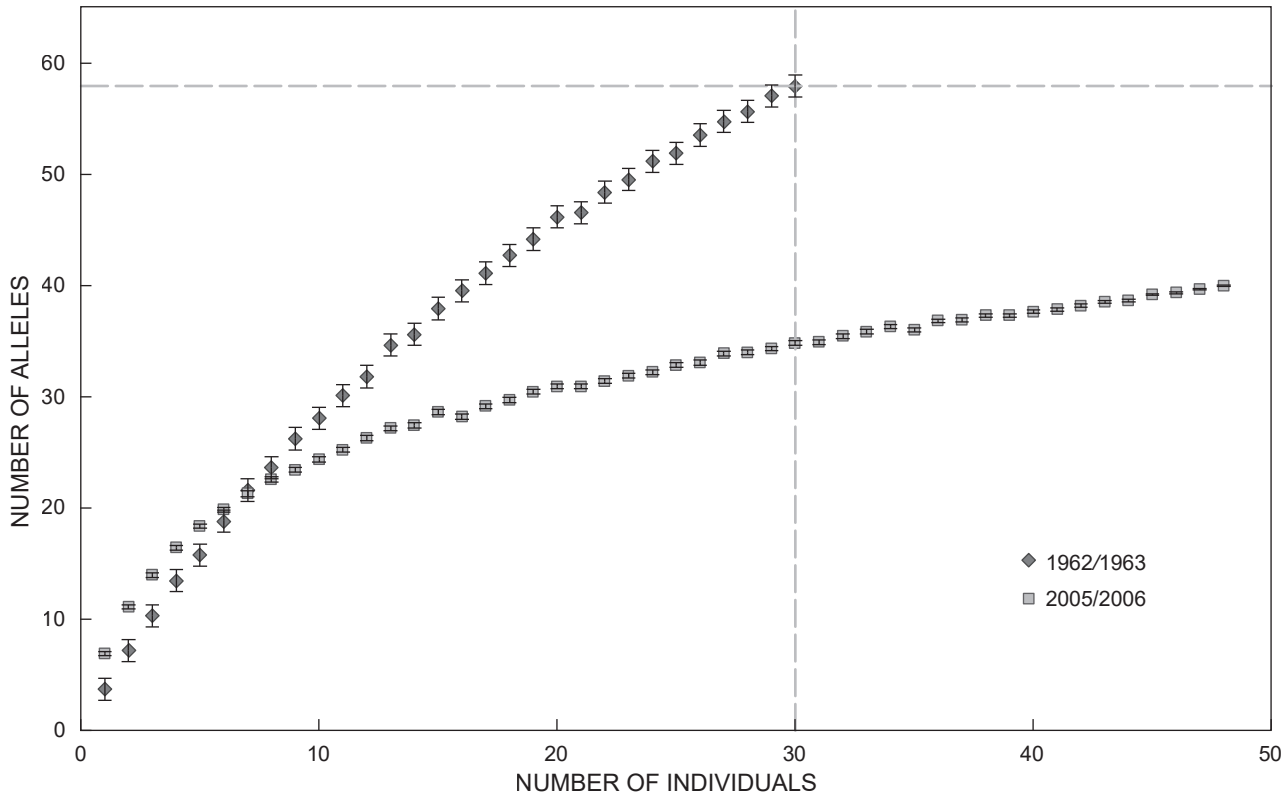

Figure 2: Changes in the number of alleles with an increasing number of samples, calculated using a resampling method (GENCLONE 1.0) to compare the genetic diversity of archived (1962/1963) and recent (2005/2006) sample groups of *Polysteganus undulosus* characterised by large difference of sample sizes. Resampling method based on 30 individuals provided 35 alleles in the 2005/2006 samples (1.0% missing data), and 58 alleles for 1962/1963 (56.7% missing data). Error bars represent the confidence interval

Table 2: Estimated pairwise F_{ST} calculated according to Weir and Cockerham (1984) for the contemporary samples (2005/2006) of *Polysteganus undulosus* from South Africa, by sample locations, including (a) comparison with the archived samples from 1962/1963, and (b) comparison among simulated data, which illustrates the effect of missing data on genetic differentiation. Note that the F_{ST} values remain the same with an increased amount of missing data. The symbol *** indicates significance at $p < 0.001$

(a)		Contemporary samples 2005/2006			Archived samples 1962/1963
		Hibberdene	Port Edward	Kei Mouth	
Contemporary samples 2005/2006	Rocky Bay	0.013	−0.004	−0.006	0.210***
	Hibberdene		0.004	0.005	0.196***
	Port Edward			−0.007	0.224***
	Kei Mouth				0.238***
(b)		Amount of missing data			
		Sim. 5%	Sim. 10%	Sim. 20%	Sim. 50%
Simulated dataset	Sim. 0%	−0.016	−0.016	−0.016	−0.014

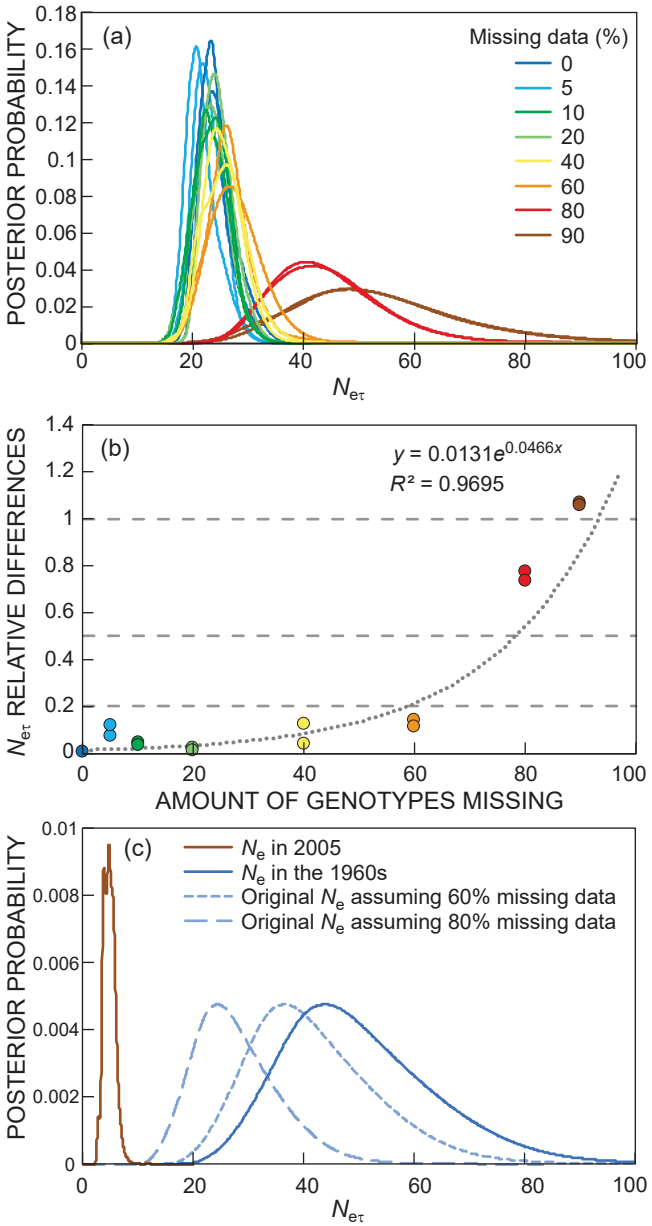

Figure 3: Estimates of the effective population size (N_e) of *Polysteganus undulosus* using simulated and empirical datasets. (a) N_e estimates of simulated datasets for varying levels of missing data, ranging from 0% missing data to 90% missing data. (b) Relative difference between estimates and values of N_e used to simulate the dataset according to the increasing amount of missing data (from 0% to 90%); the coloured circles represent the level of missing data. (c) N_e estimates for the 1962/1963 and 2005/2006 samples. (Note: N_e estimates for the 1962/1963 samples are shown as is and with correction assuming 60% and 80% missing data)

after the 60% threshold, crossing 100% when missing data were >90%.

Using the results of the simulation study conducted on our observed N_e estimates, we assessed the bias in our estimates caused by the amount of missing data in our mid-collapse samples (57% missing data). For this amount of missing data, the bias is close to 16% inflation, which means that the retrocalculated expected value (unbiased) of N_e is 36.86. This new value is marginally different from the original N_e of 43.88 and remains far greater than the post-collapse N_e estimates, yet their probabilities were still non-overlapping.

To compare N_e estimates to historical population levels and catch data, the pristine N_e was calculated to be 161.4 for 1900, using the assumption that the stock was 25% of the pristine population in the 1960s (Mann 2007) with an N_e of 36.86 (Figure 4).

Discussion

Despite challenges in amplifying genotypes from archived samples of *P. undulosus*, our study reveals a clear temporal decline in genetic diversity parameters, significant genetic differences between temporal samples, and a substantial decrease in N_e over 43 years. We show that missing data could not explain the results obtained, highlighting the robustness of our findings. This decline underscores the impact of overfishing on genetic diversity and the potential effect on adaptability, which emphasises the need for effective conservation measures.

Amplification success of archived samples

A key challenge encountered while investigating the consequences of overfishing on the stock of the Critically Endangered sparid *P. undulosus* was the difficulty of amplifying markers and obtaining reliable data from archived scale samples. While preparing the scales for ageing as part of the study by Ahrens (1964), the chemical treatment applied included an immersion step with 2% caustic soda (NaOH) solution for 24 h. Caustic soda is highly corrosive and will lead to the denaturation and hydrolysis of DNA (Gates 2009; Wang et al. 2014). This explains the low yield and DNA fragmentation of the samples. Despite these challenges, genotypic data from a limited number of archived samples (n = 30 fish) were obtained. Only 43% of the loci tested provided reliable data, and using these loci, the samples still had a significant proportion of missing genotypes (56.7%). To evaluate the robustness of the genetic inferences made from these data, we also designed a strategy to evaluate the effects of missing data on these inferences.

Substantial temporal decline in genetic diversity in Polysteganus undulosus

The present study is the first to investigate the genetic consequences of overexploitation on the population of *P. undulosus*. The results demonstrate a drastic genetic decline that was consistent with the heavy exploitation experienced during the 20th century (Mann et al. 2014). Despite the limitations of the current study, which include a small sample size of only 30 archived individuals and a significant amount of missing data, the decline in genetic diversity over time was still detected using innovative approaches. The first approach involved a rarefaction procedure that consisted of random resampling of individuals multiple times. By doing this, pseudo-samples with standardised sample sizes were created using the program GENCLONE (Arnaud-Haond and Belkhir 2007),

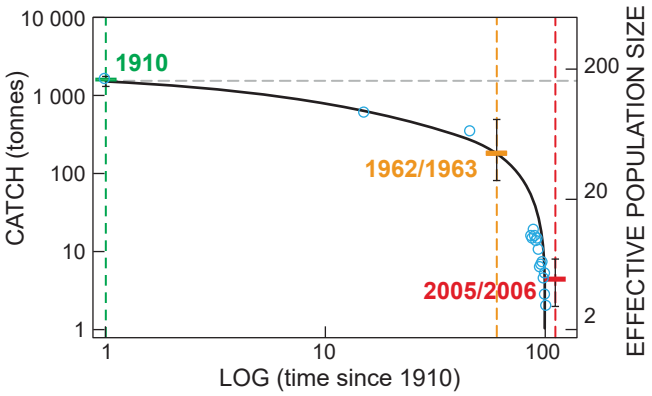

Figure 4: Catch data since the start of commercial exploitation of *Polysteganus undulosus* in 1910, and estimates of effective population size (N_e) (with 95% confidence intervals) in 1962/1963 and 2005/2006, as well as for the pristine population in 1910 (indicated in green and by the horizontal grey dashed line). This value was calculated assuming that the mid-collapse estimate in 1962/1963 represents 25% of the original pristine N_e

and these pseudo-samples allowed for comparison of the mid-collapse samples (n = 30) to the post-collapse samples (n = 87). It revealed a 40% decrease in allelic diversity between the archived and contemporary samples (Table 1). Our results illustrate that many unique alleles present in the archived samples have likely been lost in the current population due to a strong, lasting bottleneck between the mid-collapse and post-collapse periods. This is indicative of the substantial decline in genetic diversity that happened over the 88 years of exploitation during the 20th century. The decrease in genetic diversity for *P. undulosus* is significant when compared with the findings of other studies that likewise investigated declines in marine fish species. Pinsky and Palumbi (2014) compiled data from 140 fish species, revealing that overharvested populations exhibited, on average, 12% lower allelic richness compared with species where overharvesting was not recorded. As part of their research, Pinsky and Palumbi (2014) estimated that the 12% loss in allelic richness was underestimated by a factor of three (for an actual loss of allelic richness of 36%) to four (actual loss at 48%), which is clearly illustrated by the 40% loss in allelic richness observed in *P. undulosus*. These results highlight the scale of the detrimental effect of overexploitation on the genetic diversity and the adaptability of *P. undulosus*.

Temporal genetic differences between the mid-collapse and post-collapse samples

Significant genetic differences between temporal samples were observed which further indicates a genetic decline in the population. These differences were expected owing to the change in genetic diversity resulting from the strong genetic drift linked to the bottleneck, while spatial genetic differences were not anticipated in this case. A well-documented example of overexploitation leading to genetic differences within the same area in marine fish involves the Atlantic cod *Gadus morhua* in the northwestern Atlantic (Hutchinson et al. 2003). The Atlantic cod was heavily overfished for centuries and this intense fishing pressure led to a significant reduction in its population size. This population decline, combined with the selective removal of larger individuals, led to genetic changes over time, including shifts in allele frequencies. These genetic changes can have implications for the long-term viability and resilience of the population, as well as its ability to recover from overexploitation.

It is worth mentioning that the limited number of samples in the current study and the large amount of missing genotypic data were potential factors influencing allelic frequency, thereby causing potential biased genetic differences. To test this, a simulation study was conducted, removing a varying fraction of genotypes to mimic missing data (up to 60%). The results revealed that the missing data alone could not explain the significant genetic differences observed between the mid-collapse and post-collapse samples (Table 2), supporting the conclusion that the drift linked to the genetic decline was the primary cause of the changes within the genetic composition of the *P. undulosus* stock. Genetic drift causing such a level of genetic differentiation is not common in exploited marine species, but strong population bottlenecks have been previously observed to cause similar examples of genetic differentiation in other study models, such as in the Hainan gibbon *Nomascus hainanus* (Bryant et al. 2016).

Temporal change in effective population sizes in Polysteganus undulosus

The isolation-with-migration model was used to estimate the effective population size (N_e) of *P. undulosus* over a 43-year period between the mid-collapse and post-collapse periods. The results revealed a substantial 10-fold decrease in N_e, ranging from 43.88 in 1962/1963 to 4.76 in 2005/2006. Simulation-based analyses indicated that missing genotypes in the archived samples had a minimal impact on the N_e estimates, inflating them by only 16% when considering the number of missing genotypes affecting our data (56.7%). These findings provide strong evidence of a significant decline in N_e of *P. undulosus*, highlighting the detrimental genetic consequences of overfishing. In comparison to other studies, such as those conducted on summer flounder *Paralichthys dentatus* (Hoey et al. 2022), slinger seabream *Chrysoblephus puniceus* and santer seabream *Cheimerius nufar* (Coscia et al. 2016), the calculated N_e of *P. undulosus* is particularly low and falls below the thresholds suggested by Frankham (1995) for long-term evolutionary potential. The lowest effective population size likely occurred in 1998, just prior to the moratorium on fishing, and it is uncertain whether we were already above this value with the post-collapse samples, although other studies have shown encouraging post-collapse recovery (e.g. Hoey et al. 2022). The results emphasise the urgent need for conservation measures to increase the effective population size of *P. undulosus* and thus enhance its population viability.

To provide perspective on the historical changes in population size, we retrocalculated N_e in the pristine state of the *P. undulosus* population in the early 1900s. Using available catch data (1910–1998), stock assessment from the 1960s (25% of pristine population), and the N_e estimate

from the same period, the retrocalculated Ne values ranged from 147 to 176. This suggests a significant decline over the course of 88 years of intensive fishing, with an N_e that declined to at least 3% of the pristine population by 2005/2006. These results align with estimates of stock assessment that showed that the spawner biomass per recruit was below 5% by 1996 (Chale-Matsua et al. 2001) and evaluated to be at ~5% of the pristine population in 2005/2006 (Mann 2007). Importantly, the observed decline in genetic diversity and effective population size also aligns with the pattern observed in the catch data, reinforcing the detrimental impact that overfishing had on the species. At the start of exploitation in 1910, the annual catch reached 1 550 t, and from there it dropped to ~170 t by 1963. During 1997 (the last year that the fishery was still open before the implementation of the moratorium), the total reported commercial catch was only 2.6 t (0.1% of maximum catch: National Marine Linefish System, unpublished data).

Conclusions

This study provides compelling evidence of the genetic consequences of overfishing on *P. undulosus*, demonstrating a significant decline in the species' genetic diversity and effective population size over time, and significant genetic differences between the mid-collapse and post-collapse samples. Despite limitations in the sample size and number of amplifying microsatellites, and problems with the sample quality, the study still identifies significant biological effects and highlights the need for effective conservation measures.

The extremely low N_e and genetic diversity of *P. undulosus* in the 2005/2006 sample emphasise the urgency of implementing conservation strategies to ensure the species' long-term survival and restore its genetic diversity. The moratorium has now been in place for at least 25 years, and there is anecdotal information from fishermen, supported by limited baited remote underwater video (BRUV) surveys (A Bernard, SAIAB, unpublished data), that the abundance of *P. undulosus* is increasing. Based on the absence of catch data (due to the moratorium), it is recommended that a follow-up study be undertaken to assess the current N_e of *P. undulosus*, which will help inform future management and conservation strategies.

This study provides a valuable reference for understanding the historical trajectory of *P. undulosus*, for informing future conservation efforts and for future assessments of genetic recovery. The framework we have developed in this study serves as a baseline for future research and underscores the importance of assessing the robustness of statistical methods for small sample sizes and problematic samples. The development of genomic approaches can enhance the robustness of analyses for precious samples such as these, and it becomes urgent to undertake a new survey of this population almost 20 years since the last one. Overall, our findings provide crucial insights for conservation and management efforts and establish a crucial genetic baseline for future assessments of the stock's genetic recovery. It emphasises the need to protect and restore the genetic diversity of *P. undulosus* and other vulnerable marine species facing similar threats.

Acknowledgements — We thank the National Research Foundation (NRF) for financial support. We also thank the Oceanographic Research Institute (ORI) for the samples obtained for this study. This work is based on research supported by the NRF of South Africa. Genetic analyses in the present work were performed using the supercomputer Lengau Cluster at the Centre for High Performance Computing, CSIR Campus, Cape Town, South Africa. Any opinion, finding and conclusion expressed in this material is that of the authors and the NRF does not accept liability in regard thereto.

ORCID

Paulette Bloomer: https://orcid.org/0000-0002-6357-0153
Thierry Hoareau: https://orcid.org/0000-0003-1644-0775
Bruce Mann: https://orcid.org/0000-0002-7130-6301
Carel Oosthuizen: https://orcid.org/0000-0003-2747-9894

References

Agata K, Alasaad S, Almeida-Val VMF, Álvarez-Dios JA, Barbisan F, Beadell JS et al. 2011. Permanent genetic resources added to Molecular Ecology Resources Database, 1 December 2010–31 January 2011. *Molecular Ecology Resources* 11: 586–589.

Ahrens RL. 1964. A preliminary report on the biology of the seventy-four *Polysteganus undulosus* (Regan) in Natal waters. MSc thesis, University of Natal, South Africa.

Arnaud-Haond S, Belkhir K. 2007. GENCLONE: A computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes* 7: 15–17.

Baker CS, Hamner RM, Cooke J, Heimeier D, Vant M, Steel D, Constantine R. 2013. Low abundance and probable decline of the Critically Endangered Maui's dolphin estimated by genotype capture-recapture. *Animal Conservation* 16: 224–233.

Beacham TD. 1983. Variability in median size and age at sexual maturity of Atlantic cod, *Gadus morhua*, on the Scotian shelf in the Northwest Atlantic Ocean. *Fisheries Bulletin* 81: 303–321.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 1996–2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Montpellier, France: Laboratoire Génome, Populations, Interactions, Université de Montpellier II. Available at https://kimura.univ-montp2.fr/genetix.

Bryant JV, Gottelli D, Zeng X, Hong X, Chan BPL, Fellowes JR et al. 2016. Assessing current genetic status of the Hainan gibbon using historical and demographic baselines: implications for conservation management of species of extreme rarity. *Molecular Ecology* 25: 3540–3556.

Chale-Matsau JR, Govender A, Beckley LE. 2001. Age, growth and retrospective stock assessment of an economically extinct sparid fish, *Polysteganus undulosus*, from South Africa. *Fisheries Research* 51: 87–92.

Connell A. 2012. 'Marine fish eggs and larvae from the east coast of South Africa', originally posted May 2007. Available at http://fisheggs-and-larvae.saiab.ac.za.

Cornuet J, Pudlo P, Veyssier J, Dehne-Garcia A, Gautier M, Leblois R et al. 2014. DIYABC v2.0: A software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics* (Oxford, England) 30: 1187–1189.

Coscia I, Chopelet J, Waples RS, Mann BQ, Mariani S. 2016. Sex change and effective population size: implications for population genetic studies in marine fish. *Heredity* 117: 251–258.

Estoup A, Largiader CR, Perrot E, Chourrout D. 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology* 5: 295–298.

Frankham R. 1995. Conservation genetics. *Annual Review of Genetics* 29: 305–327.
Frankham R. 2021. Suggested improvements to proposed genetic indicator for CBD. *Conservation Genetics* 22: 531–532.
Furlan E, Stoklosa J, Griffiths J, Gust N, Ellis R, Huggins RM, Weeks AR. 2012. Small population size and extremely low levels of genetic diversity in island populations of the platypus, *Ornithorhynchus anatinus*. *Ecology and Evolution* 2: 844–857.
Garratt PA. 1996. Threatened fishes of the world: *Polysteganus undulosus* Regan, 1908 (Sparidae). *Environmental Biology of Fishes* 45: article 362.
Garza JC, Williamson EG. 2001. Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology* 10: 305–318.
Gates KS. 2009. an overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. *Chemical Research in Toxicology* 22: 1747–1760.
Golbuu Y, Friedlander AM. 2011. Spatial and temporal characteristics of grouper spawning aggregations in marine protected areas in Palau, western Micronesia. *Estuarine, Coastal and Shelf Science* 92: 223–231.
Griffiths MH. 2000. Long-term trends in catch and effort of commercial linefish off South Africa's Cape Province: snapshots of the 20th century. *South African Journal of Marine Science* 22: 81–110.
Hare MP, Nunney L, Schwartz MK, Ruzzante DE, Burford M, Waples RS et al. 2011. Understanding and estimating effective population size for practical application in marine species management. *Conservation Biology* 25: 438–449.
Hauser L, Adcock GJ, Smith PJ, Bernal Ramirez JHB, Carvalho GR. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences of the United States of America* 99: 11742–11747.
Hey J. 2010. Isolation with migration models for more than two populations. *Molecular Biology and Evolution* 27: 905–920.
Hoban SM, Paz-Vinas I, Aitken S, Bertola LD, Breed MF, Bruford MW et al. 2021. Effective population size remains a suitable, pragmatic indicator of genetic diversity for all species, including forest trees. *Biological Conservation* 253: article 108906.
Hoey JA, Able KW, Pinsky ML. 2022. Genetic decline and recovery of a demographically rebuilt fishery species. *Molecular Ecology* 31: 5684–5698.
Hutchinson WF, Oosterhout C, Rogers SI, Carvalho GR. 2003. Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proceedings of the Royal Society of London B* 270: 2125–2132.
Jones MW, McParland TL, Hutchings JA, Danzmann RG. 2001. Low genetic variability in lake populations of brook trout (*Salvelinus fontinalis*): a consequence of exploitation? *Conservation Genetics* 2: 245–256.
Jorgensen C, Enberg K, Dunlop ES, Arlinghaus R, Boukal DS, Brander K et al. 2007. Ecology: managing evolving fish stocks. *Science* 318: 1247–1248.
Kenchington EL. 2003. *The effects of fishing on species and genetic diversity*. Wallingford, Oxon, UK: CAB International.
Kinziger AP, Hellmair M, McCraney WT, Jacobs DK, Goldsmith G. 2015. Temporal genetic analysis of the Endangered tidewater goby: extinction–colonization dynamics or drift in isolation? *Molecular Ecology* 24: 5544–5560.
Laikre L, Hoban S, Bruford MW, Segelbacher G, Allendorf FW, Gajardo G et al. 2020. Post-2020 goals overlook genetic diversity. *Science* 367: 1083–1085.
Laikre L, Hohenlohe PA, Allendorf FW, Bertola LD, Breed MF, Bruford MW et al. 2021. Authors' reply to Letter to the Editor: Continued improvement to genetic diversity indicator for CBD. *Conservation Genetics* 22: 533–536.
Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics* 11: 355–373.
Mann BQ. 2007. Reassessment of the seventy-four *Polysteganus undulosus* stock after a ten-year moratorium. Unpublished Report No. 244. Durban, South Africa: Oceanographic Research Institute.
Mann BQ, Fennessy ST. 2013. *Polysteganus undulosus*. In: Mann BQ (ed.), *Southern African marine linefish species profiles. Special Publication No*. 9. Durban, South Africa: Oceanographic Research Institute. pp 259–261.
Mann BQ, Buxton CD, Pollard D, Carpenter KE, Sadovy Y. 2014. *Polysteganus undulosus.* The IUCN Red List of Threatened Species 2014: e.T170206A1293194. Downloaded on 4 December 2014.
Marandel F, Lorance P, Berthelé O, Trenkel V, Waples RS, Lamy J. 2019. Estimating effective population size of large marine populations, is it feasible? *Fish and Fisheries* 20: 189–198.
Marandel F, Charrier G, Lamy JB, Le Cam S, Lorance P, Trenkel VM. 2020. Estimating effective population size using RADseq: effects of SNP selection and sample size. *Ecology and Evolution* 10: 1929–1937.
Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* (*Oxford, England*) 28: 2537–2539.
Penney AJ, Mann-Lang JB, van der Elst RP, Wilke CG. 1999. Long-term trends in catch and effort in the KwaZulu-Natal nearshore line fisheries. *South African Journal of Marine Science* 21: 51–76.
Pichler FB, Baker CS. 2000. Loss of genetic diversity in the endemic Hector's dolphin due to fisheries-related mortality. *Proceedings of the Royal Society B: Biological Sciences* 267: 97–102.
Pinsky ML, Palumbi SR. 2014. Meta-analysis reveals lower genetic diversity in overfished populations. *Molecular Ecology* 23: 29–39.
Reid K, Hoareau TB, Bloomer P. 2012. High-throughput microsatellite marker development in two sparid species and verification of their transferability in the family Sparidae. *Molecular Ecology Resources* 12: 740–752.
Sadovy Y, Liu M. 2004. Report on current status and exploitation history of reef fish spawning aggregations in Eastern Indonesia. *Western Pacific Fisher Survey Series*, vol. 6. Fallbrook, California: The Society for the Conservation of Reef Fish Aggregations.
Safina C, Klinger DH. 2008. Collapse of bluefin tuna in the western Atlantic. *Conservation Biology* 22: 243–246.
Sambrook J, Fritsh ER, Maniatis T. 1989. *Molecular cloning: a laboratory manual* (2nd edn). Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
Silva CNS, Gardner JPA. 2015. Emerging patterns of genetic variation in the New Zealand endemic scallop *Pecten novaezelandiae*. *Molecular Ecology* 24: 5379–5393.
van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.
Wang X, Lim HJ, Son A. 2014. Characterization of denaturation and renaturation of DNA for DNA hybridization. *Environmental Health and Toxicology* 29: e2014007.
Wang L, Wan ZY, Lim HS, Yue GH. 2016. Genetic variability, local selection and demographic history: genomic evidence of evolving towards allopatric speciation in Asian seabass. *Molecular Ecology* 25: 3605–3621.

Manuscript received December 2023 / revised July 2024 / accepted July 2024

Appendix 1: Twelve microsatellite loci (i.e. 6 species-specific and 6 heterologous) used in determining genetic variation among *Polysteganus undulosus* sampled along the coast of southern Africa. Superscripts at locus names indicate inclusion in the three respective multiplexes

Locus	Label	Primer sequences (5′–3′)		Repeat unit	Fragment size range (bp)	Species and reference (GenBank accession no.)
		Forward	Reverse			
PU015[1]	VIC	ATG GTG GAT GAA TGA AGG A	CAT TCA ACC ATC ATA C	$(GGAT)_4$	119–139	*P. undulosus*; this study (KY353254)
PU065[1]	PET	GTT ATG TGA GAG CCA TCC AA	AAC ACC TGT CTG AAC TG	$(AATG)_4$	104–116	*P. undulosus*; this study (KY353255)
PU082[1]	NED	GCA GAC GGC TTT ACG AGT AT	CGG ATC AGT GGC AAA TCC TT	$(AAAT)_4$	99–119	*P. undulosus*; this study (KY353256)
PU086[2]	5-FAM	GCG CCT GGA TGT GAA GTT	AAG ACA CTC GCT GCC TGA GGA	$(AGCC)_6$	141–169	*P. undulosus*; this study (KY353257)
PU126[2]	PET	CAG CTG CTA ACA GTC GGT AA	TAC GGT AGT CAA CGC CGT TT	$(GTTT)_6$	96–116	*P. undulosus*; this study (KY353258)
PU165[2]	VIC	GCC ACC TGA ACA GAC AAG AA	GTT TTG TAC CGC ATG GC	$(ACAG)_6$	131–167	*P. undulosus*; this study (KY353259)
CL011[1]	VIC	TTT TGT CCA TTA CAG AGT CAT GC	AAT GTT TCC CAA ATG CAT CC	$(CA)_{16}$	208–232	*Cobitis lutheri*; Agata et al. (2011)
PA535[1]	5-FAM	GTC GAG GCT GTA AAC AGG AC	CGT GGC TTT GGT TAT TTC TTG C	$(TATC)_{17}$	101–161	*Pagrus auriga*; Reid et al. (2012)
PBt001[1]	PET	AGG TTC CCC ACA GAA GGT C	AGT ACC TGG GAA ACA GCC C	$(AGAT)_9$	215–235	*Pachymetopon blochii*; Reid et al. (2012)
PBt003[2]	PET	TTG GGG AAA AGG AGA GGC G	CCG ACT CGC TCT GTA TGT TG	$(GATA)_{17}$	162–230	*Pachymetopon blochii*; Reid et al. (2012)
PBt011[2]	NED	CAC AGA CGG GAA TGC ACA C	AGG GTG TCA GGA CAG AAC G	$(TATC)_{17}$	163–247	*Pachymetopon blochii*; Reid et al. (2012)
LLtr004[3]	PET	CGC TCA TTG ATT GGA GCC C	ACA CTG GAA CGA AAC TGG TTG	$(CAG)_{11}$	208–241	*Lithognathus lithognathus*; Reid et al. (2012)

Appendix 2: Factorial correspondence analysis illustrating the lack of genetic differences among *Polysteganus undulosus* at four South African localities, using the 2005/2006 contemporary sample group