Population genetics of the teleost orange roughy, *Hoplostethus atlanticus*, and insights into their visual adaptations to the deep-sea environment

By

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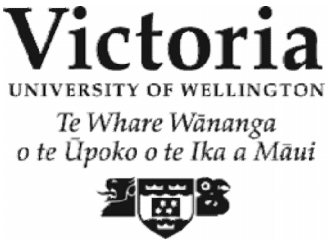

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Abstract

The orange roughy, *Hoplostethus atlanticus*, has been one of the main targeted species in deep-sea fisheries worldwide. It occurs at depths of 450 – 1800 m and is abundant off the coasts of New Zealand, Australia, Namibia, Chile, and in the Northeast Atlantic Ocean. Like many other deep-sea fishes, orange roughy is vulnerable to over exploitation because they grow slow reaching maturity at about 30 years and live for more than 100 years. Their fecundity is low, which means they have low productivity. The individuals form predictable and dense spawning aggregations close to seamounts, plateaus and canyons. The trawl fishery for orange roughy started in seamounts around New Zealand in the late 1970s and progressively expanded off the coast of other countries and to the high seas (out of any Economic Exclusive Zone). Most stocks have been fished down to or below 30% pre-exploitation levels; as a consequence, fisheries have been closed or catches largely reduced. Currently, the only large scale fisheries operate off New Zealand.

For effective fisheries management it is essential to define real biological units or "stocks". There has been considerable research into the levels of population differentiation of orange roughy using a range of techniques at different geographic scales to attempt to differentiated stocks. However, there is no consensus about the level of connectivity among populations. In the present study, I investigated the levels of population differentiation in orange roughy using two types of neutral molecular markers at a global and fine-scales. Both markers revealed high levels of genetic diversity which is likely related with historically large population sizes. The analyses of 546 cytochrome *c* oxidase subunit I (COI) sequences revealed a lack of global genetic differentiation among samples from New Zealand, Australia, Namibia, and Chile. However, low but significant differentiation was found between the Southern hemisphere sites and two Northeast Atlantic sites. Mismatch distribution and Bayesian analyses indicated the occurrence of expansion events in orange roughy during the Pleistocene period. A data set of nine microsatellite DNA loci genotyped from 812 individuals, showed a predominant lack of significant genetic differentiation across the Tasman Sea and at a fine-scale around New Zealand. At a global scale, differentiation was low but significant across the Southern hemisphere; and the highest values of differentiation were detected between the Southern hemisphere sites and the Northeast

Atlantic Ocean. The predominant lack of differentiation at the regional and fine-scale and the low differentiation within the Southern hemisphere is probably the result of stepping-stone dispersal of long-lived adults that are able to spawn many times in their life.

Most orange roughy studies have been oriented to fisheries aspects, but other kind of studies as the genetic divergence and phylogenetic relationships among *Hoplostethus* species are lacking. Using available COI sequences, I conducted a phylogenetic study including *H. atlanticus, H. crassispinus, H. gigas, H. japonicus H. latus*, and *H. mediterraneus*. As expected, the inter species divergence was much higher than the intra species divergence. Phylogenetics analyses showed that *H. latus, H. crassispinus, H. japonicus,* and *H. mediterraneus* form a separate clade from *H. atlanticus* and *H. gigas*. The position of *H. gigas* was not well defined with the nucleotide data. However, at the amino acid level, non-synonymous substitutions differentiated *H. atlanticus* from all the other species. This was correlated with morphological characteristics presented elsewhere.

A candidate gene approach was attemped using the rhodopsin gene; however, there was almost no variation among partial sequences of individuals from distant sites. Instead, this gene was used to investigate the molecular basis for visual adaptations in orange roughy to the bathypelagic light environment. It is known that certain amino acid replacements in the rhodopsin gene of vertebrates shift the λ_{max} value of the pigment to perceive different light conditions. To compare and identify critical amino acid sites that are known to be involved in spectral tuning, I obtained partial rhodopsin sequences of other 18 marine teleost habiting at different depths (1 – 1,175 m) and, thus, different light environments. A phylogenetic analysis was conducted to determine whether particular rhodopsin gene sequences correlate with the depths occupied by the species. I identified four critical amino acid replacements that have been involved in the spectral tuning of rod pigments. Orange roughy presented the same amino acid combination at two critical sites already reported for the deep-sea congener silver roughy, which was not found in any of the other species. This likely reflects an adaptation to the light available (i.e. bioluminescence) in the bathypelagic environment. The phylogeny was weakly related to the maximum depth of the species, probably because there are selectively neutral (i.e. inherited by ancestry) and non-neutral changes (i.e. influenced by natual selection) among the rhodopsin sequences of the species being considered.

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Varela AI, Ritchie PA, Smith PJ (In Preparation) Critical amino acid replacements in the rhodopsin gene of the deep-sea teleost orange roughy (*Hoplostethus atlanticus*): comparison with other teleost species occupying different light environments. Intended for submission to New Zealand Journal of Marine and Freshwater Research

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Chapter 1

General Introduction

The orange roughy, *Hoplostethus atlanticus* Collet 1889 (Teleostei: Trachichthyidae), has been one of the main targeted species in deep-sea fisheries worldwide (Pitcher 2010). Its biological characteristics and their aggregating behaviour, makes orange roughy vulnerable to over exploitation (Clark 1996; Branch 2001; Baker et al. 2009). The fishery for orange roughy started around New Zealand in the late 1970s (Clark 1996) and progressively expanded off the coast of other countries and to the high seas (out of any Economic Exclusive Zone) (reviewed by Branch 2001). Many stocks have been over-fished; consequently, fisheries have been closed or catches largely reduced (see Clark 2009; Niklitscheck et al. 2010; Foley et al. 2011). Currently, the only large scale fisheries operate off New Zealand (Clark 2009; Dunn and Forman 2011).

There are 28 valid species within the genus *Hoplostethus*, one of them has been recently described (Moore and Dodd 2010). The orange roughy is the only *Hoplostethus* species with commercial value. The silver roughy, *H. mediterraneus* is caught as by-catch in bathyal trawling and has low commercial value (Madurell and Cartes 2005). While there has been extensive research on orange roughy, less attention has been given to the other *Hoplostethus* species, with the only exception of the silver roughy, *H. mediterraneus*. Studies on this species have been mainly focused in the diet, feeding habits and bathymetric distribution (e.g. Madurell and Cartes 2005; Jones 2009; Katsanevakis and Maravelias 2009; Fanelli and Cartes 2010). There are no studies about the molecular phylogeny of *Hoplostethus* species (Moore and Dodd 2010). Kotlyar (1986) proposed a phylogenetic tree for the subgenera of *Hoplostethus*, but this was based on geographic distribution and morphology only.

Most studies of orange roughy have been orientated to fisheries aspects. There are no studies about their adaptation to the light environment of the deep-sea, except by Pankhurst (1987), who studied the retinal morphology of orange roughy and other mesopelagic and demeresal teleost. It has been reported that orange roughy have a pronounced lateral line (Paulin 1979) and functional eyes with highly specialized retina

(Pankhurst 1987), these characteristics may allow them to detect movement of conspecifics in aggregations in almost complete darkness to respond quickly, displaying avoidance behaviour to predators and fishing gear (Koslow 1996; Branch 2001). The high degree of retinal specialization of orange roughy has been correlated with a visual adaptation to dim-light (Pankhurst 1987). However, the molecular basis for this visual adaptation to the light environment of the deep-sea has never been studied.

1.1 Biology and distribution of orange roughy

The orange roughy is a widely distributed deep-sea fish. The species is found along continental slopes and seamounts at depths of 450 – 1,800 m across the Southern hemisphere and in the Northeast Atlantic Ocean (Horn et al. 1998; Branch 2001). They form dense aggregations on seamounts for spawning and feeding (Clark et al. 2000). It has been suggested that dense aggregations may also form adjacent to the bottom in areas with low currents acting as "resting sites" (Lorance et al. 2002). Orange roughy are opportunistic feeders, juveniles fed mainly on bentho and meso-pelagic crustaceans and mature individuals consume predominately fish and squid (Bulman and Koslow 1992). Rates of food consumption are high in orange roughy and their metabolism is substantially higher than the typical non-migratory deepwater fishes, and similar to mesopelagic migratory species (Bulman and Koslow 1992; Koslow 1996). The bodily composition is also similar to that of active mesopelagic fishes, high in lipid and protein and low in water (Koslow 1996). The high metabolic costs for orange roughy have been related to its exceptionally slow growth (Bulman and Koslow 1992) and with its strong locomotory performance to maintain position around seamounts; an environment characterized by strong and variable currents (Koslow 1996).

As other deep-sea fishes, the orange roughy is a "*K*-selected" species (Koslow 1996). They grow slowly, reaching maturity at about 30 years (Fenton et al. 1991; Francis and Horn 1997) and living for more than 100 years (Mace et al. 1990; Fenton et al. 1991). The fecundity is low, and therefore, they have low productivity (Pankhurst and Conroy 1987). Because of its long life span, orange roughy individuals may spawn many times; however, intermittent spawning in females has been reported related to low food availability coupled with the cost of participating in spawning migrations and

aggregations around seamounts (Bell et al. 1992). Egg development takes ~ 10 days and they sink at the end of their development hatching near the bottom (Zeldis et al. 1995; Zeldis et al. 1998), which likely results in low passive dispersal.

1.2 Deep-sea fisheries

The collapse and depletion of many inshore and shelf fish stocks in the 1900s and the increasing technology over the past decades increased the development of deep-sea fisheries, targeting, in particular, seamount-associated teleost species (Morato et al. 2006; Pitcher et al. 2010). The most widely used method to catch fishes on seamounts is bottom trawling, where a net with heavy rollers is dragged along certain areas of the seamounts having a major effect over the seafloor and its fauna (see Clark 2010). Fisheries for teleosts mainly occur on and around seamounts in the North Pacific Ocean along the Emperor and Hawaiian Ridges, in the Southwest Pacific around New Zealand, New Caledonia and Australia, in the Southeast Pacific off Chile, in the Northeast Atlantic Ocean around the Azores and on the Mid Atlantic Ridge, and in the South Atlantic (Smith 2007). Recently, Pitcher (2010) identified eight major targeted seamount fish species worldwide. One of the first and most exploited deep-sea fishes is the orange roughy. The others are the alfonsino *Beryx splendens*, the cardinalfish *Epigonus telescopus*, the oilfish *Ruvettus pretiosus*, the black scabbard fish *Aphanopus carbo*, the armorhead *Pseudopentaceros wheeleri*, the abyssal grenadier *Coryphaenoides armatus* and the smooth oreo *Pseudocyttus maculatus.* Other exploited seamount-associated fishes include the roundnose grenadier *Coryphaenoides rupestris*, the black oreo *Allocyttus niger*, the spiky oreo *Neocyttus rhomboidalis*, the toothfish *Dissostichus* spp. and *Sebastes* spp. (Smith 2007).

The extraction of seamount fish species by large-scale fisheries increased rapidly reaching a peak on catches of about 1.2 million tonnes per year in the mid 1990s, then catches decreased sharply showing a key feature of deep-sea fisheries termed “boom-and-bust” (reviewed by Pitcher et al. 2010). The un-sustainability and risk of depletion of most deep-sea fisheries is explained by the general characteristics of exploited deep-sea species. They are usually “*K*-selected” exhibiting slow grow, extreme longevity, late maturity and low fecundity; which coupled with their aggregating behaviour around

seamounts, explain their lack of resiliency and vulnerability to overexploitation (Koslow et al. 2000; Watson and Morato 2004; Morato et al. 2006; Smith 2007). The management of fish resources should ensure the preservation of genetic diversity as this is the basis for all the others levels of diversity and it is essential for the evolutionary adaptation of the species to environmental changes and other pressures (Laikre et al. 2005). This is particularly relevant for deep-sea exploited species as they are less resilient than shelf species (Smith et al. 2007).

1.3 Fisheries management and population genetics

For fisheries management and conservation plans it is crucial to determine the connectivity among populations to attempt to differentiate stocks (Begg et al. 1999; Grant and Waples 2000). There are several definitions of the term "stock" in fisheries management, but it usually refers to biological units large enough to be self-recruiting (Hilborn and Walters 1992). Several techniques have been used to study the connectivity among marine fish populations such as tag-recapture studies (e.g. Hurst et al. 1999), morphometrics (e.g. Mamuris et al. 1998), parasites (e.g. Moore et al. 2011), otoliths (e.g. Correia et al. 2011), and population genetics analyses using neutral molecular markers (e.g. Broderick et al. 2011). Laikre et al. (2005) stressed the importance of determining the genetic population structure of exploited fish species; this is the distribution of genetic variation within and between populations. According to these authors, knowledge of this structure may reduce the risk of depletion of genetic resources by considering groups of genetically homogenous individuals as the basic units for management. Based on the amount of genetically effective migration (gene flow) Laikre et al. (2005) defined three types of genetic structure for the identification of biological (genetics) units for fisheries management, 1) distinct populations: gene flow is small enough to generate genetic divergence among geographically close populations; 2) continuous change: genetic composition change over space. Gene flow is larger among geographically close populations resulting in an isolation by distance (IBD) pattern; and 3) no differentiation: gene flow is extensive and the species behaves as a single panmictic unit over the geographic region under consideration. Some species may present a combination of these three types of genetic structure.

Most studies to determine the genetic structure of marine (and terrestrial) species have been conducted using selectively neutral molecular markers, since they have a great potential to estimate gene flow (see Holderegger et al. 2006). More recent studies have also incorporated or used loci under selection to estimate differentiation due to local adaptation (e.g. Fevolden and Pogson 1997; Hemmer-Hansen et al. 2007; André et al. 2011). To date, the most commonly employed neutral markers have been mitochondrial DNA (mtDNA) sequences and microsatellite DNA loci (Hellberg 2009). Since mitochondrial DNA is usually transmitted maternally, does not recombine and evolves faster than nuclear (encoding) DNA, mtDNA sequences have been widely used in phylogenetic and phylogeographic studies (Avise 1998a). For population genetics analyses, one advantage of mtDNA over microsatellite DNA analyses is that the population size of mtDNA is about one quarter of the nuclear DNA, and therefore, it is expected to be more sensitive to genetic drift. On the other hand, microsatellite DNA loci have a higher resolution and power to detect genetic differentiation than others multilocus markers (i.e. AFLPs, RAPDs, allozymes); the main advantages of microsatellites respect to mtDNA sequences is that they are multiple independent loci and have a higher mutation rate allowing estimations of levels of genetic structure on ecological/contemporary time scales (reviewed by Selkoe and Toonen 2006).

Marine fish species typically exhibit higher genetic diversity than freshwater and anadromous fish species as measured with molecular markers; a pattern that has been related to larger evolutionarily effective population sizes of marine fishes and to the existence of fewer barriers to dispersal in the marine realm compared to freshwater systems (Ward et al. 1994; DeWoody and Avise 2000; McCusker and Bentzen 2010). However, the general pattern of high genetic diversity in many marine fish species does not imply that fishing has not affected the levels of genetic variation of populations since changes in this measure will be apparent only after several generations for species with large population sizes. This is particularly relevant for long-lived species, because they would have slow genetic drift and any reductions in genetic diversity are unlikely to be detected yet. For example, Chapman et al. (2011) suggested that one of the main hypotheses to explain the absence of genetic bottleneck in the critically endangered smalltooth sawfish *Pristis pectinata* is the longevity of this elasmobranch.

Genetic techniques provide an indirect measure of population connectivity and have been widely used because it is difficult to measure dispersal directly, particularly in the marine environment (Avise 1998b; Lowe and Allendorf 2010). The limitation of genetic

approaches is that genetic connectivity does not necessarily reflect demographic connectivity, and the later is of most interest for fisheries management. As defined by Lowe and Allendorf (2010), genetic connectivity is "the degree to which gene flow affects evolutionary processes within populations" and demographic connectivity is "the degree to which population growth and vital rates are affected by dispersal". Therefore, genetic estimates should be combined with inferences from other techniques, especially with tag-recapture studies (Lowe and Allendorf 2010). However, tag-recapture studies are very difficult to conduct for deep-sea species and a holistic approach using available techniques should be applied to effectively define stocks (Begg and Waldman 1999) as performed for the pelagic horse mackerel *Trachurus trachurus* (Abaunza et al. 2008). Dunn and Devine (2010) followed a holistic approach to determine the stock structure of orange roughy on the Chatham Rise, a complex topographic system located off the east coast of New Zealand. Using catch data, differences in life history characteristics and genetic data among others, Dunn and Devine (2010) determined the existence of two stocks on the Chatham Rise. The application of a holistic approach to other areas would likely results in better management for this species.

1.4 Population differentiation studies in orange roughy

There have been several population differentiation studies in orange roughy to attempt to differentiate management units or "stocks" using a range of techniques applied at different geographical scales. Lester et al. (1988) using parasite analyses discriminated a number of stocks among New Zealand and Australian samples. Similarly, morphometrics and otolith micro-chemistry analyses found significant differentiation among Australian populations (Elliott et al. 1995; Edmonds et al. 1991). However, genetic studies have reported contradictory results for orange roughy. The first population genetic studies for orange roughy were conducted with allozymes. Smith (1986) found high levels of genetic connectivity among samples from New Zealand and the Northeast Atlantic Ocean. Similarly, Elliott and Ward (1992) reported lack of differentiation among New Zealand and Australian samples. In contrast, using restriction enzyme analysis of mtDNA, Smolenski et al. (1993) found genetic differentiation among samples from New Zealand and Australia. Later, significant genetic structure was also reported among sites around New Zealand with RFLP and

allozyme analyses (Smith et al. 1996; Smith and Benson 1997). Covering a higher geographical scale and using microsatellite DNA markers, Oke et al. (2002) found lack of genetic differentiation among samples from New Zealand, Australia, and the Northeast Atlantic Ocean, although there was a pattern of IBD. Genetic homogeneity has been also reported with microsatellite DNA loci at a lower geographical scale among Northeastern Atlantic samples (White et al. 2009a). However, significant differentiation was reported using microsatellite DNA loci and otolith analyses in a complex topographic system located in the Northeast Atlantic Ocean (Carlsson et al. 2011). Considering all the above, a broad study about levels of population differentiation in orange roughy at global and fine-scales using one technique and a large sampling size would be highly valuable to help to determine the levels of connectivity among populations.

Therefore, this study used two types of neutral molecular markers, mtDNA sequences and microsatellite DNA loci, to investigate the genetic structure of orange roughy using samples from most of its distribution range (global-scale) including multiple sites around New Zealand (fine-scale). A candidate gene approach (i.e. genes subject to natural selection) was also attempted to investigate adaptive population divergence in orange roughy, since there are several examples of population differentiation at candidate genes without differentiation at neutral markers in marine fishes (e.g. Pogson and Fevolden 2003; Hemmer-Hansen et al. 2007; André et al. 2011). The only candidate gene that was successfully amplified for orange roughy samples was the rhodopsin gene, which is involved in visual adaptations at inter and intra-specific levels in marine fishes (e.g. Yokoyama and Takenaka 2004; Yokoyama 2008; Larmuseau et al. 2009b, 2010, 2011). However, there was almost no variation among partial rhodopsin sequences in orange roughy (i.e. only one site presented a transition and there were no transversions), even for samples from distant regions (e.g. New Zealand and the Northeast Atlantic Ocean). Given this, partial sequences of the rhodopsin gene were instead used to study the molecular basis for visual adaptations of this species to the bathypelagic light environment. As stated before, the high degree of retinal specialization of orange roughy has been correlated with a visual adaptation to dim-light (Pankhurst 1987); however, the molecular basis for this visual adaptation has never been studied in orange roughy. This is important to understand how deep-sea species have adapted to the light available in the bathypelagic environment (Yokoyama 2008).

1.5 Deep-sea environment and visual adaptations

Orange roughy habits the largest ecosystem on Earth. The deep-sea extends over the 65% of the World's surface (Sverdrup et al. 1942). It is commonly considered that the deep-sea begin at the shelf break, which occurs at a depth of 200 m in most of the Oceans, marking a transition between shallow to deep-sea fauna (Thistle 2003). The deep-sea ocean is characterized by extreme environmental conditions. Salinity and pressure are high, while temperature and food availability are low (Sanders et al. 1965; Thistle 2003). However, topographic features, as most seamounts, have a complex topography and are biological "hot-spots" characterized by high production due to Taylor columns (parcels of water that retain microorganisms) and upwelling of nutrient rich water (Rogers 1994). They provide habitat to support a range of epifaunal and suspension-feeding invertebrates (Ramirez-Llodra et al. 2010), attracting high abundance and diversity of top-level predators as fishes (Dower and Brodeur 2004). Early studies (e.g. Sanders 1968) considered that the deep-sea was a physically stable environment. However, over the past decades increasing technology and sampling efforts have shown that the deep-sea is a complex and dynamic environment (Stuart et al. 2003). There are gradients, habitat shifts, and high species diversity (Rex et al. 1993; Levin et al. 2001; Ramirez-Llodra et al. 2011).

One striking difference of the deep-sea environment respect to the sea surface is the lack of photosynthetically-usable light below about 250 m, which results in lack of primary production in most deep-sea ecosystems (Thistle 2003). Light intensity decreases exponentially with depth because photons are absorbed or scattered by the water column (Levine and MacNichol 1982). Many marine organisms, therefore, have developed visual adaptations to the spectral quality of the deep-sea (reviewed by Crescitelli 1991), while others lack of vision and have evolved other senses as chemoreception and mechanoreception (Thistle 2003). To adapt to the dim-light and bioluminescence of the deep-sea, many visual fish species have lost cone photoreceptors and have only rod photoreceptors (see Hunt et al. 2001), because rods mediate vision at low light intensities (reviewed by Yokoyama 2008). Visual pigments consist of an opsin protein and a chromophore, and are characterized by the wavelengths of maximal absorption (λ_{max}) (Bowmaker 1995). Rhodopsin pigments (λ_{max} = ~ 500 nm) are usually expressed in rods (Yokoyama et al. 1999) and certain amino acid replacements in the opsin protein explain changes in the λ_{max} of the pigment to perceive different light

environments, a phenomenon known as spectral tuning (reviewed by Yokoyama 2008). The molecular basis for spectral tuning has been studied in several shallow and deep-sea fish species, allowing the identification of critical amino acid sites, called "tuning sites" (e.g. Hope et al. 1997; Yokoyama et al. 1999; Hunt et al. 2001; Yokoyama and Takenaka 2004).

1.6 Demographic history and DNA sequence analyses

The analyses of DNA sequences are also suited to make inferences about the past demographic history of the species (Rogers and Harpending 1992; Drummond et al. 2005). The molecular clock hypothesis assumes that DNA sequences change at a near constant rate through time at intra-specific levels. Demographic events and the time-frame when they occurred can be inferred from DNA sequences analyses (Grant and Waples 2000). Glaciations events during the Pleistocene period (~ 1.6 Myr to 10,000 years before present) resulted in major demographic changes, which in turn had a great influence in the intra-specific genetic diversity in many marine and freshwater species (reviewed by Grant and Waples 2000). Two measures of diversities among intra-specific DNA sequences have been used to categorize marine fishes into four groups depending on different combinations of small and large values of haplotype and nucleotide diversity (Grant and Bowen 1998). Low values for both measures are characteristic of species that have had catastrophic reductions in population's sizes (bottleneck events or founder events). Low nucleotide diversity and medium-high levels of haplotype diversity are expected for species that have suffered bottleneck events followed by sudden expansion in populations that have rapidly grown. When both measures are high it is assumed that populations have been large and stable over long periods of time or that isolated and divergent populations have become into contact. Finally, low levels of haplotype diversity and high nucleotide diversity is typical of large ancient populations, which suffered drastic reductions in size or that have become isolated. Marine fishes are usually within one the first three categories and the last group may be most common in inshore or freshwater species, since gene flow is more restricted than in coastal or oceanic environments (reviewed by Grant and Waples 2000).

1.7 The aims and structure of this thesis

The aims of this thesis were to: (i) use neutral molecular markers to study the genetic diversity, differentiation and past demographic history of orange roughy populations using large sample sizes at a global and fine-scales, (ii) conduct a phylogenetic study of *Hoplostethus* species, and (iii) study the molecular basis for visual adaptation of orange roughy to the bathypelagic light environment.

In Chapter 2, I present my results about levels of global genetic differentiation and demographic history of orange roughy using mitochondrial DNA sequences. In Chapter 3, I show the results of microsatellite DNA analyses that were conducted to determine the genetic structure of orange roughy at a global and fine-scales. In Chapter 4, I show the results of phylogenetic analyses of *Hoplostethus* species using the mitochondrial DNA sequences obtained by me for orange roughy and silver roughy (*H. mediterraneus*) and sequences of congener species available from a public data base (GenBank). In Chapter 5, I present the findings of critical amino acid sites replacements in the rhodopsin gene of orange roughy, which are known to be involved in spectral tuning. Rhodopsin sequences were also obtained for other 18 species to compare to other teleost species habiting different light environments. Each chapter has been written as independent manuscripts, and hence there is some repetition in the introductions and methodological sections. Finally, in Chapter 6, I conclude with a summary and general discussion of the main findings of this thesis.

Chapter 2

Low levels of global genetic differentiation and population expansion in the deep-sea teleost *Hoplostethus atlanticus* revealed by mitochondrial DNA sequences

2.1 Abstract

The orange roughy *Hoplostethus atlanticus* is a well known commercial species with a global distribution. There is no consensus about levels of connectivity among populations despite a range of techniques having been applied. I used cytochrome *c* oxidase subunit I (COI) and cytochrome *b* (cyt *b*) sequences to study genetic connectivity at a global scale. Pairwise Φ_{ST} analyses revealed a lack of significant differentiation among samples from New Zealand, Australia, Namibia, and Chile. However, low but significant differentiation (Φ_{ST} = 0.02-0.13 $P < 0.05$) was found between two Northeast Atlantic sites and all the other sites with COI. AMOVA and the haplotype genealogy confirmed these results. The prevalent lack of genetic differentiation is probably due to active adult dispersal under the stepping-stone model. Demographic analyses suggested the occurrence of two expansion events during the Pleistocene period.

2.2 Introduction

Deep-sea fishes (i.e. > 200 m) live in the largest habitat on Earth. Many deep-sea fishes are widely distributed, and have a circumglobal distribution, e.g. the alfonsino *Beryx decadactylus*. Some bathyal fishes inhabit continental slopes, the slopes of oceanic islands, and seamounts that are separated by extensive areas of deep ocean. The dispersal of deep-sea fishes may be less constrained by their habitat than that of coastal fishes. However, while there are few obvious barriers in the deep-sea, migration of individuals could be limited by ocean currents and bathymetric features, as reported for the demersal fish *Brosme brosme* (Knutsen et al. 2009). There are several examples of genetic differentiation among deep-sea fish populations at different geographical scales, including the spikey oreo *Neocyttus rhomboidalis* (Elliott et al. 1998), the bluemouth *Helicolenus dactylopterus* (Aboim et al. 2005), the Patagonian toothfish *Dissostichus eleginoides* (Smith and McVeagh 2000; Rogers et al. 2006), and the Cape hake *Merluccius paradoxus* (von der Heyden et al. 2007). However, there are also species that exhibit panmixia at regional, oceanic, and inter-oceanic scales such as the armorhead *Pseudopentaceros wheeleri*, the wreckfish *Polyprion americanus*, the alfonsino *Beryx splendens*, and the oreos *Allocytus niger* and *Pseudocyttus maculatus* (Martin et al. 1992; Sedberry et al. 1996; Hoarau and Borsa 2000; Smith et al. 2002b).

The orange roughy, *Hoplostethus atlanticus,* has a cosmopolitan distribution (Paulin 1979). It occurs at depths of 450-1800 m and is locally abundant off the coasts of New Zealand, Australia, Namibia, and in the Northeast Atlantic Ocean (Horn et al. 1998; Branch 2001) and off Chile (Labbé and Arana 2001). Orange roughy aggregate near prominent topographic features such as seamounts, plateaus and canyons, especially during spawning and feeding periods (Clark et al. 2000). It is thought that adults migrate hundreds of kilometres to spawn, as mature fish are widespread, but spawning occurs in just a few specific areas (Francis and Clark 1998). Orange roughy fisheries started in 1979 around New Zealand (Clark 1996) and later developed off Australia (1980s), South Africa (Namibia), and in the Northeast Atlantic Ocean in the 1990s (Branch 2001), and in the South Indian Ocean and off Chile in 1999 (Branch 2001; Labbé and Arana 2001). Orange roughy grow slowly, live longer (100+ years) (Mace et al. 1990; Fenton et al. 1991), mature later (~30 years) than most other marine teleosts (Fenton et al. 1991; Francis and Horn 1997; Horn et al. 1998) and has low productivity due to its low fecundity (Pankhurst and Conroy 1987). All these life history

characteristics make orange roughy vulnerable to over-exploitation. Most orange roughy stocks have been fished down to or below 30% of pre-exploitation levels and most fisheries have shown a pattern of rapidly decreasing catches (reviewed by Branch 2001). As part of the management for this species, which is found in several Economic Exclusive Zones and occurs in international waters, it is important to determine levels of connectivity among populations.

A range of indirect techniques have been applied to estimate connectivity among orange roughy populations, as tag-recapture studies have been unsuccessful in this deep-sea species (Branch 2001). In general, studies using morphometrics, otolith micro-chemistry, and parasite analyses have found differences between populations of orange roughy within ocean basins (e.g. Elliott et al. 1995; Edmonds et al. 1991; Lester et al. 1988). On the other hand, genetic studies, with different molecular markers applied at different geographical scales, have reached contrary conclusions about differentiation among populations, and wide scale connectivity among populations has not been rejected. Smith (1986) using allozyme analyses found low genetic differentiation among samples from the Tasman Sea, Southwest Pacific Ocean, and Northeast Atlantic Ocean. Similarly, Elliott and Ward (1992) showed a lack of genetic subdivision among six sites off southern Australia and one site off the eastern coast of New Zealand. Using a restriction enzyme analysis of mitochondrial DNA, Smolenski et al. (1993) surveyed samples from seven locations in Australia, New Zealand and South Africa, and found evidence for genetic separation among southeastern Australian and New Zealand samples. Genetic structure was found among different sites off the coast of New Zealand by Smith et al. (1996) using RFLP analysis and by Smith and Benson (1997) with allozyme loci. In another study based on multiple techniques, Smith et al. (2002a) also found evidence for differentiation among populations off the west coast of New Zealand with the analysis of otolith shape, fish size, and restriction digest of the whole mitochondrial genome. However, mitochondrial DNA polymorphism of the control region and two microsatellite loci revealed lack of differentiation among New Zealand sites (Smith et al. 2002a). A study by Oke et al. (2002) using microsatellite markers showed no significant differentiation within Australian waters, and no evidence of genetic differentiation between samples from Australia and New Zealand, and Australia and the Northeast Atlantic, although both regional comparisons revealed a significant pattern of isolation by distance (Oke et al. 2002). Recently, White et al. (2009a) used 14 microsatellite loci to test the hypothesis that the life-history characteristics of orange

roughy should generate philopatry and the evolution of population genetic structure at a relatively small geographical scale. However, they found panmixia among five samples from the Northeast Atlantic, with some sampling sites separated by over 2000 km, but significant differentiation between the pooled Northeast Atlantic samples and one sample from Namibia. Contrary to White et al. (2009a), Carlsson et al. (2011) recently reported low but significant differentiation within a complex topographic system in the Northeast Atlantic, mainly due to genetic differences between samples from a flat area and seamount sites. The authors suggested that differences in seafloor structures (seamounts and flats) may provide discrete spawning structures with limited gene flow between them.

The application of molecular markers allows not only inferences about levels of genetic connectivity among populations, but they also enable the study of the demographic history of species, in which expansion and contraction events can be related to global climatic changes (e.g. Larmuseau et al. 2009a).

The main goal of this study was to use molecular markers to determine levels of genetic variation within and differentiation among orange roughy populations at a global scale. The second goal was to determine the demographic history of the species and to relate this to past climatic events. I used DNA sequence data from the mitochondrial cytochrome *c* oxidase subunit I (COI) and cytochrome *b* (cyt *b*) genes and samples collected from New Zealand, Australia, Namibia, Chile, and the Northeast Atlantic. Large samples from multiple locations around New Zealand were used to determine within region variation.

2.3 Materials and Methods

2.3.1 Sampling and DNA extractions

Tissue samples were obtained from 546 orange roughy specimens from 13 sites in five regions: New Zealand, Australia, Namibia, Chile, and the Northeast Atlantic Ocean (Fig. 2.1). Muscle samples were collected from fresh fish and frozen at -20°C. Samples from northern New Zealand and from Lord Howe Rise (outside the Economic Exclusive Zone (EEZ) of New Zealand,) were taken by New Zealand Ministry of Fishery

Observers. Samples from all other sites in New Zealand, Australia and from Namibia were from a frozen tissue collection held at NIWA (National Institute of Water & Atmospheric Research Ltd., New Zealand). Samples from Chile and the Northeast Atlantic Ocean were supplied by researchers undertaking studies in their respective locations.

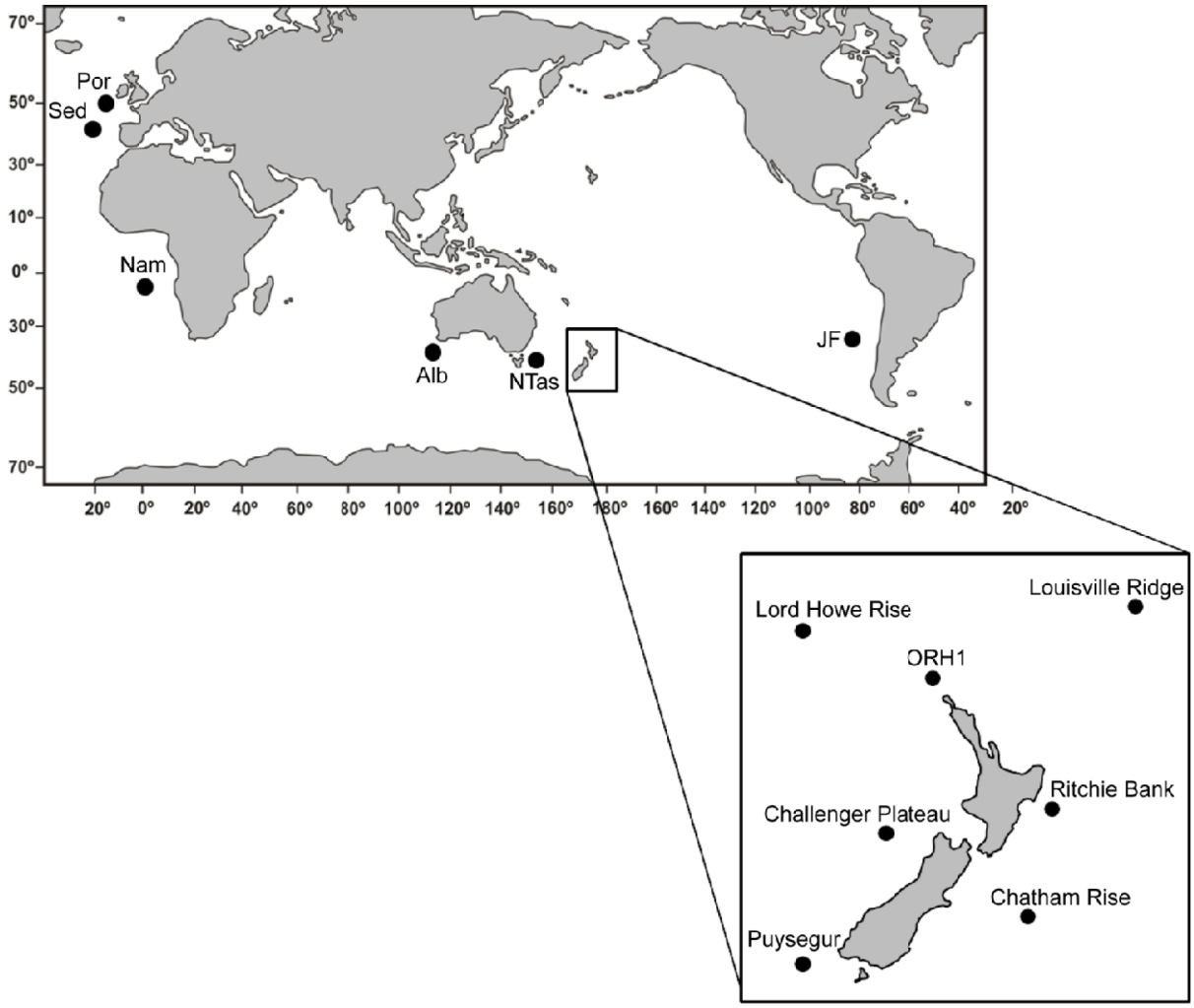


Fig. 2.1 Map showing the 13 sampling sites across the five regions surveyed for orange roughy. Codes as follow: Por = Porcupine Bank, Sed = Sedlo (Northeast Atlantic), Nam = Namibia, Alb = Albany, NTas = North Tasmania (Australia), JF = Juán Fernández Archipelago (Chile), ORH1 = Northern New Zealand

DNA extractions were performed using proteinase K digestion followed by salt extraction. The remaining pellet was washed twice with ethanol and resuspended in 100 µL TE buffer. DNA samples were stored at 4°C.

2.3.2 PCR amplification, sequencing and alignment

A 630 bp fragment of the cytochrome *c* oxidase subunit I (COI) gene was amplified by PCR using the primers developed by Ward et al. (2005) with the modification of two bases in the reverse primer based on the complete mitochondrial sequence of *Hoplostethus japonicus* (GenBank: AP002938.1). The primers used were: FishF1-5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1(a)-5'-TATACTTCGGGG TGGCCAAAGAATCA-3'. A 416 bp fragment of the cytochrome *b* gene (cyt *b*) was amplified in 95 individuals from the five regions. The forward and reverse primers (Palumbi et al. 1991 and Kocher et al. 1989, respectively) were modified based on the complete mitochondrial sequence of *H. japonicus.* The primers used were tGludg(a)-5'-TGACTTGAAAAACCACCGTTG-3' and Cyb2(a)-5'-CCCTCAAAAGGATATTTGT CCTCA-3'.

Reactions of 10 µL total volume for partial amplification of both genes consisted of ~20 ng of DNA, 1X PCR buffer (160mM $(NH_4)_2SO_4$, 670mM Tris-HCl, 0.1% stabilizer), 2 mM $MgCl_2$, 0.6 µM of each primer, 0.2 mM of each dNTPs, 1.5 U of *Taq* polymerase and 0.4 mg mL^{-1} of Bovine Serum Albumin (BSA). PCR cycles were performed on a Eppendorf Mastercycler ep gradient S, as follow: 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 57°C for COI and 48°C for cyt *b* for 30 s, 72°C for 1 min and a final extension at 72°C for 10 min. PCR products were purified with ExoSAP-IT following manufacturer's instructions. The DNA sequences of the purified products were determined using an ABI3730 Genetic Analyzer. Initially, DNA sequences were obtained in both directions which resulted in high quality sequences, subsequently only the forward primer direction was sequenced for both the COI and cyt *b* genes. Sequences were aligned using the software Geneious 5.1.7 (Biomatters Ltd.) using the Geneious alignment option (Drumond et al. 2010). Alignments were verified through translation into amino acid sequences.

2.3.3 Data analyses

Genetic diversity indices, the number of polymorphic (segregating) sites (*S*), the number of haplotypes (N_h), haplotype diversity (h), and nucleotide diversity (π), were calculated in DnaSP 5.10 (Librado and Rozas 2009) for both genes. The software ARLEQUIN 3.11 (Excoffier et al. 2005) was used to estimate pairwise genetic divergence between populations using the fixation index Φ_{ST}. The significance of the Φ_{ST} values was evaluated by 20,000 permutations. Further analyses were only performed for COI sequences. The pattern of Isolation By Distance (IBD) was evaluated for New Zealand sites using a Mantel Test with 10,000 permutations comparing linearized Φ_{ST} with the shortest marine distances (ln m) between sites. Geographic distances were estimated using Google Earth v.4.3. ARLEQUIN 3.11 was also used to perform an AMOVA analysis with pairwise difference as the distance method and 20,000 permutations.

A haplotype network was first constructed using the median-joining approach implemented in the NETWORK 4.6 software (Bandelt et al. 1999). The resulting network had many loops that were only resolved using both, the Frequency >1 criterion and the criteria based on coalescent theory (Crandall and Templeton 1993). However, only haplotypes present in two or more individuals were represented in the network. The alternative method described by Salzburger et al. (2011) was implemented to obtain a complete haplotype genealogy without reticulations. A phylogenetic reconstruction was estimated with a Maximum Likelihood (ML) approach implemented in the program PhyML 3.0 (Guindon et al. 2010) using default settings and the best fit model obtained with jModelTest (Posada 2008). The ML tree was then converted into a haplotype genealogy using the Haploviewer software (Salzburger et al. 2011).

A mismatch distribution analysis was performed in ARLEQUIN and DnaSP to infer the demographic history. A unimodal distribution is indicative of a sudden population expansion in the past whereas a multimodal curve is most typical of populations in equilibrium whose sizes have been constant over a long period of time (Rogers and Harpending 1992). The demographic parameters τ, θ_0 and θ_1 were obtained from the mismatch distribution analysis. The time since the last expansion (t) can be estimated from $\tau = 2ut$ where τ is an index of time since expansion expressed in units of mutational time and u is the mutation rate considering $u = \mu k$ (μ is the mutation rate per nucleotide and k is the number of nucleotides obtained by PCR) (Rogers and Harpending 1992). Two mutations rates were applied for the COI sequences, the one

proposed by Brown et al. (1979) of 2%/Myr for the vertebrate mitochondrial genome based on mammalian data and that calibrated by Bermingham et al. (1997) of 1.2%/Myr for the COI gene in marine fishes. The parameters θ_0 and θ_1 correspond to the mutation parameter before and after population growth and is defined as $2Nu$ for mitochondrial loci, where N is the effective female population size and u is the mutation rate per generation. ARLEQUIN was also used to obtain the sum of square deviations (SSD) between the observed and the expected distribution of pairwise differences and to calculate the Harpending's raggedness index (H_r) (Harpending 1994). Both statistics were used to test the null hypothesis of population expansion. Tajima's (D) (Tajima 1989) and Fu's (F_S) (Fu 1997) neutrality tests were also performed in ARLEQUIN. Negative and significant D and F_S values are also indicative of population expansion.

I also performed Bayesian skyline analyses to infer changes in effective population size (N) through time. This analysis allows the discovery of complex demographic signatures that are not possible to recover with simple demographic models (Drummond et al. 2005). The settings to construct the skyline plot were implemented in BEAST v1.6.1 (Drummond and Rambaut 2007). All analyses were run under the HKY+I+G model that was selected by jModelTest (Posada 2008) using both, the Bayesian and the Akaike information criterion. The analyses were run for 100,000,000 iterations sampled every 10,000 iterations with a burn-in of 10%. The parameter m that represents the number of grouped intervals was set to 10. The mutation rate used was 1.2%/Myr (Bermingham et al. 1997) with an uncorrelated lognormal relaxed clock model (Drummond et al. 2006). All operators were optimized automatically. Two independent runs were combined using LogCombiner v.1.6.1 (Drummond and Rambaut 2007). The skyline plot was then visualized in TRACER v1.5 (Rambaut and Drummond 2007).

2.4 Results

2.4.1 Genetic diversity and population structure

Analyses of the COI gene sequences for 546 individuals revealed very high levels of diversity. In the 630 bp fragment, 82 sites were polymorphic and produced 114 haplotypes of which 71 were unique (recorded in one individual only). The haplotype

and nucleotide diversities ranged from 0.810 to 0.940 and from 0.0034 to 0.0049 respectively in each site (Table 2.1). Analyses of the cyt *b* gene sequences for 95 individuals also showed high levels of variation. In the 416 bp fragment, 37 sites were polymorphic and there were 37 haplotypes of which 24 were unique. The haplotype and nucleotide diversities ranged from 0.819 to 0.965 and from 0.0063 to 0.0099 respectively (Table 2.2). Sequences of all haplotypes were deposited in GenBank under Accession Numbers: JN580075- JN580188 for the COI gene and JN580189-JN580225 for the cyt *b* gene. The haplotype frequencies in each sampling site/area are provided in Appendixes A and B for the COI and cyt *b* genes, respectively.

Table 2.1 Sampling sites grouped in five regions and COI genetic diversity indices. *n*: number of samples, *S*: number of segregating sites, N_h: number of haplotypes, *h*: haplotype diversity, π: nucleotide diversity. The standard deviations of haplotype and nucleotide diversity values are given between brackets

Regions	Sampling sites	Code	*n*	*S*	N_h	*h*	Π
New Zealand	Northern New Zealand	ORH1	41	23	23	0.926 (0.028)	0.00430 (0.00036)
	Ritchie Bank	Rit	40	23	22	0.931 (0.026)	0.00466 (0.00039)
	Chatham Rise	Chat	46	31	24	0.926 (0.025)	0.00406 (0.00044)
	Puysegur	Puy	48	25	21	0.890 (0.030)	0.00408 (0.00040)
	Challenger Plateau	Cha	30	24	19	0.926 (0.034)	0.00494 (0.00051)
	Lord Howe Rise	LHR	40	27	23	0.940 (0.023)	0.00420 (0.00044)
	Louisville Ridge	Lou	40	21	22	0.933 (0.022)	0.00399 (0.00038)
Australia	North Tasmania	NTas	25	11	10	0.810 (0.056)	0.00342 (0.00035)
	Albany	Alb	46	25	23	0.916 (0.024)	0.00411 (0.00035)
Namibia	Namibia	Nam	45	19	16	0.872 (0.032)	0.00413 (0.00038)
Chile	Juan Fernández	JF	52	19	14	0.839 (0.035)	0.00403 (0.00036)
Northeast	Porcupine Bank	Por	51	16	12	0.831 (0.038)	0.00466 (0.00043)
Atlantic	Sedlo	Sed	42	19	13	0.837 (0.039)	0.00445 (0.00049)
	Total		546	82	114	0.902 (0.008)	0.00433 (0.00012)

Table 2.2 Samples sizes for the five regions and genetic diversity indices of the cyt *b* gene. *n*: number of samples, *S*: number of segregating sites, N_h: number of haplotypes, *h*: haplotype diversity, π : nucleotide diversity. The standard deviations of haplotype and nucleotide diversity values are given between brackets

Regions	*N*	*S*	N_h	*H*	π
New Zealand	19	15	10	0.819 (0.083)	0.00628 (0.00126)
Australia	19	17	14	0.965 (0.028)	0.00922 (0.00072)
Namibia	18	15	9	0.863 (0.057)	0.00817 (0.00111)
Chile	19	15	12	0.936 (0.037)	0.00922 (0.00099)
Northeast Atlantic	20	15	8	0.821 (0.072)	0.00998 (0.00100)
Total	95	37	37	0.907 (0.020)	0.00863 (0.00045)

None of the pairwise Φ_{ST} values showed significant differentiation for the cyt *b* gene ($P > 0.05$, data not shown). The pairwise Φ_{ST} analysis of the COI sequences between sites resulted in low values revealing a lack of significant genetic differentiation for most comparisons (Table 2.3). However, Φ_{ST} values were highest for comparisons between the Northeast Atlantic sites and all the other sites and all were significant ($P < 0.05$). Significant differences after Bonferroni correction between few pairs of sites are shown in Table 2.3.Within regions, the only significant differentiation was between Chatham Rise and Puysegur in New Zealand ($\Phi_{ST} = 0.031$, $P = 0.027$) and there was an absence of IBD for New Zealand sites (Mantel test, $P = 0.853$). In the absence of significant differentiation within the regions, sites were pooled by region to perform a new Φ_{ST} analysis. The revised analysis on pooled samples showed significant differentiation (after Bonferroni correction) among the Northeast Atlantic region and all other regions (Table 2.4).

The AMOVA for the COI data revealed that almost all the variation was within sites (97.88%) with a significant overall Φ_{ST} value among sites ($\Phi_{ST} = 0.021$, $P = 0.0003$), which is influenced by the slight differentiation between the Northeast Atlantic sites and all the others sites. This was confirmed performing a new AMOVA without the Northeast Atlantic sites, which showed that the overall Φ_{ST} among sites was very low and non significant ($\Phi_{ST} = 0.0003$, $P = 0.425$).

Table 2.3 Pairwise Φ_{ST} values for the COI gene between sampling sites (below diagonal) and *P* values (above diagonal). Codes as in Table 2.1. Significant Φ_{ST} values ($P < 0.05$) are shown in bold. Values with * indicate significant differentiation after Bonferroni correction ($P < 0.0006$). The pairwise difference was the distance method and 20,000 permutations were performed

	New Zealand							Australia		Namibia	Chile	Northeast Atlantic	
	ORH1	Rit	Chat	Puy	Cha	LHR	Lou	NTas	Alb	Nam	JF	Por	Sed
ORH1		0.6779	0.1776	0.6567	0.6917	0.6617	0.3829	0.6949	0.6932	0.6893	0.3029	0.0034	0.0237
Rit	-0.008		0.0586	0.8959	0.7516	0.3678	0.2764	0.7105	0.4929	0.8402	0.4687	0.0108	0.0474
Chat	0.008	0.021		0.0278	0.5808	0.9765	0.4301	0.0542	0.1114	0.1060	0.0232	0.0001	0.0002
Puy	-0.007	-0.012	**0.031**		0.3649	0.2062	0.1622	0.9497	0.4922	0.7699	0.6656	0.0089	0.0473
Cha	-0.009	-0.011	-0.005	-0.001		0.8937	0.8875	0.4346	0.3121	0.7113	0.2418	0.0021	0.0091
LHR	-0.007	0.001	-0.013	0.007	-0.013		0.9030	0.2258	0.5740	0.4159	0.1298	0.0005	0.0051
Lou	-0.001	0.004	-0.002	0.010	-0.014	-0.011		0.1918	0.3161	0.4953	0.1487	0.0005	0.0048
NTas	-0.012	-0.013	0.032	-0.019	-0.005	0.008	0.012		0.2875	0.4726	0.7318	0.0302	0.0489
Alb	-0.008	-0.004	0.013	-0.004	0.002	-0.005	0.002	0.003		0.5189	0.3047	0.0044	0.0497
Nam	-0.008	-0.012	0.015	-0.010	-0.010	-0.002	-0.004	-0.006	-0.005		0.3743	0.0048	0.0442
JF	0.001	-0.004	0.036	-0.008	0.006	0.013	0.012	-0.015	0.001	-0.001		0.0064	0.0259
Por	**0.068**	**0.052**	***0.134**	**0.050**	**0.089**	***0.101**	***0.097**	**0.053**	**0.063**	**0.063**	**0.058**		0.2574
Sed	**0.038**	**0.028**	***0.093**	**0.027**	**0.060**	**0.062**	**0.062**	**0.041**	**0.026**	**0.032**	**0.039**	0.005	

Table 2.4 Pairwise Φ_{ST} values for the COI gene between regions (below diagonal) and *P* values (above diagonal). Significant Φ_{ST} values after Bonferroni correction ($P < 0.005$) are shown in bold. The pairwise difference was the distance method and 20,000 permutations were performed

	New Zealand	Australia	Namibia	Chile	Northeast Atlantic
New Zealand		0.4534	0.6956	0.1049	0.0001
Australia	-0.001		0.6036	0.5508	0.0016
Namibia	-0.004	-0.006		0.3676	0.0050
Chile	0.008	-0.005	-0.001		0.0046
Northeast Atlantic	**0.071**	**0.046**	**0.047**	**0.047**	

Haplotypes from the different sites were grouped by region to construct the haplotype genealogy. A star-like genealogy was obtained showing three main haplotypes of high frequency present in all five regions (Fig. 2.2). Most of the derived haplotypes were shared by more than one region. However, considering the haplotypes of frequency > 1, four haplotypes were only present in the Northeast Atlantic (frequency 4-8). Five haplotypes were restricted to New Zealand (frequency 2-5), but the New Zealand region included the greatest number of sites (7) and individuals (285) compared to two sites and 93 individuals from the Northeast Atlantic. Therefore, the finding of four haplotypes restricted to the Northeast Atlantic is more meaningful than five haplotypes restricted to the New Zealand area.

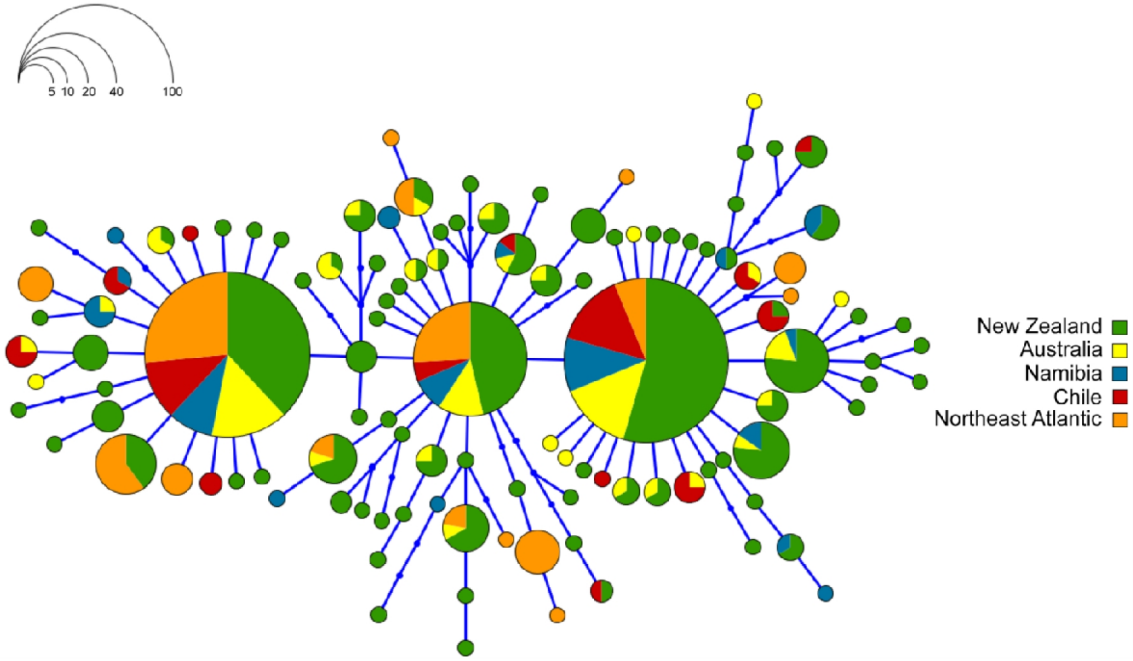


Fig. 2.2 Haplotype genealogy from Maximum Likelihood (ML) tree of the COI sequences performed in the software Haploviewer, which shows the relationship among haplotypes of the five regions. The model of nucleotide substitution for ML was HKY + G (0.744) + I (0.654). The circles represent the haplotypes. The scale shown on the upper left side of the figure indicates the relationship between circle sizes and the frequency of the haplotypes. Lines connecting the circles indicate a mutational step and dots in the lines represent putative mutational steps between haplotypes

2.4.2 Demographic history

High levels of haplotype diversity (mean 0.902 for COI and 0.907 for cyt *b*) and low nucleotide diversity (mean 0.0043 for COI and 0.0086 for cyt *b*) are indicative of a population bottleneck followed by rapid population growth and accumulation of mutations (Grant and Bowen 1998). This interpretation was supported by negative and significant Tajima's *D* and Fu's *F*s values in each site. Few sites showed negative but not significant values (Table 2.5). The overall Tajima's *D* statistic was negative but not significant (D = -1.49, P = 0.078). However, the overall Fu's *F*s, which is more sensitive to population demographic expansion, was significantly negative (Fs = -11.94, P = 0.033) (Table 2.5).

Table 2.5 Values and probabilities of Tajima's *D* and Fu's *F*s for all the sampling sites

Regions	Sampling sites	Tajima's *D*	*P*	Fu's *F*s	*P*
New Zealand	Northern New Zealand	-1.66	0.028	-18.58	< 0.001
	Ritchie Bank	-1.54	0.034	-15.77	< 0.001
	Chatham Rise	-2.15	0.003	-19.92	< 0.001
	Puysegur	-1.79	0.022	-13.75	< 0.001
	Challenger Plateau	-1.73	0.017	-12.98	< 0.001
	Lord Howe Rise	-1.99	0.004	-19.29	< 0.001
	Louisville Ridge	-1.63	0.037	-17.97	< 0.001
Australia	North Tasmania	-0.87	0.204	-3.39	0.031
	Albany	-1.80	0.016	-17.73	< 0.001
Namibia	Namibia	-1.29	0.082	-6.73	0.004
Chile	Juan Fernández	-1.25	0.098	-4.08	0.056
Northeast Atlantic	Porcupine Bank	-0.54	0.359	-1.73	0.258
	Sedlo	-1.19	0.110	-3.30	0.076
	Mean	-1.49	0.078	-11.94	0.033

The mismatch analysis produced a unimodal distribution (Fig. 2.3) consistent with a population expansion event. This was also supported by the sum of square distributions values and low levels of Harpending's raggedness indexes in all sites without exceptions (mean SSD = 0.009, P = 0.431 and mean H_r = 0.042, P = 0.503, Table 2.6). Additional evidence for population expansion in orange roughy was the star-like shape of the haplotype genealogy (Fig. 2.2) and the values of θ_0 and θ_1 for all populations that ranged from zero to 0.05 and from 5.85 to 99999 respectively (Table 2.6). The values of θ_0 and θ_1 also suggest that there was a major reduction before the expansion. The mean value of τ was 3.19 and the time since the last expansion was estimated between 211,000 and 126,000 years ago (Middle - Late Pleistocene). Interestingly, the skyline plot revealed two expansion events (Fig. 2.4). According to the plot, a fast population growth occurred between ~150,000 and 75,000 years ago (Late Pleistocene) and a second but smaller expansion event took place less than 25,000 years ago (end of Pleistocene).

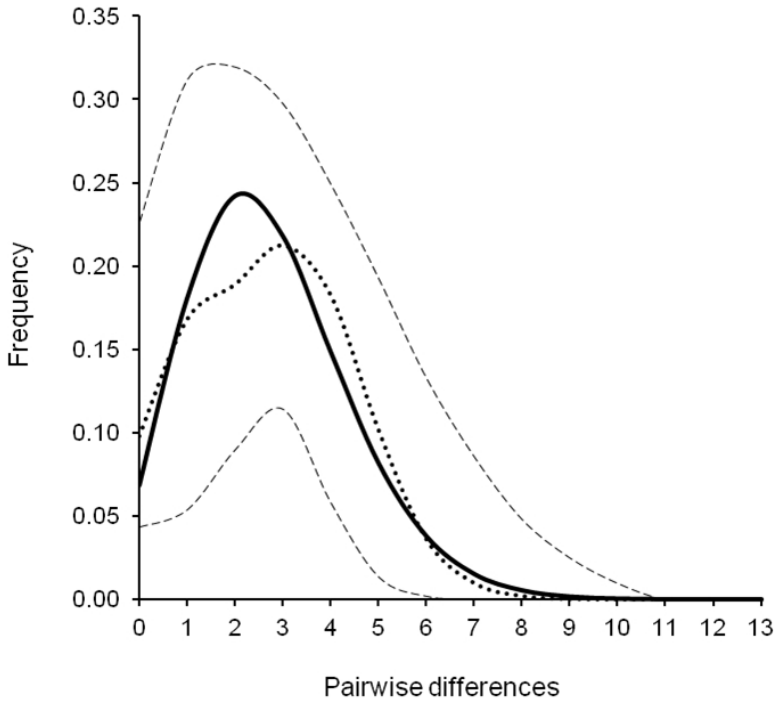


Fig. 2.3 Mismatch distribution graphic of the COI sequences. The solid line indicates the expected frequency of the pairwise differences between sequences under the sudden expansion model. The dotted line indicates the observed frequency and the dashed lines the 95% confidence intervals (CI). SSD = 0.00941, P = 0.431. F_S = -11.94, P = 0.033

Table 2.6 Mismatch distribution values for all the sites. τ : Time since expansion expressed in units of mutational time, θ_0: population size before expansion, θ_1: population size after expansion, SSD: Sum of squared distribution and H_r: Harpending's raggedness index

Regions	Sampling sites	Mismatch distribution			Goodness-of-fit	
		τ (95% CI)	θ_0	θ_1	SSD (*P*)	H_r (*P*)
New Zealand	Northern New Zealand	2.97 (1.54-3.65)	0 (0-0.92)	379.38 (12.07-99999)	0.007 (0.160)	0.039 (0.312)
	Ritchie Bank	3.36 (1.38-4.62)	0 (0-1.24)	27.50 (7.47-99999)	0.005 (0.335)	0.029 (0.538)
	Chatham Rise	2.63 (1.38-4.62)	0 (0-0.89)	48.44 (6.51-99999)	0.006 (0.210)	0.043 (0.362)
	Puysegur	2.94 (0.87-4.32)	0.02 (0-0.87)	12.66 (4.36-5.68)	0.001 (0.829)	0.020 (0.868)
	Challenger Plateau	3.64 (1.47-4.91)	0 (0-1.35)	35.00 (9.17-99999)	0.022 (0.083)	0.071 (0.075)
	Lord Howe Rise	2.69 (1.63-3.29)	0 (0-0.70)	2515.00 (8.59-99999)	0.001 (0.607)	0.034 (0.561)
	Louisville Ridge	2.55 (1.53-3.25)	0.02 (0-0.38)	99999 (9.61-99999)	0.001 (0.776)	0.038 (0.466)
Australia	North Tasmania	2.88 (0.60-4.76)	0 (0-59)	8.36 (3.20-99999)	0.031 (0.150)	0.089 (0.218)
	Albany	2.79 (1.28-3.81)	0.05 (0-0.99)	38.13 (7.94-99999)	0.002 (0.641)	0.027 (0.697)
Namibia	Namibia	3.21 (0.96-5.04)	0.01 (0-0.91)	10.49 (4.09-99999)	0.006 (0.499)	0.026 (0.701)
Chile	Juan Fernández	3.63 (0.64-6.26)	0 (0-0.83)	6.02 (2.88-99999)	0.009 (0.526)	0.027 (0.759)
Northeast	Porcupine Bank	4.75 (0.79-7.92)	0 (0-1.06)	5.85 (3.06-99999)	0.010 (0.599)	0.023 (0.832)
Atlantic	Sedlo	3.52 (0.79-5.79)	0.01 (0-1.08)	8.65 (4.06-99999)	0.021 (0.182)	0.075 (0.147)
	Mean	3.19 (1.12-4.69)	0 (0-0.91)	7930.34 (6.39-99999)	0.009 (0.431)	0.042 (0.503)

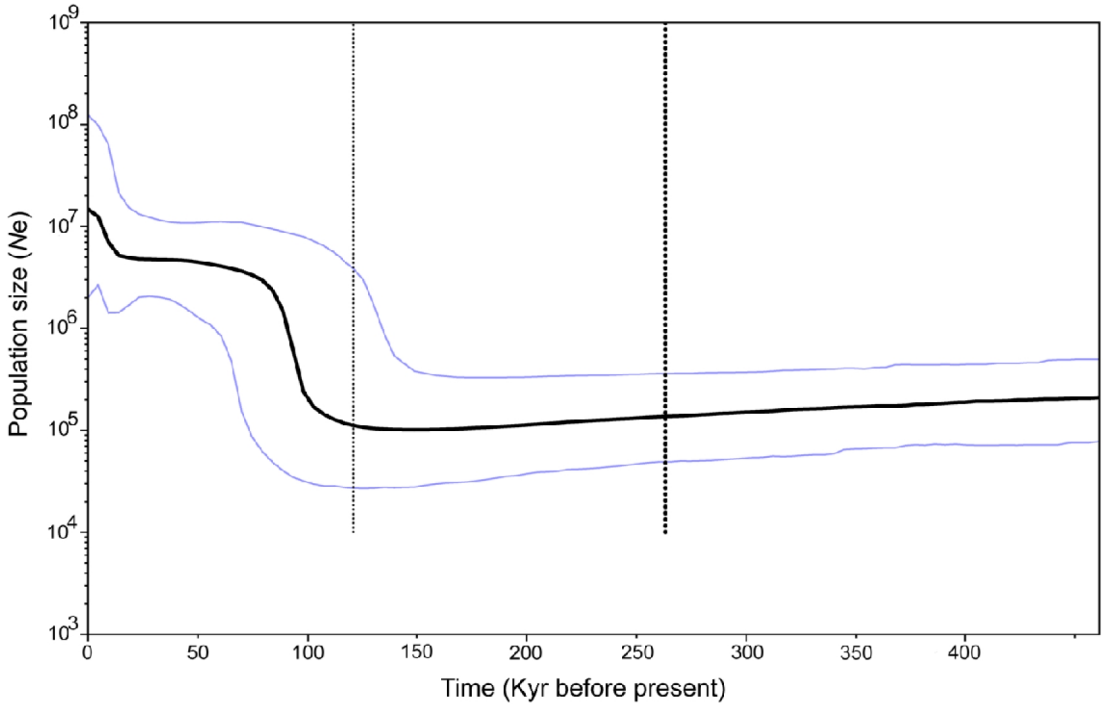


Fig. 2.4 Bayesian skyline plot showing change of population size through time using a mutation rate of 1.2%/Myr. The thick line indicates the median estimate and the thin lines denote the 95% upper and lower confidence limits (95% HPD). The verticals dotted lines indicate the maximum time when the root height is the lower 95% HPD and the upper 95% HPD

2.5 Discussion

2.5.1 Genetic diversity and structure

Analyses of the COI and cyt *b* sequences in orange roughy revealed high haplotype diversity which is indicative of a large historical effective population size (McCusker and Bentzen 2010). This pattern of high haplotype diversity is common in marine fishes and consistent with previous reports for cyt *b* sequences in orange roughy by Baker et al. (1995) and in the bluemouth *Helicolenus dactylopterus* by Aboim et al. (2005). Similarly, high values of diversity have been reported for mtDNA d-loop sequences in other deep-sea fishes such as the thornyhead rockfishes *Sebastolobus alascanus, S. altivelis* and *S. macrochir*, the cape hake *Merluccius capensis*, the Alaskan Pacific halibut *Hippoglossus stenolepis*, and the alfonsino *Beryx decadactylus* (Stepien et al. 2000; von der Heyden et al. 2007; Nielsen et al. 2010; Friess and Sedberry 2011).

The lack of genetic differentiation among orange roughy populations from the South Atlantic Ocean and South Pacific Ocean is surprising given the large geographic distances between the regions surveyed in this study. Genetic homogeneity among distant orange roughy populations was also found by Smith (1986), Elliott and Ward (1992) and Oke et al. (2002) with different molecular markers. At a regional scale (i.e. within New Zealand) and between regions, our results are consistent with those reported in a study of microsatellite markers (White et al. 2009a), which found panmixia in orange roughy within the Northeast Atlantic, but significant differentiation between samples from the Northeast Atlantic and one sample from Namibia. A lack of genetic differentiation has been also reported for other deep-sea fishes, as the armorhead *Pseudopentaceros wheeleri* in the North Pacific Ocean (Martin et al. 1992; Yanagimoto et al. 2008). The larvae of the armorhead remain pelagic for 1.5 to 2.5 years and have a neustonic distribution with high potential for dispersion by wind-driven currents (Boehlert and Sasaki 1988; Mundy and Moser 1997). Similarly, genetic homogeneity was reported among populations of the wreckfish *Polyprion americanus* in the North Atlantic (Sedberry et al. 1996), which is probably related to the long period that juvenile stages spend in the pelagic environment. Several other deep-sea species show genetic homogeneity among populations and have pelagic larval and juvenile stages, that last for several months and could be passively transported by ocean currents (Hoarau and Borsa 2000; von der Heyden et al. 2007; Friess and Sedberry 2011).

In contrast, orange roughy have a relatively short larval period of ~ 10 days (Zeldis et al. 1998), and coupled with spawning around seamounts, where Taylor columns (closed circulation cells) can retain eggs and larvae (Rogers 1994), long distant juvenile dispersal is unlikely. The characteristics of orange roughy that might contribute to panmixa over such large distances might relate to their longevity and active adult dispersal. It is generally recognized that some deepwater teleosts (e.g. *Coryphaenoides* spp., *Sebastolobus altivelis*) have reduced bone and musculature for neutral buoyancy and low metabolism as an adaptation to the deep-sea environment (Koslow 1996). However, the metabolism of orange roughy is substantially higher than the typical non-migratory deepwater fishes, and similar to shallow water active species (Koslow 1997), suggesting a high capacity for active migration of adults. Based on this fact, I agree with White et al. (2009a) that there is potential for gene flow under a stepping stone model, especially considering the longevity of orange roughy and the opportunity to spawn many times in a lifetime. Rogers et al. (2006) reported that the lack of differentiation between two Southern Ocean sites in the Patagonian toothfish could be due to stepping stone links via oceanic ridge systems and seamounts. Although the geographic distance among orange roughy sites surveyed here is much larger than between the two sites considered by Rogers et al. (2006), there could be systems connecting ridges or seamounts were orange roughy is found that may promote dispersal of adults.

Nevertheless, the two Northeast Atlantic sites (41°N and 50°N) showed some degree of genetic differentiation from all the other regions. Oceanographic characteristics may be important for explaining this pattern. Tsuchiya et al. (1992) reported an abrupt water-mass boundary at 41°N at the Azores-Biscay Rise. It is possible that this oceanographic boundary restricts the north-south dispersal of orange roughy ~41°N and may help explain our findings. Low but significant differentiation between sites in the Azores region (38-39°N) and one site off Madeira (33°N) was detected for the deep-sea species *Pagellus bogaraveo* with mitochondrial DNA sequences (Stockley et al. 2005). Further studies are needed to determine if oceanographic characteristics at the Azores-Biscay Rise around 41°N represent a barrier for dispersal of deep-sea teleosts.

When considering a fine scale of comparison, it is notable that Carlsson et al. (2011) using microsatellites and otolith chemistry found shallow but significant genetic differentiation in orange roughy samples within a complex topographic system in the Northeast Atlantic. The contrast between our results (lack of genetic differentiation at a regional scale, i.e within New Zealand) and the results of Carlsson et al. (2011) could be

explained by the different signals revealed by different types of DNA markers. Although, as mentioned before, White et al (2009a) using microsatellites markers found no significant differentiation in a fine scale study in the Northeast Atlantic. It is possible that the findings of Carlsson et al. (2011) are related to differences in the particular topographic system, as the genetic differences were largely between one flat site and seamount sites.

The genetic structure of orange roughy, as measured here with mtDNA markers, suggest that homogeneity prevails over large geographic areas and there is no measurable genetic divergence among populations. However, this does not necessarily mean that populations are demographically connected. Genetic differentiation may not be detected with selectively neutral markers especially when population sizes are large, and when populations have recently diverged. Indeed the COI gene typically shows low divergence among widely distributed populations in marine fishes (Ward et al. 2009). The genetic homogeneity found in this study may result from 1) panmixia; 2) recent differentiation that is undetected with mitochondrial markers; 3) few migrants per generation that maintain genetic connectivity, but not necessarily demographic connectivity. Nevertheless, the mitochondrial DNA markers employed here have clearly shown high genetic diversity, which is related with historical large population size (McCusker and Bentzen 2010), and genetic connectivity among orange roughy populations in the past, even though more recent events may have changed these patterns.

2.5.2 Implications for fisheries management in New Zealand

Currently there are five areas that are treated separately for assessment and management of orange roughy in New Zealand. Each area (ORH) may consist of one or more stocks. In this study I included samples from Northern New Zealand (ORH1), Ritchie Bank (ORH2A), Chatham Rise and Puysegur (ORH3B), Challenger Plateau (ORH7A) and two sites outside the EEZ, Lord Howe Rise and Louisville Ridge. I found no significant differences among sites and no pattern of IBD. The COI data did not reject a single genetic-stock model for orange roughy in New Zealand waters. However, there was one exception; the Chatham Rise sample (east of central New Zealand) was significantly different to the Puysegur sample (south of New Zealand) at the 5% level (Φ_{ST} = 0.031,

P = 0.027), but not after Bonferroni correction. While additional markers are desirable to determine the extent of differentiation among these sites, the data presented here provide additional genetic support for management of the Puysegur stock as a separate unit to the Chatham Rise stock within ORH3B. It is notable that earlier studies of mtDNA RFLPs's found significant divergence between these sites (Smith et al. 1996; 1997).

2.5.3 Demographic history

The pattern of high haplotype diversity versus low nucleotide diversity found in all the sites and for the total pooled sites indicates that there was a drastic reduction in population size in the past followed by sudden expansion (Grant and Bowen 1998). Similar results have been found in other fishes, *Helicolenus dactylopterus*, *Merluccius capensis*, and *Glyptocephalus stelleri* (Aboim et al. 2005; von der Heyden et al. 2007; Xiao et al. 2010).

Taking into account both, mismatch distribution and skyline analyses and the mutation rates of 2% and 1.2%/Myr (Brown et al. 1979; Bermingham et al. 1997), two expansion events were detected for orange roughy. The first was estimated to have occurred between 200,000 to 75,000 years ago during the Middle - Late Pleistocene period and the second of lower magnitude less than 25,000 years ago at the end of the Pleistocene. The timing of the population expansion events largely depends on the mutation rate used, therefore the precise timing of these events is uncertain, but they most likely occurred during the Pleistocene period which span from ~1.6 Myr to 10,000 years before present. The Pleistocene was punctuated by a series of large glacial-interglacial changes (Imbrie et al. 1992). Over the last ~175,000 years, marine ecosystems responded to the climatic events, with changes in species distributions and abundances and changes in productivity (Webb and Bartlein 1992). There were also changes in oceanic circulation, for example, Rasmussen et al. (2003) found that during a period 160,000 to 10,000 years ago the Labrador Sea -one of the main areas for deep water formation in the North Atlantic Ocean today- underwent rapid variations in deep and surface water circulation coinciding with ice rafting events. It has been inferred that glaciations-interglaciations events and associated changes in the marine environment may have had great effects in the demographic history of many marine fishes as the

coastal species *Glyptocephalus stelleri* and *Pennahia argentata* (Xiao et al. 2010; Han et al. 2008) and the deep-sea species as *Beryx decadactylus* (Friess and Sedberry 2011).

2.6 Conclusion

Using mitochondrial DNA sequences I found little evidence of genetic differentiation at both global (between Oceans) and local (within New Zealand) scales. Only the Northeast Atlantic sample showed some degree of genetic differentiation from all other regions. This pattern of historical genetic homogeneity may be explained by dispersal of adults under the stepping-stone model. These findings provide new evidence supporting previous studies that have reported high levels of connectivity in deep-sea species. On the other hand, demographic analyses indicated that there were two population expansion events in orange roughy, one during the Middle-Late Pleistocene and the second at the end of the Pleistocene period.

Chapter 3

Global and fine-scale population structure in the commercially exploited deep-sea teleost orange roughy (*Hoplostethus atlanticus*)

3.1 Abstract

The widely distributed teleost orange roughy, *Hoplostethus atlanticus*, has been one of the main species targeted in deep-sea fisheries. While morphometric, parasite and otolith analyses have generally found differentiation among populations within ocean basins, genetic techniques have shown contradictory results at different geographical scales. Here, I used nine microsatellite DNA loci to study genetic diversity and differentiation in orange roughy at a global scale using samples from New Zealand, Australia, Namibia, Chile, and the Northeast Atlantic Ocean. Additionally, temporal genetic variation was assessed for eight sites in Northern New Zealand sampled in two different years. The expected heterozygosity was high in all the sites, suggesting high levels of genetic diversity in orange roughy. Overall, I detected low but significant differentiation at the global scale: across the Southern hemisphere and between the Southern hemisphere regions and the Northeast Atlantic Ocean. However, genetic homogeneity was found between New Zealand and Australia. The present data does not showed temporal variation in orange roughy from Northern New Zealand. A pattern of isolation by distance at the global scale suggests stepping-stone migration, which is likely the result of active adult dispersal. This study revealed levels of genetic differentiation at the global scale that were undetected with mitochondrial DNA sequences analyses.

3.2 Introduction

The marine environment was once considered a largely "open" system with few boundaries to dispersal of marine organisms. However, increasing research has revealed that many marine species are not panmictic. Despite the potential for widespread dispersal of swimming adults and/or pelagic early life stages, many studies have reported restricted gene flow at different geographic scales in marine fishes. Oceanographic and topographic features, coupled with behavioural characteristics provide barriers for dispersal in marine species. For example, Fauvelot and Borsa (2011) found high levels of genetic divergence in the widely-distributed Spanish mackerel *Scomberomorus commerson* and suggested that the high migrating ability of this fish may be associated with phylopatric behaviour rather than promoting wide-scale dispersal. In contrast, there are examples of species with genetically homogeneous populations over wide geographical scales; Wu et al. (2010) found that the yellowfin tuna *Thunnus albacares* exhibits no genetic differentiation between populations from the Western Pacific and Western Indian Oceans. For commercial marine fishes rates of migration are important to determine the boundaries of a "stock", which is typically considered a population or unit large enough to be self-reproducing (Hilborn and Walters 1992). Stock delineation is of major importance for stock assessment and effective fisheries management, but it is complex and usually requires a multidisciplinary approach (see Begg et al. 1999). One widely used technique to assist in the study of stock structure is the application of neutral molecular markers (e.g. González-Wangüemert et al. 2010; Broderick et al. 2011; Schunter et al. 2011). Genetic studies are particularly useful for deep-sea species for which tag-recapture techniques are difficult to apply. Population genetics studies have been conducted on deep-sea fishes such as the wreckfish *Polyprion americanus* (Ball et al. 2000), the tusk *Brosme brosme* (Knutsen et al. 2009) and the alfonsino *Beryx decadactylus* (Friess and Sedberry 2011) among others. While dispersal by larval stages and swimming adults was related to the finding of a single genetic stock in *B. decadactylus* in the North Atlantic Ocean (Friess and Sedberry 2011), genetic differentiation among North Atlantic samples of *B. brosme* was related to bathymetric barriers (Knutsen et al. 2009). At a wider scale including samples from the North and South Atlantic Ocean, the Mediterranean Sea, and the South Pacific Ocean, Ball et al. (2000) found three genetic stocks in the wreckfish *P. americanus*.

Many deep-sea fishes are *K*-selected: they are long-lived, have late maturation and low fecundity. The increased fishing pressure on deep-sea species over the last three decades (see Pitcher et al. 2010) coupled with their life history characteristics make them vulnerable to over exploitation (reviewed by Baker et al. 2009). One such species is the widely distributed deep-sea teleost orange roughy, *Hoplostethus atlanticus,* which is found between 450 and 1,800 m in the Indian Ocean, South Pacific Ocean, and the Northeast Atlantic Ocean (Horn et al. 1998; Branch 2001; Labbé and Arana 2001). Like other deep-sea fish species, orange roughy occur around seamounts especially during spawning and feeding events. It has been suggested that they could migrate hundreds of kilometres to spawn (Bell et al. 1992; Francis and Clark 1998), but they also disperse to flat areas close to seamounts (Clark 1996). Orange roughy have been one of eight major target species in global seamount fisheries (Pitcher 2010). Fisheries for orange roughy started off New Zealand in 1979 and progressively expanded to Australia, Namibia, the Northeast Atlantic Ocean, the Indian Ocean and Chile (reviewed by Branch 2001). Many stocks have been fished down to or below 30% of initial levels, and some fisheries have been closed or quotas largely reduced (see Clark 2009; Niklitscheck et al. 2010; Foley et al. 2011). Currently, the only remaining large-scale fisheries for orange roughy are around New Zealand (Clark 2009; New Zealand Ministry of Fisheries 2011).

Several studies using parasite, phenotypic traits, and genetic markers have identified population differentiation in orange roughy over a range of geographical scales (Lester et al. 1988; Edmonds et al. 1991; Smolenski et al. 1993; Elliott et al. 1995; Smith et al. 1996; Smith and Benson 1997; Smith et al. 1997; Smith et al. 2002a). Some earlier genetic studies found little differentiation at regional and global scales (Smith 1986; Elliott and Ward 1992; Oke et al. 2002). Using mitochondrial DNA sequences and large sample sizes, I found genetic homogeneity between orange roughy from sites off New Zealand, Australia, Namibia, and Chile, but low levels of genetic differentiation between these regions and the Northeast Atlantic Ocean (Chapter 2). Contrasting levels of genetic differentiation have been detected with microsatellite DNA markers among populations in the Northeast Atlantic Ocean. White et al. (2009a) detected panmixia among five sites between ~ 40°N and 56°N, some separated by over 2,000 km, while Carlsson et al. (2011) detected low but significant differentiation at a smaller spatial scale within the Porcupine Bank (a complex topographic system ~ 50° N), mostly in comparisons between one flat site and seamount sites.

The main goal of the present study was to use microsatellite DNA markers over the same geographic range surveyed with mitochondrial DNA sequences (Chapter 2) to determine levels of nuclear variation and to test for global levels of genetic differentiation that might have been undetected with the mtDNA marker. The second goal was to conduct a fine-scale study around New Zealand to provide new genetic data for local stock discrimination and fisheries management. Samples from a second season for some sites in Northern New Zealand were included to assess temporal variation.

3.3 Materials and methods

3.3.1 Sampling and DNA extraction

Tissue samples from 812 individuals were available from 22 sites in five regions, New Zealand (16 sites), Australia (2 sites), Namibia (1 site), Chile (1 site) and the Northeast Atlantic Ocean (2 sites) (Fig. 3.1). Around northern New Zealand (fishery management area ORH 1), eight sites were sampled in both 2008 and 2010 (Fig. 3.1). Muscle tissue samples were taken from fresh fish and frozen at -20°C, or stored in 95% ethanol. Samples from ORH 1 were obtained by New Zealand Ministry of Fishery Observers. Samples from all other sites around New Zealand, Australia, and Namibia were made available from a frozen tissue collection held at NIWA (National Institute of Water and Atmospheric Research Ltd., New Zealand). Samples from Chile and the Northeast Atlantic Ocean were provided from tissue collections held at Universidad Austral de Chile, University College Cork, Ireland and University of the Azores, Portugal.

A small piece of tissue (~ $2mm^3$) was used for DNA extractions. Genomic DNA was obtained using a salt extraction protocol with proteinase K digestion. The remaining pellet was washed two times with ethanol and resuspended in 100 µL TE buffer. DNA samples were stored at 4°C.

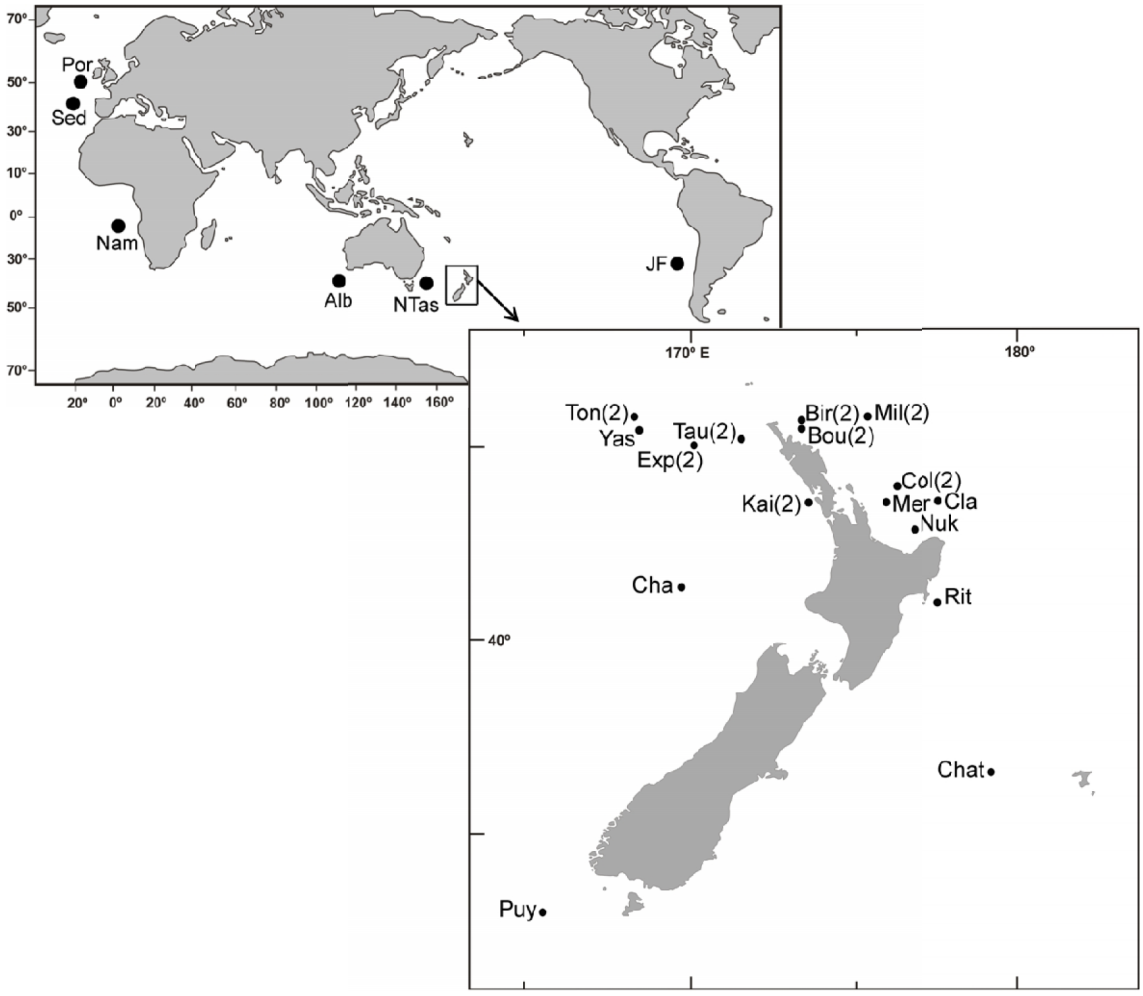


Fig. 3.1 Map showing the 22 sampling sites across the five regions surveyed. Sites sampled in both, 2008 and 2010 are indicated by (2). Codes for New Zealand sites are: Kai = Kaipara Flats, Tau = Tauroa Knoll, Exp = Explorer Hill, Ton = Tony B, Yas = Yasmin's, Bir = Birdflue, Bou = Boulder Ridge, Mil = Milan's, Nuk = Nukuhou Knoll, Col = Colville Knoll, Mer = Mercury Knoll, Cla = Clark, Rit = Ritchie Bank, Chat = Chatham Rise, Puy = Puysegur, Cha = Challenger Plateau. Codes for sites outside New Zealand are as follow: Por = Porcupine Bank, Sed = Sedlo (Northeast Atlantic), Nam = Namibia, Alb = Albany, NTas = North Tasmania (Australia), JF = Juán Fernández Archipelago (Chile).

3.3.2 PCR and genotyping

Fourteen published microsatellite DNA loci (Oke et al. 1999; White et al. 2009b) were assessed in preliminary trials to determine amplification success by polymerase chain reaction (PCR) and genotyping errors. Five loci were discarded due to technical difficulties; four of them showed a consistent presence of null alleles and one locus presented a high proportion of missing data (i.e. alleles were not possible to determine for more than 80% of the individuals). I used nine loci: *Hat2a*, *Hat3*, *Hat7*, *Hat9a*, *Hat45* (Oke et al. 1999), *HopAt2*, *HopAt4*, *HopAt5* and *HopAt11* (White et al. 2009b) across all 812 samples. Forward primers were synthesised by Applied Biosystems (Foster City, USA) with the 5'-labelled with a florescent tag: VIC, 6FAM, NED or PET. Reverse primers were synthesised by Invitrogen (Auckland, New Zealand). Final PCR reactions of 15 µL total volume consisted of ~20 ng of DNA, 1X PCR buffer (160mM $(NH_4)_2SO_4$, 670mM Tris-HCl, 0.1% stabilizer), 2 mM $MgCl_2$, 0.5 µM of each primer, 0.2 mM of each dNTPs, 1.5 U of *Taq* polymerase and 0.4 mg mL^{-1} of Bovine Serum Albumin (BSA). PCR cycles were performed in an Eppendorf Mastercycler ep gradient S using 96 well plates. The profile for primers *Hat2a*, *Hat3*, *Hat7*, *Hat9a* and *Hat45* was: 94°C for 3 min, then 10 cycles of denaturing at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 30 s followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s and a final extension at 72°C for 10 min. For primers *HopAt2*, *HopAt4*, *HopAt5* and *HopAt11* the profile was: 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 63°C for 90 s, 72°C for 1 min and a final extension at 60°C for 10 min. Final PCR products of each locus were pooled together for genotyping (three pool plexes each one with three loci). Samples were analyzed on a capillary ABI3730 Genetic Analyser (Applied Biosystems Inc.) using LIZ 500 (-250) size standard by Massey Genome Service (Palmerston North, New Zealand). Alleles were scored using GENEMAPPER 3.7 (Applied Biosystems).

3.3.3 Data analyses

The total data set (812 specimens) was used for spatial and temporal analyses of genetic differentiation. The spatial analyses were performed for 620 individuals collected from the 22 sites (Table 3.1). For the temporal analyses eight sites in northern New Zealand (ORH 1) were sampled in both 2008 and 2010, and 192 individuals were available from

2010 (Table 3.4). MICROCHECKER 2.2.3 (van Oosterhout et al. 2004) was used to detect possible scoring errors and the presence of null alleles. Pairwise tests of linkage disequilibrium (Slatkin 1994; Slatkin and Excoffier 1996) were performed running 10,000 permutations in ARLEQUIN 3.11 (Excoffier et al. 2005). Deviations from Hardy-Weinberg Equilibrium (HWE) for each locus were tested using GENEPOP 4.0 (Rousset 2008). A Bayesian approach implemented in the program BAYESCAN (Foll and Gaggiotti 2008) was used to detect loci under selection.

3.3.3.1 Spatial analyses

Diversity indices were calculated for each of the 22 sites (including the New Zealand sites sampled on 2008). The average number of alleles per locus (N_a), allelic richness (A_r), observed number of private alleles (P_a) and the inbreeding coefficient (F_{IS}) were calculated in FSTAT 2.9.3.2 (Goudet 1995; 2001). Allelic richness calculates the number of alleles independent of sample size. The observed (H_O) and expected (H_E) heterozygosities were determined with ARLEQUIN and HWE tests were performed using 100,000 steps in Markov Chain and 10,000 dememorisation steps.

The pairwise genetic divergence between sites using the fixation index F_{ST} was estimated with ARLEQUIN. The software POWSIM 4.1 (Ryman and Palm 2006) was used to estimate the statistical power of the loci to detect population differentiation at F_{ST} level of 0.005 using Chi-squared and Fisher's exact tests. Two combinations of settings were used to generate the desired F_{ST} value, N_e = 2,000, t = 20 and N_e = 10,000, t = 100; using 1,000 and 100 replicates, respectively, and the sampling sizes of each of the 22 sites. Estimates of the statistical α (type I) error were generated using samples drawn directly from the base population, omitting the drift steps (t = 0) and using 1,000 replicates. Another measure that is able to detect differentiation even when diversity is high is the estimator of actual differentiation, D_{est} (Jost 2008). D_{est} was calculated for pairwise comparisons using 1,000 bootstrap replicates in SMOGD 1.2.5 (Crawford 2010). ARLEQUIN was also used to perform AMOVA and Mantel tests. For F_{ST} and AMOVA 10,000 permutations were implemented. The Mantel tests were performed to determine the occurrence of the isolation by distance (IBD) pattern at a global scale (with sites from ORH 1 pooled together), and at a fine-scale within New Zealand, where all the sites were considered separate. Correlation between genetic differentiation

$F_{ST}/(1-F_{ST})$ and geographic distance (ln m) between sites, was assessed performing 1,000 permutations. The shortest distance between sites was estimated using Google Earth 4.3.

To visualize patterns of population differentiation, a Principal Component Analysis (PCA) was performed in P.C.A.-GEN 1.2 (http://www2.unil.ch/popgen/softwares/pcagen.htm). The program tests the significance of total inertia as well as individual axes inertia. Population structure was also evaluated with STRUCTURE 2.3.3 (Pritchard et al. 2000). The program CONVERT 1.31 (Glaubitz 2004) was used to obtain the STRUCTURE file. STRUCTURE assigns individuals probabilistically to *K* groups maximizing HWE and linkage equilibrium. Sample location was used as a prior as this improves the performance of the analysis when the F_{ST} values are low (Hubisz et al. 2009). The no admixture model and correlated allele frequencies (Falush et al. 2003) were implemented. Ten independent runs were performed for each *K* (*K* = two to 22) with 100,000 iterations and a burn-in length of 100,000. The program STRUCTURE HARVESTER (Earl and vonHoldt 2011) was used to apply the Evanno method (Evanno et al. 2005) to detect the value of *K* (number of genetic groups) that best fit the data.

3.3.3.2 Temporal analyses

Temporal analyses were conducted for the eight sampling sites around Northern New Zealand (ORH 1) sampled in 2008 and 2010 (see Fig. 3.1). The diversity indices, average number of alleles per locus (N_a), allelic richness (A_r), observed number of private alleles (P_a), inbreeding coefficient (F_{IS}), and observed (H_O) and expected (H_E) heterozygosities were calculated for each of the eight sites sampled in 2010. An AMOVA was conducted to compare samples nested from both seasons within sampling sites to evaluate the variation among sampling sites relative to variation among seasons within sampling sites. To further explore the data, variability among temporal samples was assessed with pairwise F_{ST} and D_{est} analyses. Pairwise F_{ST} values were calculated in ARLEQUIN using 10,000 permutations and D_{est} pairwise comparisons were calculated in SMOGD using 1,000 bootstrap replicates. POWSIM 4.1 was used to evaluate the statistical power of the loci to detect population differentiation at F_{ST} level of 0.005 for the temporal data set. The settings were the same as with the spatial analysis, two

combinations of settings were used, N_e = 2,000, t = 20 and N_e = 10,000, t = 100; with 1,000 and 100 replicates, respectively, and the sampling sizes of each of the 16 samples (eight sites per season).

3.4 Results

The genotypes of each individual for the spatial and temporal analyses are found in Appendixes C and D. MICROCHECKER analyses found no scoring errors. Five loci showed an excess of homozygotes suggesting the presence of null alleles, but none were consistent across sites (i.e. in two to four sites from the total 22). No tests for linkage disequilibrium were significant after Bonferroni correction (α = 0.001). According to GENEPOP, two loci (*Hat2a* and *Hat7*) showed deviations from Hardy-Weinberg expectations but only at single sites. No loci were identified as outliers under selection, although locus *Hat3* showed higher F_{ST} values in comparison with the other eight loci. Statistics per locus for each sampling site are found in Appendix E.

3.4.1 Spatial analyses

Summary statistics for each site across all loci are reported in Table 3.1. The largest number of samples was obtained from New Zealand (430), with 16 sites surveyed with 22 to 37 samples per site. The sample sizes for the other four regions, Australia, Namibia, Chile, and the Northeast Atlantic, ranged from 46 to 49. The average number of alleles per locus (N_a) ranged from 10.8 to 16.9 and allelic richness (A_r) from 9.2 to 11.2. The Porcupine Bank in the Northeast Atlantic had the lowest allelic diversity as indicated by both, N_a and A_r. The highest allelic diversity was obtained for two sites in New Zealand, where Milan's presented the highest N_a and A_r. The A_r was equally high in the Challenger Plateau. A few private alleles (between 1 and 3) were detected at 12 sites. The observed and expected heterozygosities were similar across all sites, ranging from 0.79 to 0.88 and from 0.85 to 0.88, respectively. There were no significant departures from Hardy-Weinberg equilibrium in any of the sites and concordantly, none of the F_{IS} values were significantly different from zero after Bonferroni correction (α = 0.00025, $P > 0.01$).

Table 3.1 Summary statistics for the levels of genetic variation across all loci for 22 sampling sites grouped in five regions. ORH 1 sites (see note below table) were sampled on 2008. Sample size (n), average number of alleles per locus (N_a), allelic richness (A_r), observed number of private alleles (P_a), observed heterozygosity (H_O), expected heterozygosity (H_E), Hardy-Weinberg probability (P_{HWE}) and the inbreeding coefficient (F_{IS}). $P > 0.01$ for all F_{IS} values (Bonferroni correction $\alpha = 0.00025$). Total $n = 620$

Region	Sampling sites*		Code	N	N_a	A_r	P_a	H_O	H_E	P_{HWE}	F_{IS}
New Zealand	Kaipara Flats	1A	Kai	37	15.3	10.4	—	0.85	0.86	0.446	0.013
	Tauroa Knoll	1B	Tau	24	12.6	10.1	1	0.79	0.85	0.329	0.075
	Explorer Hill	1B	Exp	24	13.8	10.8	1	0.81	0.86	0.412	0.059
	Tony B	1B	Ton	24	12.7	10.3	—	0.87	0.86	0.550	-0.019
	Yasmin's	1B	Yas	24	13.7	10.6	1	0.81	0.86	0.469	0.060
	Birdflue	1C	Bir	36	16.0	10.9	3	0.83	0.87	0.412	0.038
	Boulder Ridge	1C	Bou	22	12.7	10.4	—	0.85	0.87	0.529	0.022
	Milan's	1C	Mil	36	16.9	11.2	—	0.84	0.86	0.458	0.030
	Nukuhou Knoll	1D	Nuk	22	13.7	11.0	2	0.83	0.86	0.365	0.040
	Colville Knoll	1D	Col	24	13.8	11.1	1	0.85	0.86	0.560	0.018
	Mercury Knoll	1D	Mer	24	14.1	11.0	—	0.88	0.87	0.615	-0.013
	Clark	1D	Cla	24	14.0	10.9	—	0.84	0.88	0.344	0.046
	Ritchie Bank	2A	Rit	24	13.4	10.6	—	0.86	0.86	0.467	0.006
	Chatham Rise	3B	Chat	24	13.0	10.6	1	0.84	0.86	0.464	0.013
	Puysegur	3B	Puy	36	15.7	10.9	1	0.83	0.85	0.578	0.018
	Challenger Plateau	7A	Cha	25	14.9	11.2	1	0.87	0.87	0.497	0.000
Australia	North Tasmania		NTas	22	11.4	9.8	—	0.84	0.87	0.478	0.033
	Albany		Alb	25	13.8	10.8	1	0.86	0.86	0.498	0.000
Namibia	Namibia		Nam	48	14.7	10.0	3	0.82	0.86	0.436	0.037
Chile	Juan Fernández		JF	46	14.7	10.2	1	0.83	0.86	0.371	0.039
Northeast	Porcupine Bank		Por	23	10.8	9.2	—	0.81	0.86	0.460	0.054
Atlantic	Sedlo		Sed	26	12.0	9.7	—	0.83	0.85	0.359	0.028

*For New Zealand sampling sites, reference to main management areas for orange roughy as defined by the Ministry of Fisheries is indicated by: 1A-1D = subareas within ORH 1, 2A = ORH 2A, 3B = ORH 3B and 7A = ORH 7A

At a global scale, the F_{ST} analysis revealed a lack of differentiation in most comparisons between New Zealand and Australian sites. A few F_{ST} values were significant ($P < 0.05$), and D_{est} values between sites in these two regions ranged from 0.000 to 0.039 (Table 3.2). In general, comparisons of New Zealand sites with North Tasmania resulted in higher F_{ST} and D_{est} values than comparisons with Albany; and the F_{ST} value between North Tasmania and Albany was low but significant ($P < 0.05$, Table 3.2). On the other hand, most F_{ST} values showed significant differentiation ($P < 0.05$) between the New Zealand/Australian sites and those from Namibia, Chile, and the Northeast Atlantic (Table 3.2). The results of the POWSIM analysis for the two combinations of settings showed that the power to detect population differentiation at F_{ST} 0.005 was 1.0 (100% of the tests with both, Chi-squared and Fisher's exact tests) suggesting that the number of loci and sample sizes were sufficient to detect differentiation with a high degree of confidence. The estimates of the statistical α (type I) error were 0.062 and 0.090 with the Chi-squared and Fisher's exact test, respectively. Some comparisons were also significant after correction for multiple tests ($P < 0.0002$). D_{est} values between sites in the different regions ranged from 0.000 to 0.114 (Table 3.2). Sites were pooled by region to perform new F_{ST} and D_{est} analyses. The revised analyses on pooled samples showed significant F_{ST} values ($P < 0.005$) between regions, but not between New Zealand and Australia (Table 3.3). Similarly, D_{est} values of pooled samples ranged from 0.0125 to 0.1031 between regions, but was 0.0016 between New Zealand and Australia (Table 3.3). The F_{ST} analysis showed a lack of genetic differentiation at a fine-scale around New Zealand (Table 3.2) with only eight comparisons (out of 120) showing significant differentiation (F_{ST} = 0.006-0.010, $P <$ 0.05), and most of these corresponded to comparisons between the Northern sites and the Chatham Rise or Puysegur sites (see Fig. 3.1). Similarly, D_{est} values were low within New Zealand, ranging from 0.000 to 0.024, with a mean value of 0.002. The highest D_{est} values (0.012-0.024) were obtained in comparisons with the Chatham Rise or Puysegur sites.

Table 3.2 Pairwise F_{ST} values (below diagonal) and D_{est} values (above diagonal) between sampling sites. Codes and reference to each management area for New Zealand sites are found in Table 3.1. F_{ST} values shown in bold indicate statistically significant differences considering $P < 0.05$. Values with * indicate significant differentiation after Bonferroni correction for multiple tests ($P < 0.0002$)

	New Zealand																Australia		Nam	Chile	NE Atlantic	
	Kai	Tau	Exp	Ton	Yas	Bir	Bou	Mil	Nuk	Col	Mer	Cla	Rit	Chat	Puy	Cha	NTas	Alb	Nam	JF	Por	Sed
Kai		0.001	0.000	0.000	-0.004	0.000	0.000	-0.002	0.000	0.000	-0.001	0.002	0.003	0.000	0.000	-0.010	0.012	0.004	0.008	0.047	0.048	0.056
Tau	0.001		0.009	0.003	0.000	0.002	0.000	0.001	0.001	0.001	0.001	0.013	0.005	0.000	0.021	0.000	0.004	-0.004	0.028	0.064	0.042	0.025
Exp	-0.002	-0.001		0.001	0.000	0.000	-0.009	-0.002	0.000	0.000	0.000	0.000	-0.002	0.001	0.001	0.007	0.008	0.000	0.017	0.029	0.046	0.046
Ton	-0.002	-0.002	0.003		0.000	-0.002	0.000	0.000	0.000	-0.005	0.000	-0.001	0.000	0.012	0.020	-0.008	0.007	0.000	0.010	0.060	0.026	0.035
Yas	-0.005	-0.007	-0.005	-0.007		-0.002	0.000	0.000	0.002	0.000	0.000	0.003	0.001	0.000	0.016	-0.007	0.005	-0.005	0.005	0.057	0.046	0.045
Bir	-0.004	0.001	-0.008	-0.008	-0.010		-0.001	0.000	0.000	0.000	-0.002	0.004	0.000	0.012	0.000	-0.003	0.015	0.000	0.013	0.036	0.026	0.072
Bou	-0.001	-0.004	-0.007	-0.001	-0.002	-0.009		0.000	0.000	0.000	-0.004	0.000	0.000	0.008	0.000	-0.019	0.000	-0.001	0.012	0.013	0.022	0.027
Mil	-0.004	-0.003	-0.001	-0.002	-0.001	-0.008	0.002		0.003	0.000	0.000	0.002	0.006	0.001	0.002	0.000	0.030	0.000	0.019	0.046	0.066	0.070
Nuk	0.002	-0.001	-0.001	-0.001	0.002	-0.005	-0.001	**0.008**		0.003	0.000	0.000	0.005	0.000	0.002	0.000	0.025	-0.001	0.020	0.065	0.010	0.020
Col	-0.006	-0.005	-0.012	-0.017	-0.018	-0.001	-0.015	-0.018	-0.006		-0.003	0.003	-0.002	0.001	0.001	0.000	0.008	0.002	0.016	0.027	0.024	0.044
Mer	-0.005	-0.003	-0.001	-0.001	-0.006	-0.009	-0.005	-0.004	-0.001	-0.018		0.000	-0.001	0.012	-0.001	-0.012	0.007	0.000	0.005	0.028	0.000	0.002
Cla	0.001	0.005	0.001	-0.003	0.002	-0.004	-0.002	0.004	0.005	-0.010	-0.002		0.009	0.024	0.013	0.000	0.001	0.006	0.033	0.076	0.044	0.037
Rit	0.002	-0.001	-0.003	-0.001	-0.003	-0.004	0.001	0.003	0.003	-0.015	-0.001	0.003		0.001	0.006	-0.004	0.025	0.000	0.008	0.053	0.063	0.065
Chat	-0.001	-0.008	0.001	**0.007**	-0.003	-0.001	0.005	0.001	0.003	-0.012	**0.010**	**0.010**	0.001		0.017	0.000	0.022	0.000	0.027	0.041	0.056	0.079
Puy	-0.002	0.003	-0.002	**0.006**	0.002	-0.008	0.001	0.002	0.001	-0.012	-0.004	**0.008**	0.004	**0.007**		0.000	0.039	0.001	0.015	0.016	0.067	0.083
Cha	-0.003	-0.001	0.003	-0.005	-0.005	-0.008	-0.005	0.005	0.004	-0.017	-0.003	-0.003	-0.002	**0.007**	0.005		0.008	0.000	0.007	0.041	0.022	0.024
NTas	0.004	0.001	**0.008**	0.003	0.004	-0.001	-0.003	**0.008**	0.007	-0.007	0.004	0.001	**0.006**	**0.011**	**0.010**	0.001		0.011	0.036	0.063	0.050	0.055
Alb	0.002	-0.008	-0.005	0.001	-0.005	-0.005	-0.002	0.001	-0.001	-0.016	-0.001	**0.007**	-0.004	-0.004	0.001	0.002	**0.006**		0.011	0.039	0.073	0.037
Nam	**0.005**	**0.009**	**0.008**	**0.007**	0.004	0.001	**0.010**	***0.011**	**0.008**	-0.005	0.007	**0.012**	**0.008**	**0.008**	***0.010**	**0.011**	**0.010**	**0.007**		0.056	0.041	0.055
JF	***0.013**	***0.015**	**0.010**	***0.015**	**0.012**	**0.006**	**0.009**	***0.017**	**0.013**	-0.003	0.015	***0.016**	***0.020**	***0.013**	***0.012**	***0.021**	***0.017**	**0.011**	***0.011**		0.080	0.114
Por	**0.009**	0.004	**0.013**	**0.012**	**0.009**	0.001	**0.008**	**0.010**	0.006	-0.003	-0.002	0.006	**0.011**	***0.019**	**0.014**	**0.011**	**0.014**	**0.011**	**0.014**	***0.020**		0.000
Sed	**0.013**	0.004	**0.011**	**0.008**	**0.011**	**0.007**	**0.009**	***0.015**	**0.010**	-0.004	0.003	**0.011**	**0.014**	***0.018**	***0.015**	**0.011**	**0.011**	**0.010**	***0.019**	***0.026**	-0.003	

Table 3.3 Pairwise F_{ST} values (below diagonal) and D_{est} values (above diagonal) between the five regions. Values shown in bold indicate statistically significant differences considering Bonferroni correction ($P < 0.005$)

	New Zealand	Australia	Namibia	Chile	Northeast Atlantic
New Zealand		0.0016	0.0125	0.0418	0.0667
Australia	0.0002		0.0135	0.0461	0.0574
Namibia	**0.0082**	**0.0064**		0.0559	0.0487
Chile	**0.0140**	**0.0119**	**0.0109**		0.1031
Northeast Atlantic	**0.0111**	**0.0103**	**0.0174**	**0.0236**	

An AMOVA over all sites showed that most of the variation (99%) was within sites. A significant but weak pattern of IBD was detected within New Zealand (Mantel test, $r = 0.25$, $P = 0.02$), driven by the consistent differentiation of the Chatham Rise and Puysegur sites from the Northern sites. A clear pattern of IBD was detected at the global scale (Mantel test, $r = 0.62$, $P = 0.001$). In the PCA most of the variation was explained by axis PC1 (Fig. 3.2). In the plot, Australian sites were next to New Zealand sites and sites from Namibia, Chile and the Northeast Atlantic appeared distant from New Zealand/Australia, with the site from Chile being the most distant.

Interestingly, STRUCTURE revealed three genetic groups ($K = 3$ was the best K obtained with STRUCTURE HARVESTER, Fig. 3.3). In concordance with the analyses above, one group included all the New Zealand and Australian sites, and a second group corresponded to the Northeast Atlantic sites. However, despite the significant differentiation detected between Namibia and Chile ($F_{ST} = 0.011$ $P < 0.0002$, $D_{est} = 0.056$), the third group included the sites from Namibia and Chile (Fig. 3.4). In the PCA plot the closest site to the Chilean site was Namibia.

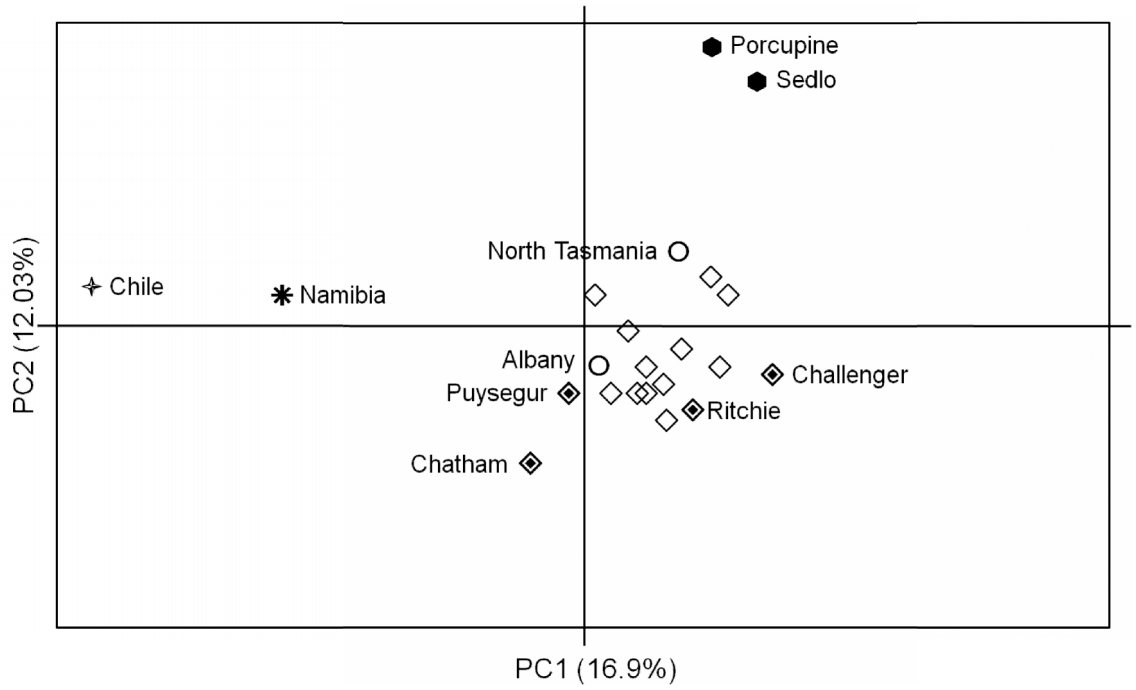


Fig. 3.2 Plot of the first two principal components axes of microsatellite DNA variation. Each symbol corresponds to one of the 22 sampling sites. ◇ : sites from the ORH 1 area in New Zealand (see Table 3.1) sampled on 2008, ◈ : other sites around New Zealand, ○ : Australian sites, ✳: Namibia, ✧ : Chile, ⬢ : Northeast Atlantic sites. The axe PC1 explains most of the variation. $P = 0.01$

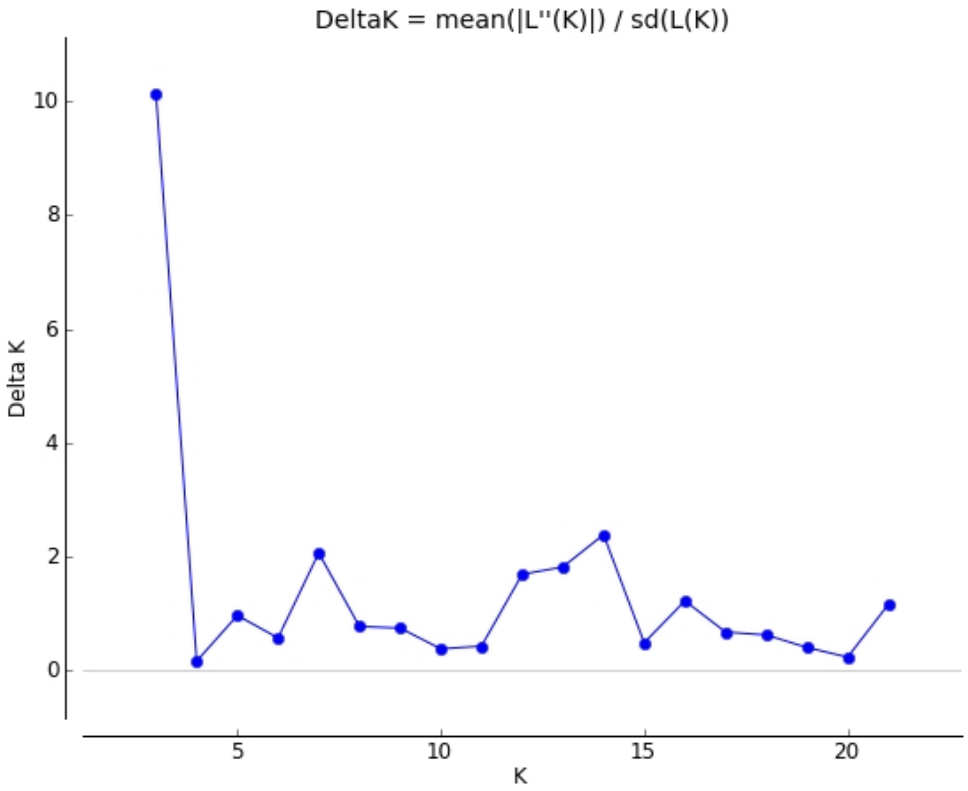
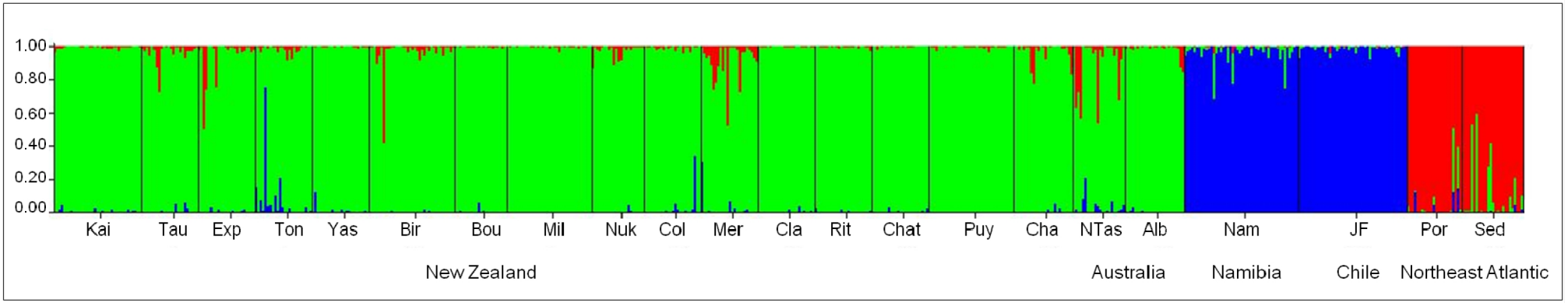


Fig. 3.3 Evanno et al. (2005) plot obtained from STRUCTURE HARVESTER. The modal value of this distribution is the best number of *K* groups for the data

Fig. 3.4 Population structure inferred by STRUCTURE for $K = 3$ (see Fig. 3.3) among the 22 sampling sites (Codes as in Table 3.1). Each colour represents a genetic group defined by STRUCTURE. Individuals are represented as thin vertical lines partitioned into coloured segments according to their probability of assignment to each genetic cluster



3.4.2 Temporal analyses

Analyses were conducted for eight sites in ORH 1 that were sampled in both 2008 and 2010 (see Fig. 3.1). A summary of the genetic indexes per site surveyed on 2010 are shown in Table 3.4 (genetic indexes for sites surveyed on 2008 are shown in Table 3.1). The mean number of alleles per locus (N_a) and the allelic richness (A_r) for each site were similar between years, particularly for the allelic richness that is independent of the sample size. The observed and expected heterozygosities were also very similar between the two years, ranging from 0.80 to 0.87 and from 0.85 to 0.87, respectively for the sites sampled on 2010. All the sites were in Hardy-Weinberg equilibrium in both years and none of the F_{IS} values were significant after Bonferroni correction (sites sampled on 2010: $\alpha = 0.00069$, P values > 0.01).

The AMOVA revealed a lack of spatial and temporal differentiation, since no variation was found among sites nor between seasons within each site sampled in 2008 and 2010. Similarly, most pairwise F_{ST} and D_{est} values were low and not significant ($P > 0.05$) (Table 3.5). None of the F_{ST} values were significant after Bonferroni correction ($P > 0.0004$). POWSIM result for the temporal data set was the same as for the spatial data set: the power to detect population differentiation at F_{ST} 0.005 was also 1.0 (100% of the tests with both, Chi-squared and Fisher's exact tests). The four F_{ST} comparisons that were significant at $P < 0.05$, also showed some of the highest D_{est} values (0.012-0.031) and six other comparisons showed D_{est} values ≥ 0.010 (see Table 3.5). It is worth noting that two sites, Milan's and Colville Knoll, showed D_{est} values ≥ 0.010 between years.

Table 3.4 Summary statistics for the levels of genetic variation across all loci for eight sampling sites from the ORH 1 area sampled in 2010. Sample size (n), average number of alleles per locus (N_a), allelic richness (A_r), observed number of private alleles (P_a), observed heterozygosity (H_O), expected heterozygosity (H_E), Hardy-Weinberg probability (P_{HWE}) and the inbreeding coefficient (F_{IS}). $P > 0.01$ for all F_{IS} values (Bonferroni correction $\alpha = 0.00069$). Total $n = 192$

Sampling sites	n	N_a	A_r	H_O	H_E	P_{HWE}	F_{IS}
Kaipara Flats	24	13.9	11.4	0.85	0.87	0.568	0.017
Tauroa Knoll	24	12.4	10.6	0.87	0.86	0.644	-0.006
Explorer Hill	24	13.1	10.9	0.82	0.85	0.460	0.035
Tony B	24	13.9	11.1	0.85	0.87	0.564	0.020
Birdflue	24	13.1	10.6	0.80	0.85	0.374	0.057
Boulder Ridge	24	13.8	11.3	0.82	0.85	0.471	0.035
Milan's	24	12.8	10.8	0.87	0.87	0.336	0.005
Colville Knoll	24	13.8	11.1	0.84	0.86	0.582	0.027

Table 3.5 Pairwise F_{ST} values (below diagonal) and D_{est} values (above diagonal) between ORH 1 sites sampled in 2008 (1) and 2010 (2). Codes and reference to each subarea are found in Table 3.1. F_{ST} values shown in bold indicate statistically significant differences considering $P < 0.05$. None of the F_{ST} values were significant after Bonferroni correction for multiple tests ($P > 0.0004$)

	Kai (1)	Kai(2)	Tau (1)	Tau (2)	Exp (1)	Exp (2)	Ton (1)	Ton (2)	Bir (1)	Bir (2)	Bou (1)	Bou (2)	Mil (1)	Mil (2)	Col (1)	Col (2)
Kai (1)		-0.0040	0.0006	0.0000	0.0004	-0.0045	0.0002	0.0000	0.0000	0.0292	0.0001	0.0038	-0.0017	0.0018	0.0000	0.0005
Kai (2)	-0.0029		0.0001	0.0000	0.0001	0.0000	-0.0001	-0.0117	-0.0001	0.0000	-0.0039	0.0000	-0.0216	0.0108	0.0002	-0.0004
Tau (1)	0.0014	-0.0001		0.0000	0.0091	-0.0033	0.0034	0.0142	0.0021	0.0048	0.0001	0.0027	0.0007	0.0178	0.0005	0.0011
Tau (2)	-0.0014	0.0019	-0.0046		-0.0040	-0.0100	0.0000	0.0000	-0.0151	0.0008	-0.0061	-0.0130	-0.0003	0.0000	0.0002	0.0012
Exp (1)	-0.0023	0.0009	-0.0003	-0.0048		0.0000	0.0015	-0.0002	0.0000	0.0026	-0.0091	-0.0006	-0.0023	0.0001	0.0003	0.0023
Exp (2)	-0.0058	-0.0007	-0.0062	-0.0086	-0.0048		-0.0001	0.0028	0.0000	0.0000	0.0000	-0.0037	0.0000	0.0010	0.0004	-0.0012
Ton (1)	-0.0019	0.0003	-0.0019	-0.0001	0.0028	-0.0017		-0.0003	-0.0024	-0.0048	0.0000	0.0000	-0.0002	0.0002	-0.0054	0.0069
Ton (2)	-0.0018	-0.0039	0.0029	0.0023	-0.0025	-0.0002	-0.0036		0.0021	0.0006	0.0000	0.0038	0.0001	0.0043	0.0004	0.0009
Bir (1)	-0.0038	-0.0066	0.0012	-0.0094	-0.0077	-0.0070	-0.0085	-0.0039		0.0074	-0.0015	-0.0002	0.0000	0.0006	0.0000	0.0000
Bir (2)	**0.0081**	0.0012	-0.0006	0.0047	0.0037	-0.0001	-0.0047	0.0015	-0.0008		0.0010	0.0015	0.0128	0.0082	0.0070	0.0107
Bou (1)	-0.0014	-0.0046	-0.0038	-0.0028	-0.0067	-0.0026	-0.0008	-0.0016	-0.0088	0.0042		0.0001	0.0001	-0.0025	0.0000	0.0000
Bou (2)	0.0009	0.0003	-0.0040	-0.0058	-0.0050	-0.0046	-0.0004	0.0055	-0.0075	0.0069	-0.0002		0.0000	0.0315	-0.0002	0.0173
Mil (1)	-0.0042	-0.0046	-0.0030	0.0004	-0.0073	-0.0055	-0.0023	0.0043	-0.0078	**0.0072**	0.0020	0.0016		0.0215	0.0000	0.0054
Mil (2)	-0.0070	-0.0029	-0.0074	-0.0090	-0.0057	0.0006	-0.0095	-0.0090	-0.0094	-0.0017	-0.0158	**0.0088**	-0.0023		0.0003	0.0037
Col (1)	-0.0065	-0.0073	-0.0046	-0.0146	-0.0124	-0.0122	-0.0170	-0.0135	-0.0001	-0.0063	-0.0153	-0.0146	-0.0182	-0.0211		0.0266
Col (2)	-0.0006	-0.0014	0.0001	0.0031	0.0023	-0.0024	0.0034	0.0006	-0.0078	0.0037	-0.0023	**0.0089**	0.0026	-0.0051	-0.0048	

3.5 Discussion

3.5.1 Microsatellite DNA diversity

Levels of genetic variation were high and similar among all the sites, with a mean expected heterozygosity (H_E) of 0.86; similar, but slightly lower mean values were reported for orange roughy by White et al. (2009*a*) (H_E = 0.72) and Carlsson et al. (2011) (H_E = 0.77). Considerably lower diversity was reported by Oke et al. (2002) (mean H_E = 0.40), which is probably related to the fact that of the six loci used by Oke et al. (2002) three have low heterozygosities and were not used in this study. High levels of genetic diversity for microsatellite DNA markers are typical of commercially exploited deep-sea teleosts, such as the redfish *Sebastes mentella* (Roques et al. 2002, H_E = 0.87), the roundnose grenadier *Coryphaenoides rupestris* (White et al. 2010, H_E = 0.77), and the blue hake *Antimora rostrata* (White et al. 2011, H_E = 0.83). A positive relationship between genetic diversity and abundance in fishes has been determined by McCusker and Bentzen (2010), especially for microsatellite DNA markers. However, the high levels of genetic diversity in orange roughy detected here and in other studies with microsatellite DNA markers (White et al. 2009a; Carlsson et al. 2011) and mtDNA markers (Chapter 2), does not rule out the possibility that commercial exploitation has impacted on the genetic composition and abundance of orange roughy populations. Fisheries for the species begun just ~ 30 years ago, and because of the long-life span of orange roughy (more than 100 years, Mace et al. 1990; Fenton et al. 1991) and late maturity (~ 30 years, Fenton et al. 1991; Francis and Horn 1997) it is unlikely that reduction in levels of genetic diversity would be detected at this early stage with selectively neutral markers, as inferred previously for orange roughy (Baker et al. 1995; Carlsson et al. 2011).

3.5.2 Global scale population structure

Overall, I detected low but significant levels of differentiation at the global scale, within the Southern hemisphere, and between the Southern hemisphere sites and the Northeast Atlantic Ocean. Genetic homogeneity prevailed between orange roughy samples from around New Zealand and Australia, although there was some differentiation between

few sites. The general lack of differentiation among sites around New Zealand and Australia was reported with allozymes, microsatellite DNA, and mitochondrial DNA sequence analyses (Elliott and Ward 1992; Oke et al. 2002; Chapter 2). However, using mtDNA RFLP's Smolenski et al. (1993) found evidence of genetic differentiation between Southeastern Australian and New Zealand samples. In another deep-sea fish, microsatellite DNA analyses showed no differentiation between New Zealand and Australian samples of the wreckfish *Polyprion americanus* (Ball et al. 2000). Significant differentiation between orange roughy sites in the Northeast Atlantic Ocean and the Southern hemisphere was also detected with mitochondrial DNA COI gene sequences (Chapter 2), and here the highest values of differentiation were between pooled sites in the Northeast Atlantic Ocean and Southern hemisphere regions (F_{ST} = 0.010-0.023 and D_{est} = 0.049-0.103). It is possible that oceanographic characteristics at 41°N restrict the north-south dispersal of orange roughy (see Discussion section in Chapter 2). The present study revealed levels of genetic differentiation within the Southern hemisphere (Australia/New Zealand, Namibia, and Chile) that were undetected with the COI gene sequences (Chapter 2). Similarly, in the deep-sea wreckfish *P. americanus* a clear pattern of differentiation between sites in the Northern and Southern hemisphere was detected with microsatellite DNA markers (Ball et al. 2000), but little evidence of genetic divergence was found with mtDNA markers (Sedberry et al. 1996).

Low, but significant values of microsatellite DNA differentiation at the global scale (pooled sites, F_{ST} = 0.006-0.023 and D_{est} = 0.012-0.103) indicate a lack of panmixia, as might be expected given the large geographic distances between the regions (~ 8,000 to ~ 20,000 km). Genetic distance was correlated with geographic distance (Mantel test, r = 0.62, P = 0.001). These findings suggest a restriction to gene flow at the global scale and some fidelity to specific regions. However, it is probable that some degree of dispersal occurs, particularly, within the Southern hemisphere. Long distance dispersal is unlikely to occur during the early life-history stages because eggs and larvae are probably retained around seamounts (Rogers 1994), and the pelagic larval period is extremely short (~ 10 days, Zeldis et al. 1998). The predominant lack of differentiation found between New Zealand and Australia, and the low levels of differentiation within the Southern hemisphere, most likely result from active stepping-stone dispersal of long-lived adults that are able to spawn many times during their life (Elliott et al. 1994; White et al. 2009a).

3.5.3 Fine-scale population structure and implications for fisheries management in New Zealand

In New Zealand orange roughy fisheries have been divided into different areas (code ORH) for assessment and management. This is the first genetic study to include samples from sites around Northern New Zealand (ORH 1, 12 sites); and considering the other 4 sites sampled around New Zealand, this study included samples from most of the orange roughy fishing grounds inside the New Zealand Economic Exclusive Zone (see Table 3.1), except for the area ORH 7B (off the west coast of the South Island). In general, I found a lack of differentiation within New Zealand as revealed by STRUCTURE and PCA analyses. The spatial F_{ST} analysis showed that only 6.7% of the comparisons were significant ($P < 0.05$) among New Zealand sites. Similarly, in the spatial D_{est} analysis only 10% of the comparisons showed values higher than 0.01. Significant differentiation was found between the Chatham Rise and Puysegur sites ($F_{ST} = 0.007$, $P = 0.02$, $D_{est} = 0.017$), and most other examples of significant differentiation involved these two sites (see Table 3.2). Previous studies using mtDNA RFLPs's (Smith et al. 1996; 1997), and mitochondrial DNA sequences (Chapter 2) found significant divergence between Chatham Rise and Puysegur; while an allozyme study detected significant differentiation between samples from the east coast of New Zealand and samples of the Chatham Rise (Smith and Benson 1997). Together these combined studies indicate that there is a restriction to dispersal among these sites supporting the current management of the Chatham Rise and Puysegur as separate stocks within ORH 3B. In the spatial F_{ST} analysis only one comparison showed significant differentiation ($P < 0.05$) that did not include the Chatham Rise or Puysegur sites, this was the comparison between Milan's from ORH 1C and Nukuhou Knoll from ORH 1D.

The temporal analyses conducted for eight sites within ORH 1 showed a lack of significant differentiation between sites and years, suggesting temporal stability in genetic variation and panmixia for orange roughy at this fine-scale. Genetic homogeneity in orange roughy has been also found at a fine-scale in the Northeast Atlantic Ocean (White et al. 2009a). However, the present findings should be treated carefully because it may be the result of highly variable loci and sample sizes of less than 50 specimens per site. Temporal differentiation in orange roughy has been detected in previous studies. Smith and Benson (1997) found spatial and temporal differentiation at one allozyme locus among multiple samples from the Chatman Rise surveyed in

winter and summer seasons in the same year. Some degree of temporal differentiation was also detected at one site off South Australia with mtDNA RFLP's, that corresponded with biological, reproductive and catch-rate data (Smolenski et al. 1993). The subtle temporal differentiation detected by Smith and Benson (1997) and Smolenski et al. (1993) may be related with the inference that mature females are able to spawn many times in their life time, but they do not spawn every year, and may not participate in annual migrations to spawning sites (Bell et al. 1992; Zeldis et al. 1997). A pattern of temporal variation in orange roughy cannot be ruled out by the present data and should be further explored in studies including larger sample sizes.

There is some uncertainty about the current levels of dispersal among sites and the general lack of genetic differentiation that I have detected among New Zealand sites and between New Zealand and Australian sites. These findings could be explained by high levels of gene flow by adults actively dispersing around New Zealand and across the Tasman Sea. However, they could also be explained by low levels of dispersal, since genetic connectivity can be maintained by the dispersal of only a few individuals, but populations are not considered demographically coupled until about 10% of the individuals are regularly exchanged (see Lowe and Allendorf 2010). Another possibility is that distinct populations have recently diverged, and it is too early to detect the lack of gene flow with microsatellite DNA markers, particularly considering that genetic drift has probably been very weak in orange roughy populations due to their historically large sizes, and because of the species' longevity and late maturation. Future research applying higher resolution markers such as single nucleotide polymorphisms (SNPs) and/or studies investigating genes under selection may help to disentangle the degree of population connectivity at a fine-scale (around New Zealand) and at a regional scale (between New Zealand and Australia).

3.6 Conclusion

In the present study I found high levels of microsatellite DNA diversity in orange roughy populations, which likely reflect large historical population sizes. Low, but significant differentiation was detected at the global scale, with the highest values of differentiation between the Northeast Atlantic Ocean and the Southern hemisphere sites. No significant divergence was found in most comparisons between New Zealand and Australia. At a fine-scale within New Zealand, there was a lack of spatial divergence, but low levels of differentiation were detected between few sites off Northern New Zealand and two sites off the east and south coast of the South Island. Temporal analyses also revealed a predominant pattern of genetic homogeneity. Overall, the high levels of diversity and the low levels of genetic differentiation detected in this study simile findings for other deep-sea fishes.

Chapter 4

Genetic divergence and phylogenetic relationships of six species of the genus *Hoplostethus* based on Cytochrome *c* Oxidase I (COI) sequences

4.1 Abstract

The genetic divergence and the molecular phylogenetic relationships among *Hoplostethus* species have not been proposed yet. Using available COI sequences within the barcode region, I conducted a phylogenetic study including *H. atlanticus, H. crassispinus, H. gigas, H. japonicus H. latus*, and *H. mediterraneus*. Representatives of other three genera, *Aulotrachichthys prosthemius*, *Gephyroberyx darwinni* and *Paratrachichthys macleayi* were added to the analysis to place the phylogenetic position of the genus *Hoplostethus* within the family Trachichthyidae. The inter species divergence (7.37 - 13.57%) was much higher than the intra species divergence (0.0 - 0.65%). Kimura 2-Parameter (K2P) distance, Maximum Likelihood (ML) and Bayesian analyses showed that *H. latus, H. crassispinus, H. japonicus,* and *H. mediterraneus* form a separate clade from *H. atlanticus* and *H. gigas*. The position of *H. gigas* was not well defined with the nucleotide data. With K2P distance *H. gigas* was sister to *H. atlanticus*, but not with ML and Bayesian analyses. At the amino acid level, non-synonymous substitutions clearly differentiated *H. atlanticus* from all the other species. This was correlated with morphological characteristics presented elsewhere. The genus *Hoplostethus* was monophyletic. According to ML and Bayesian analyses, the *Hoplostethus* clade was a derived group respect to the other three genera considered here.

4.2 Introduction

The marine fishes within the family Trachichthyidae are represented by eight genera, *Aulotrachichthys*, *Gephyroberyx*, *Hoplostethus*, *Optivus*, *Parinoberix*, *Paratrachichthys*, *Sorosichthys* and *Trachichthys* (www.fishbase.org), with most of the species belonging to the genus *Hoplostethus.* Currently, there are 28 valid species of *Hoplostethus* (Moore and Dodd 2010), which are commonly called roughies or slimeheads. They mainly inhabit the continental slope and seamounts in tropical, subtropical, and temperate waters of the Atlantic, Indian, and Pacific oceans (Kotlyar 1996 in Kotlyar 2010; Table 4.1). Some of the main characteristics of the species in the genus *Hoplostethus* are a high body (1/3 – 1/2 standard body length), a large head with bony crests covered with fine spines, large eyes, large transverse mouth, fine seta-like teeth in the jaws and presence or absence of a keel on the abdomen, among others (Kotlyar 1986). The pigmentation of the body is either light (red, orange or brownish) or dark (black and grey) (Kotlyar 1986). *Hoplostethus* specimens are of relatively small size, the maximum length recorded among the species range from 7.5 cm (*H. rifti*) to 75 cm (*H. atlanticus*) (Table 4.1). All *Hoplostethus* are deep-sea species (i.e. maximum depth recorded > 300 m, Table 4.1).

The amount of scientific research on each *Hoplostethus* species typically corresponds to their commercial value. As stated in the previous chapters, many studies have been conducted in the highly valuable species *H. atlanticus* (orange roughy) and a few others on *H. mediterraneus* (silver roughy) which has lower commercial value. Published studies are generally lacking for the other *Hoplostethus* species. Moreover, there are no studies on the genetic divergence among *Hoplostethus* species; and phylogenetic relationships within the genus *Hoplostethus* or the family Trachichthyidae have not been proposed yet (Moore and Dodd 2010). Kotlyar (1986) considered that *Hoplostethus* species were grouped in four subgenera and proposed a phylogenetic tree for the subgenera based in geographic distribution and a few morphological characters (Kotlyar 1986).

A suitable molecular marker to determine genetic divergence and phylogenetic relationship among species is the Cytochrome *c* Oxidase I (COI) gene. In a specific region of the COI gene, the inter species variation is much greater than the intra species variation and is the marker most often used for species identification ("DNA barcoding") in fish (e.g. Ward et al. 2005; Cawthorn et al. 2011; Lakra et al. 2011;

Zhang 2011; Zhang and Hanner 2011) and in many other animal groups (see Bucklin et al. 2011).

The first goal of the present study was to determine intra and inter species genetic divergence and to propose phylogenetic relationships for some of the *Hoplostethus* species using available Cytochrome *c* Oxidase I (COI) sequences. The species included in the analyses were *H. atlanticus, H. crassispinus, H. gigas, H. japonicus H. latus*, and *H. mediterraneus*. The second goal was to place the phylogenetic position of the genus *Hoplostethus* within the Trachichthyidae family using available COI sequences. Representatives from three other genera were included, *Aulotrachichthys prosthemius*, *Gephyroberyx darwinni* and *Paratrachichthys macleayi.*

Table 4.1 Valid *Hoplostethus* species to date

Scientific name	Author	Max length (cm)	Depth range or recorded depth (m)			Distribution
H. abramovi	Kotlyar, 1986	19.0		300		Western Indian Ocean
H. atlanticus	Collet, 1889	75.0	450	–	1800	South Pacific, Indian and Northeast Atlantic Ocean
H. cadenati	Quéro, 1974	30.0	70	–	1000	Eastern Atlantic and Western Indian Ocean
H. confinis	Kotlyar, 1980	12.3	290	–	330	Eastern Indian Ocean
H. crassispinus	Kotlyar, 1980	25.4	280	–	600	Northwest Pacific Ocean
H. druzhinini	Kotlyar, 1986	13.1	330	–	445	Western Indian Ocean
H. fedorovi	Kotlyar, 1986	15.9	500	–	520	Western Central Pacific Ocean
H. fragilis	(de Buen, 1959)	–	250	–	300	Southeast Pacific Ocean
H. gigas	McCulloch, 1914	52.0	237	–	311	Eastern Indian and Southwest Pacific Ocean
H. intermedius	(Hector, 1875)	18.0	200	–	680	Indo-West Pacific Ocean
H. japonicus	Hilgendorf, 1879	12.6	336	–	605	Northwest Pacific Ocean
H. latus	McCulloch, 1914	53.0	146	–	586	Eastern Indian Ocean
H. marisrubri	Kotlyar, 1986	12.2	510	–	520	Western Indian Ocean
H. mediterraneus	Cuvier, 1829	42.0	100	–	1175	Mediterranean Sea, Northeast Atlantic, Indian and South Pacific Ocean
H. melanopterus	Fowler, 1938	14.0	600	–	1500	Western Central Pacific, Eastern Atlantic and Western Indian Ocean
H. melanopus	(Weber, 1913)	25.0	400	–	914	Indo-West Pacific and Southeast Atlantic Ocean
H. mento	(Garman, 1899)	12.0	–		–	Eastern Pacific Ocean
H. metallicus	Fowler, 1938	–	55	–	550	Western Central Pacific Ocean
H. mikhailini	Kotlyar, 1986	14.7	273	–	525	Southeast Atlantic and Western Indian Ocean
H. occidentalis	Woods, 1973	17.3	485	–	550	North and South Atlantic Ocean
H. pacificus	Garman, 1899	–		703		Southeast Pacific Ocean
H. ravurictus	Gomon, 2008	14.1	250	–	1000	Eastern Indian Ocean
H. rifti	Kotlyar, 1986	7.5	480	–	500	Western Indian Ocean
H. robustispinus	Moore and Dood, 2010	34.0	648	–	660	Pacific Ocean
H. rubellopterus	Kotlyar, 1980	10.5	800	–	875	Indian Ocean
H. shubnikovi	Kotlyar, 1980	20.3	800	–	875	Indian Ocean
H. tenebricus	Kotlyar, 1980	21.0	825	–	885	Western Indian Ocean
H. vniro	Kotlyar, 1995	19.6	1050	–	1080	Eastern Central Atlantic Ocean

Note: Valid names as in Moore and Dodd (2010). Max length, depth range and distribution obtained from FishBase (www.fishbase.org). The symbol (–) indicates that no value has been recorded for max length or depth range.

4.3 Materials and methods

4.3.1 Data collection

The 114 COI haplotypes produced by 546 orange roughy specimens (see Chapter 2) were included in the phylogenetic analyses.

Tissue samples of *H. mediterraneus* were obtained from a total of 45 specimens from New Zealand (21), Portugal (8), France (8) and Italy (8). Samples were from a frozen tissue collection held at NIWA. DNA extractions were performed using proteinase K digestion followed by salt extraction. The remaining pellet was washed twice with ethanol and resuspended in 100 µL TE buffer. DNA samples were stored at 4°C. COI sequences of *H. mediterraneus* were obtained following the same primers and protocol used for *H. atlanticus* (see subsection 2.3.2 PCR amplification, sequencing and alignment of Materials and methods, Chapter 2). The 45 samples produced 12 haplotypes (GenBank Accession Numbers: JX049153-JX049164).

Five COI sequences of *H. crassispinus* were available (supplied by PJ Smith) and resulted in two haplotypes. A GenBank search on March 2012 was conducted to obtain all the available COI sequences of *Hoplostethus* species. Sequences of other three *Hoplostethus* species were found on GenBank. The used haplotypes were two of *H. gigas* (DQ108107.1, DQ108106.1), two of *H. latus* (DQ108099.1, DQ108097.1) and one of *H. japonicus* obtained from the complete mitochondrial sequence (NC_003187.1). In total, 133 COI haplotypes of *H. atlanticus, H. mediterraneus, H. crassispinus, H. gigas, H. latus* and *H. japonicus* were aligned using the software Geneious 5.1.7 and the Geneious alignment option (Drummond et al. 2010). COI sequences of *Aulotrachichthys prosthemius* (DQ648438), *Gephyroberyx darwinni* (DQ108114) and *Paratrachichthys macleayi* (EF609430) were added to the alignment to place the phylogenetic position of the genus *Hoplostethus* within Trachichthyidae. The alignment was truncated at each extreme to generate equal length sequences. Geneious was also used to translate the nucleotide sequences into amino acid sequences using the vertebrate mitochondrial genetic code. The translation was performed to determine synonymous and non-synonymous changes and the percentages of variation at each codon position. The amino acid sites were numbered according to the bovine sequence (*Bos taurus*, AAQ06595.1).

4.3.2 DNA sequence analyses

Pairwise distance among haplotypes was determined by the Kimura 2-Parameter (K2P) method (Kimura 1980) using the software MEGA 5.05 (Tamura et al. 2011).

A neighbour-joining (NJ) phylogram was constructed in MEGA using the K2P method to compute the evolutionary distances. The robustness of the topology was evaluated with 10,000 bootstrap replications. Phylograms were also constructed using Maximum Likelihood (ML) and Bayesian criterions in MEGA and MrBayes3.1.2 (Ronquist and Huelsenbeck 2003), respectively. The software jModelTest (Posada 2008) was used to obtain the model of molecular substitution that best fit the data. The selected model under the Akaike information criterion (AIC) was TVM+G. The Hasegawa-Kishino-Yano model with a proportion of invariable sites and a gamma distribution (HKY+I+G) was used in the ML and Bayesian analyses since it was ranked 3rd among the 88 models compared with the AIC criterion and was available in MEGA and MrBayes. For ML analyses, support for the nodes was obtained from 1,000 bootstrap replications. Bayesians analyses were performed using 1,000,000 iterations and sampling every 10 generations ensuring that the average standard deviation of split frequencies was < 0.01. The first 25% of the saved trees were discarded as burn-in.

4.4 Results

4.4.1 Genetic divergence

The proportion of A, C, G, and T bases over all 133 *Hoplostethus* COI sequences was 23.5%, 30.0%, 17.8% and 28.7%, respectively. The GC content of the sequences was 47.8%. The total length of the nucleotide sequences was 609 bp (within the barcode region of ~ 655 bp) and this region codes for 203 amino acids. Considering the bovine sequence as reference, the *Hoplostethus* amino acid sequences obtained started at site 32 and ended at site 234.

The total length of the nucleotide sequences contained 151 variable sites. From the total of variable sites, 93.38% were synonymous changes at the third codon positions and 6.62% of the changes occurred at the first codon positions. No changes were

observed in the second codon positions. From the total of 10 changes at the first codon positions, three were non-synonymous. At one of those nucleotide sites, *H. atlanticus* had guanine giving the codon GTT and the amino acid Valine at the amino acid site 83. All the other species had adenine giving the codon ATT and the amino acid Isoleucine at site 83. In the two other first codon positions that were variable, *H. atlanticus* presented timine resulting in the codon TCC and in the amino acid Serine at sites 114 and 187. All the other species instead had guanine which resulted in the codon GCC and the amino acid Alanine in sites 114 and 187.

The K2P distance within species ranged from zero to 0.65% and among species ranged from 7.37% (between *H. japonicus* and *H. crassispinus*) to 13.57% between (*H. crassispinus* and *H. gigas*) (Table 4.2).

Table 4.2 K2P (%) distances within species (diagonal) and among species (below diagonal)

	H. gig.	*H. atla.*	*H. lat.*	*H. cras.*	*H. jap.*	*H. med.*
H. gigas	0.00					
H. atlanticus	7.74	0.65				
H. latus	9.29	12.89	0.33			
H. crassispinus	13.57	14.48	7.94	0.30		
H. japonicas	11.20	10.10	7.73	7.37	---	
H. mediterraneus	10.26	12.55	9.77	10.45	9.19	0.40

4.4.2 Phylogenetic relationships

The phylogenetic analyses performed using K2P distance, ML and Bayesian criterions resulted in similar topologies (Fig. 4.1 and 4.2). The NJ phylogram obtained with K2P distance showed that *H. atlanticus* and *H. gigas* form a separate clade, which was well supported by bootstrap analysis (81%). A good supported clade (92%) was composed by the other four *Hoplostethus* species. Within this clade, *H. latus* and *H. crassispinus*

grouped together with *H. japonicus* closer to *H. latus*/*H. crassispinus* than to *H. mediterraneus.* The representative species of other three genera, *A. prosthemius*, *G. darwinni* and *P. macleayi,* grouped together and where clearly separated from *Hoplostethus* species, with *A. prosthemius* sister to *G. darwinni* (Fig. 4.1).

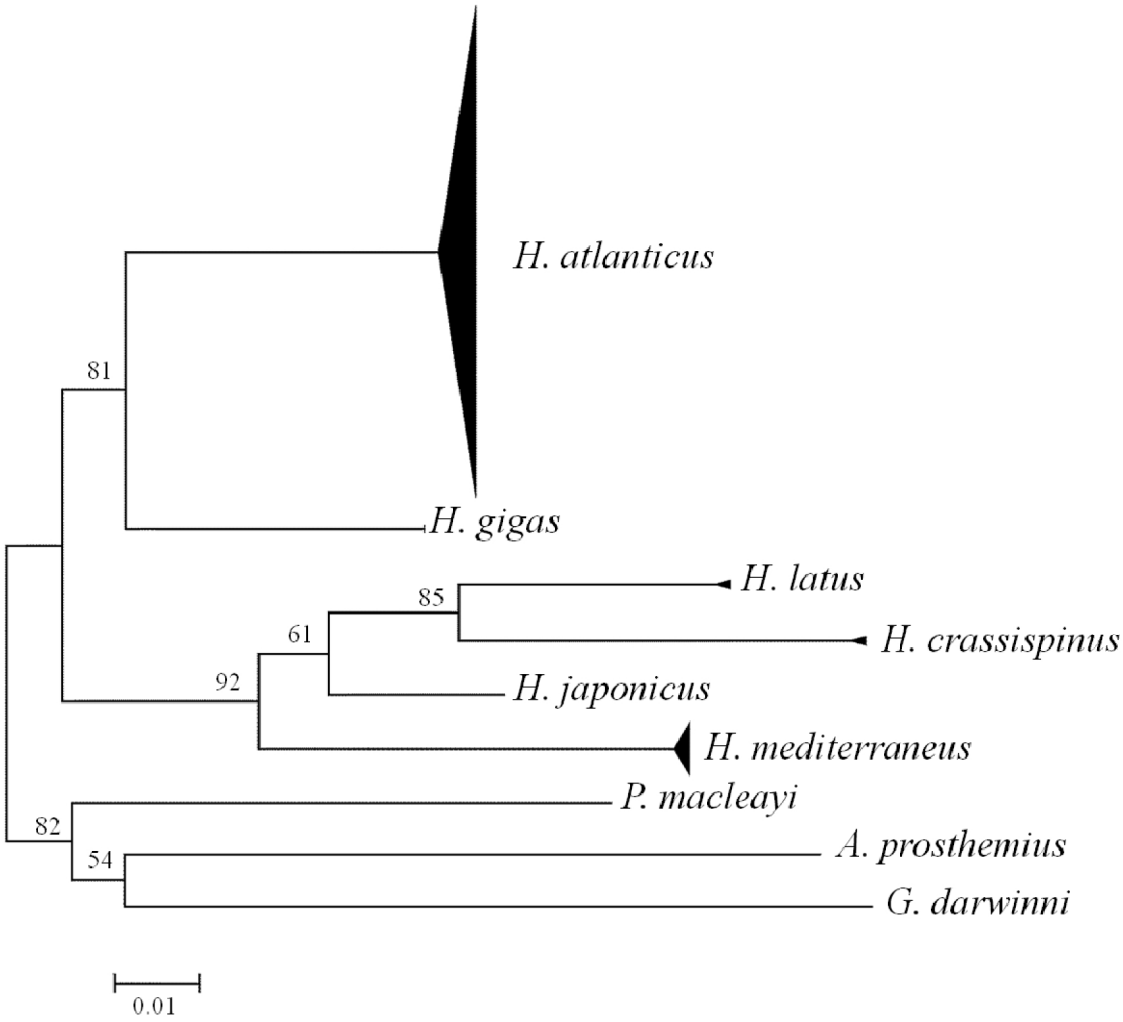


Fig. 4.1 Kimura 2-Parameter distance neighbour-joining phylogram of COI sequences from six *Hoplostethus* species and representatives from other three genera, *Paratrachichthys macleayi, Aulotrachichthys prosthemius*, *Gephyroberyx darwinni*. Numbers at nodes indicate bootstrap values (%)

The phylograms obtained with ML and Bayesian criterions resulted in a similar topology (only the ML tree is shown with Bayesian posterior probabilities [PP] included in the corresponding nodes). The only difference between ML and Bayesian phylograms was that *A. prosthemius* and *P. macleayi* were grouped as sister taxa in the ML tree (Fig. 4.2); however, the phylogenetic relationship between these species was not resolved with the Bayesian analysis (Bayesian tree not shown).

There were few differences between ML/Bayesian phylograms (Fig. 4.2), compared to the K2P phylogram (Fig. 4.1). First, in the ML/Bayesian trees *G. darwinni* appeared in a basal position separated from all the other species; in the K2P tree instead, *G. darwinni* was a sister taxa to *A. prosthemius*. The second difference is related with the relationship between *Hoplostethus* species. In the K2P tree *H. atlanticus* and *H. gigas* were sister taxa (bootstrap = 81%), but in the ML/Bayesian trees, *H. atlanticus* and *H. gigas* were not grouped together, which had a medium-low support value (bootstrap = 38%, PP = 0.78). In the ML/Bayesian trees *H. gigas* appeared closer to the other *Hoplostethus* species, however, this relationship had a low support value (bootstrap = 22%, PP = 0.52). The phylogenetic relationships among *H. latus*, *H. crassispinus*, *H. japonicus,* and *H. mediterraneus* were the same in K2P, ML and Bayesian analyses (Fig. 4.1 and 4.2).

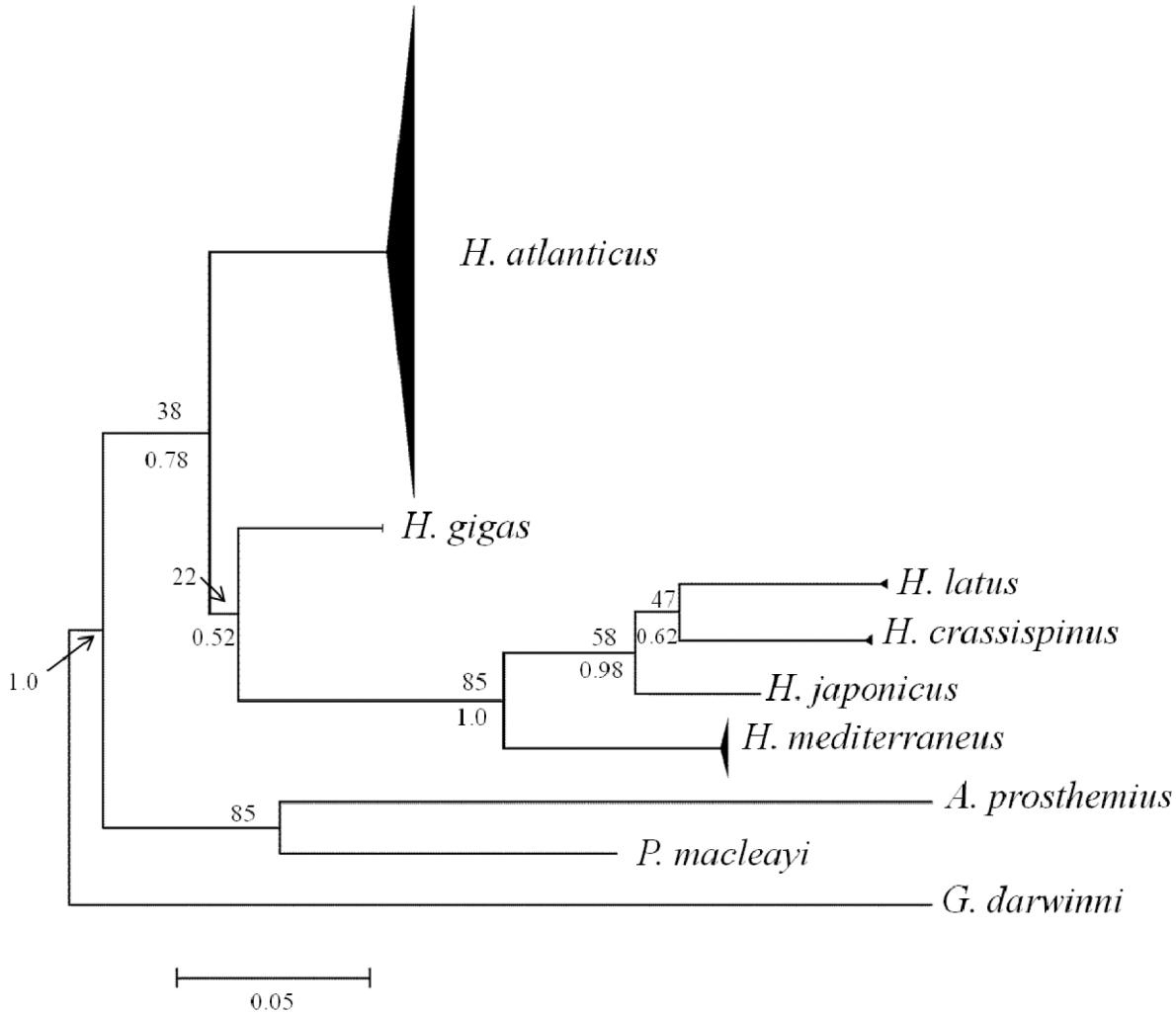


Fig. 4.2 Maximum Likelihood phylogram of COI sequences from six *Hoplostethus* species and representatives from other three genera, *Paratrachichthys macleayi, Aulotrachichthys prosthemius*, *Gephyroberyx darwinni*. Numbers above and below the branches indicate bootstrap values (%) and Bayesian posterior probabilities (PP), respectively

4.5 Discussion

4.5.1 Genetic divergence

Most of the variation across the COI sequences occurred in third codon positions, which is consistent with the expectation of a degenerated genetic code. Few variable sites occurred at first codon positions and none at second codon positions. This pattern was also reported by Ward and Holmes (2007) for many species of marine fishes. Considering the three non-synonymous changes at first codon positions, it is notable that only *H. atlanticus* presented different amino acids respect to the other five *Hoplostethus* species. The amino acidic site 83 was reported as a high variable site by Ward and Holmes (2007). In that study, it was also determined that site 83 have valine or isoleucine, both aliphatic amino acids. In the present study, the two other non-synonymous sites produced the amino acids Serine or Alanine at sites 114 and 187, which are hydroxyl and aliphatic amino acids, respectively. As pointed out by Ward and Holmes (2007), amino acid substitutions are most common among physicochemical similar amino acids than among dissimilar amino acids. As only *H. atlanticus* had amino acids of different physicochemical characteristics at sites 114 and 187 compared to the other species, there may be a particular adaptation in the expression of the COI gene in *H. atlanticus*. This could be related to a unique characteristic in *H. atlanticus* in respect to the other *Hoplostethus* species considered here, for example, *H. atlanticus* is the only one able to occur deeper than 1,200 m.

The analysis of the genetic distance as measured by the K2P method among the COI haplotypes of *Hoplostethus* species showed that the intra-species divergence was much lower than the inter-species divergence (intra-species divergence was not possible to estimate for *H. japonicus* as there was only one haplotype available). The marked difference between intra and inter-species divergence is widely recognized for a specific region of COI gene making this of one the main characteristics for the generalized use of the COI gene as the molecular marker for DNA barcoding (Hebert et al. 2003). Although only two haplotypes per species were available to estimate intra-specific divergence in *H. gigas, H. latus* and *H. crassispinus*, the marked difference between intra-species divergence respect to inter-species divergence was also found in *H. mediterraneus* (12 haplotypes) and *H. atlanticus* (114 haplotypes). The intra-species divergence in *H. mediterraneus* was 0.40% and the divergence respect to the other

species ranged from 9.19 to 12.55%. Similarly, the intra-species divergence in *H. atlanticus* was 0.65%, diverging from the other species in 7.74 to 14.48%. The average intra (five species) and inter (six species) divergence among *Hoplostethus* species were 0.34% and 10.30%, respectively. These values are within the ranges reported for marine fishes: 0.10 - 0.39% and 4.58 - 17.6% for the intra and inter-species divergence, respectively (Ward et al. 2005; Cawthorn et al. 2011; Lakra et al. 2011; Zhang 2011; Zhang and Hanner 2011).

4.5.2 Phylogenetic relationships

All the phylogenetic analyses based on K2P distance, Maximum Likelihood and Bayesian criterions showed that *H. latus, H. crassispinus, H. japonicus,* and *H. mediterraneus* form a separate clade from *H. atlanticus* and *H. gigas*. Within the former, *H. latus* and *H. crassispinus* were sister taxa and *H. japonicus* was close to *H. latus* and *H. crassispinus* with *H. mediterraneus* being the most divergent species in the clade. The position of *H. gigas* was not well defined with the nucleotide data. With the K2P distance criterion this species was sister to *H. atlanticus* with a good support value. Instead Maximum Likelihood and Bayesian analyses showed that *H. gigas* was a separate taxa from *H. atlanticus*. On the other hand, and as stated before, at the amino acid level, non-synonymous substitutions at three sites in first codon positions clearly differentiated *H. atlanticus* from all the other species with *H. gigas* presenting the same amino acids at those positions than *H. latus*, *H. crassispinus H. japonicus* and *H. mediterraneus*. The differentiation of *H. atlanticus* from all the other five species at the amino acid level and the clear differentiation *H. atlanticus* from *H. latus, H. crassispinus, H. japonicus,* and *H. mediterraneus* at the nucleotide level could be correlated with morphological characteristics. Kotlyar (1986) considered that *H. atlanticus* was part of the subgenus *Macrohoplostethus* and that *H. gigas, H. crassispinus H. japonicus,* and *H. mediterraneus* were part of the subgenus *Hoplostethus* (*H. latus* was not recognized in that study). According to Kotlyar (1986) these two subgenera were morphological differentiated by the size and thickness of the scales in the ventral keel, the number of soft rays in the dorsal fin, the number of vertebrae, the morphology of the pyloric caeca, and the number of terminal appendages.

Therefore, I found a correlation between the results of the phylogenetic analyses presented here for six *Hoplostethus* species and general morphologic characteristics presented elsewhere.

The analyses conducted here support the monophyly of the genus *Hoplostethus*. Also, Maximum Likelihood and Bayesian analyses showed that the *Hoplostethus* clade seems to be a derived group respect to the phylogenetic positions of other three genera, *Aulotrachichthys*, *Gephyroberyx* and *Paratrachichthys*.

4.6 Conclusions

A specific region of the COI gene is widely used to differentiate species (reviewed by Bucklin et al. 2011) even congeneric species (Hebert et al. 2003). In this study I found that this COI region is highly effective in differentiate species of the genus *Hoplostethus* given the marked difference between intra and inter-species divergence. The COI marker was also useful to determine the phylogenetic relationships of the species under study. Overall, taking into account the different methods employed, the phylogenetic relationship among *H. atlanticus, H. latus*, *H. crassispinus H. japonicus,* and *H. mediterraneus* was well defined. However, the phylogenetic position of *H. gigas* respect to the others species was uncertain. Future studies using nuclear molecular markers may help to determine the phylogenetic position of *H. gigas.*

This is the first study conducted to determine the genetic divergence and the molecular phylogenetic relationship among species of the genus *Hoplostethus*. Nevertheless, the un-availability of samples of other congeners resulted in the inclusion of only six out of 28 valid *Hoplostethus* species. A further study using the barcode region of the COI gene in combination with nuclear markers and including all the species within the genus is needed to resolve the evolutionary relationship among these deep-sea congeners.

Chapter 5

Critical amino acid replacements in the rhodopsin gene of the deep-sea teleost orange roughy (*Hoplostethus atlanticus*): comparison with other teleost species occupying different light environments

5.1 Abstract

Critical amino acid replacements in opsin proteins shift the maximal absorbance of visual pigments to perceive different photic environments (spectral tuning). Here I studied the molecular basis for spectral tuning of the rhodopsin (RH1) pigment in the deep-sea fish orange roughy, which is important to understand how deep-sea species have adapted to the dim-light available in the bathypelagic environment. To compare and identify critical amino acid changes I also obtained partial rhodopsin sequences of 18 species of marine teleost habiting different light environments. I identified replacements at four critical amino acid sites that have been numbered according to the bovine RH1 pigment: 194, 195, 292 and 299. All the species had the substitutions P194R and H195A. The combination 292S/299A was only found in the deep-sea congeners silver roughy and orange roughy. This likely reflects an adaptation of these species to the bathypelagic light environment. All the epipelagic species studied and the epi-mesopelgic species blue cod, had the combination 292A/299S, except the red gurnard (292A/299A) and the spotty (292S/299S). It is possible that the combination 292A/299S is an adaptation to longer wavelengths of light in comparison with the deeper species. This is the first study in determine partial rhodopsin in all the species under study, except by the hoki and silver roughy. Further studies are needed to determine all the critical amino acid replacements in the rhodopsin gene of these species and to measure the maximal absorbance (λ_{max}) of the corresponding RH1 pigments.

5.2 Introduction

Many animal groups have evolved visual pigments that are often specialized and enable them to perceive their surrounding environment. A diverse range of visual pigments have arisen, which indicate the adaptive significance that light sensitive molecules have for organisms living in different photic environments (see Yokoyama 2008). Visual pigments consist of an opsin protein attached to a chromophore via a Schiff base linkage and a conserved lysine residue. The opsin protein in vertebrates is composed of a single polypeptide chain of 340 – 370 amino acids that forms seven α-helical transmembrane (TM) regions connected by cytoplasmic and luminal loops (Dratz and Hargrave 1983). Each pigment shows a characteristic peak of maximal absorbance (λ_{max}); the location of this peak depends on the chromophore (11-*cis*-retinal or 11-*cis*-3,4-dehydroretinal) and the interactions between the chromophore and the specific amino acid sequence of the opsin protein (Bowmaker 1995). In most vertebrates, rod photoreceptors are highly sensitive to dim-light and cone photoreceptors mediate vision at higher light intensities (reviewed by Yokoyama 2008).

In terrestrial environments and in the surface of the Oceans the sun radiation is dominated by photons of the visual spectrum from ultraviolet with wavelengths of ~ 300 nm, to infrared with wavelengths of ~ 1,100 nm (Levine and MacNichol 1982; Bowmaker 1995). In the Oceans, the intensity and spectral composition of the light decreases with depth due to absorption by water molecules and suspended particles; the light becomes monochromatic towards blue (~ 470 nm), while ultraviolet and infrared light are reduced (Bowmaker 1995; Warrant and Locket 2004). The epipelagic zone up to ~ 150 – 200 m receives bright sunlight, further down to about 1,000 m the mesopelagic zone is characterized by dim-light bioluminescence (Douglas et al. 1998). After 1,000 m, in the dark bathypelagic zone, the light comes from point-source bioluminescent flashes (Warrant and Locket 2004). Over 80% of the marine species living deeper than 200 m produce bioluminescence for diverse functions such as intra and inter-specific communication, camouflage, startling predators and attracting prey (Douglas et al. 1998). Organisms usually produce blue/green bioluminescence; however, some produce far-red illumination (Douglas et al. 1998; Turner et al. 2009). Deep-sea fishes (i.e. > 200 m) have visual systems adapted to perceive the limited spectrum of light; one typical feature is the loss of cone photoreceptors resulting in rod-only retina (see Hunt et al. 2001).

The opsin genes were first isolated and characterized in bovines and later in humans (Nathans and Hogness 1983, 1984). Since then, many opsin genes from a variety of vertebrate species have been studied including the measure of the λ_{max}s of the corresponding visual pigments (see Yokoyama 2008). Modifications of the λ_{max}s values have allowed organism to adapt to diverse light environments. These pigments are divided into five groups: rhodopsins (RH1, λ_{max} = ~ 500 nm), RH1-like (RH2, λ_{max} = 470 – 510 nm), short wavelength-sensitive type 1 (SWS1, λ_{max} = 360 – 420 nm), SWS type 2 (SWS2, λ_{max} = 440 – 455 nm), and middle and long wavelength-sensitive (M/LWS, λ_{max} = 510 – 570 nm) (Yokoyama 1999 and references therein). RH1 pigments are usually expressed in rods and the four other classes of pigments in cones (Yokoyama et al. 1999). Certain amino acid changes in the opsin protein shift the λ_{max} values of the visual pigments making possible a phenomenon known as "spectral tuning" in which a chromophore attains different absorption spectra when attached to different opsins (Yokoyama 2008). The critical amino acid sites (tuning sites) that shift λ_{max} values have been identified according to the site numbers of the bovine RH1 pigments and specific replacements in many marine fishes shift the λ_{max} values of the RH1 pigments towards the blue (e.g. Yokoyama 1999; Yokoyama et al. 1999; Hunt et al. 2001; Yokoyama and Takenaka 2004; Yokoyama 2008). Hunt et al. (2001) studied the rhodopsin gene sequence of 28 deep-sea fish species; most of the λ_{max} values of the rod pigments of those species vary from 490 to 477 nm.

The molecular basis for spectral tuning of rod pigments has never been studied in the teleost orange roughy*, Hoplostethus atlanticus*. This deep-sea fish inhabit depths of 450 – 1,800 m between the mesopelagic and the bathypelagic zones. It is likely that orange roughy exhibits visual adaptations to dim-light and bioluminescence. Studying retinal morphology of mesopelagic and demersal teleost, Pankhurst (1987) found that orange roughy have retinal adaptations that maximise visual sensitivity. Hunt et al. (2001) identified tuning sites in the congener species *H. mediterraneus* that occurs at 100 – 1,175 m and reported that the rod visual pigment of this species have a λ_{max} value of 479 nm, likely an adaptation to the blue-light environment and blue luminescence.

The first goal of this study was to determine whether orange roughy have critical amino acid replacements that are known to cause a shift in the λ_{max} value of RH1 pigments. To achieve this, I sequenced a partial sequence of the rhodopsin gene of orange roughy. To compare and identify critical amino acid changes I also sequenced the rhodopsin sequence of 18 species of marine teleost habiting at different depths (1 –

1,175 m) and, therefore, different light environments. The second goal was to conduct a phylogenetic analysis of partial sequences of the rhodopsin gene among all the species under study. I hypothesized that, 1) the species would have amino acid replacement that are known to be implicated in shift the λ_{max} of RH1 pigments towards the blue; 2) orange roughy would have particular set of amino acid replacements that is not found in the shallower species due to an adaptation to the bathypelagic light environment; and 3) the arrangement of the phylogenetic tree would correspond with the depths occupied by species.

5.3 Materials and methods

5.3.1 Sampling and DNA extractions

Tissue samples from a total of 127 specimens were obtained from 19 teleost species belonging to 16 families (Table 5.1). Sample size per species ranged from two to 19 and sampling areas included New Zealand, Australia, Namibia, Chile, the Mediterranean Sea and the Northeast Atlantic Ocean (Table 5.1). Muscle samples were collected from fresh fish and frozen at -20°C or stored in 95% ethanol.

DNA extractions were performed using proteinase K digestion followed by salt extraction. The remaining pellet was washed twice with ethanol and resuspended in 100 µL TE buffer. DNA samples were stored at 4°C.

Table 5.1 Sample size (*n*) and sampling area of 19 teleots species used for partial amplification of the rhodopsin gene. The common names of each species are those used in New Zealand. Total *n* = 127

Common names	Scientific name (Family)	*n*	Sampling area
Butterfish	*Odax pullus* (Odacidae)	5	New Zealand
Kahawai	*Arripis trutta* (Arripidae)	2	New Zealand
Yellowbelly flounder	*Rhombosolea leporina* (Pleuronectidae)	3	New Zealand
Sand flounder	*Rhombosolea plebeia* (Pleuronectidae)	3	New Zealand
Sole	*Peltorhamphus latus* (Pleuronectidae)	3	New Zealand
Blue moki	*Latridopsis ciliaris* (Latridae)	5	New Zealand
Spotty	*Notolabrus celidotus* (Labridae)	10	New Zealand
Red gurnard	*Chelidonichthys kumu* (Triglidae)	4	New Zealand
Snapper	*Pagrus auratus* (Sparidae)	10	New Zealand
Jack mackerel	*Trachurus murphyi* (Carangidae)	10	Chile
Blue cod	*Parapercis colias* (Pinguipedidae)	13	New Zealand
Tarakihi	*Nemadactylus macropterus* (Cheilodactylidae)	4	New Zealand
Blue warehou	*Seriolella brama* (Centrolophidae)	4	New Zealand
Hapuku	*Polyprion oxygeneios* (Polyprionidae)	5	New Zealand
Hoki	*Macruronus novaezelandiae* (Merlucciidae)	12	New Zealand, Australia, Chile
Giant stargazer	*Kathetostoma giganteum* (Uranoscopidae)	5	New Zealand
Red cod	*Pseudophycis bachus* (Moridae)	5	New Zealand
Silver roughy	*Hoplostethus mediterraneus* (Trachichthyidae)	5	New Zealand, Mediterranean Sea, Northeast Atlantic
Orange roughy	*Hoplostethus atlanticus* (Trachichthyidae)	19	New Zealand, Australia, Namibia, Chile, Northeast Atlantic

5.3.2 PCR amplification, sequencing and alignment

Six primers pairs developed for teleost species (Chen et al. 2003) were tested for partial amplification of the rhodopsin gene. Un-specific DNA fragments were observed in gel electrophoresis for all primer pairs. Successful optimization was obtained for one primer pair. The primers used were: Rh545 -5'- GCAAGCCCATCAGCAACTTCCG-3' and Rh1073r -5'- CCRCAGCACARCGTGGTGATCATG-3' (Chen et al. 2003).

Final PCR reactions of 10 µL total volume consisted of ~20 ng of DNA, 1X PCR buffer (160mM (NH4)2SO4, 670mM Tris-HCl, 0.1% stabilizer), 1.5 mM $MgCl_2$, 0.6 µM of each primer, 0.2 mM of each dNTPs, 1.5 U of Taq polymerase and 0.4 mg mL^{-1} of Bovine Serum Albumin (BSA). PCR cycles were performed on a Eppendorf Mastercycler ep gradient S, as follow: initial denaturing at 95°C for 2 min, followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 58°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 5 min. PCR products were purified with ExoSAP-IT following manufacturer's instructions. The nucleotide sequences were determined using an ABI3730 Genetic Analyzer. The software Geneious 5.1.7 (Biomatters Ltd.) was used to align the sequences using the Geneious alignment option (Drumond et al. 2010). Each species had a unique nucleotide sequence (except for within orange roughy), with few heterozygotes sites (see results). The nucleotide sequence of each species was translated into amino acid sequences in Geneious using the standard genetic code. The amino acid sequences of the 19 species were aligned to the bovine rhodopsin sequence (*Bos taurus*, GenBank accession number: NP_001014890) to determine potential sites for spectral tuning.

5.3.3 Data analyses

A phylogenetic analysis was performed to determine if the phylogeny of the partial sequences of the rhodopsin gene was related with the depth range of the species (Table 5.2). A Bayesian analysis was conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using the bovine sequence as the outgroup. The program was set to determine the best fixed-rate model for the amino acid sequences during the analysis. The analysis was performed using 5,000,000 iterations and sampling every 10

generations. The standard deviation of split frequencies index fall to 0.004. The 25% of the trees were discarded as burn-in.

Table 5.2 Depth range and geographic distribution of the 19 teleost species. The information was obtained from FishBase (www.fishbase.org) and the literature as indicated. Scientific names of the species can be found in Table 5.1

Species	Depth range (m)	Distribution	References
Butterfish	0 – 40	New Zealand	Trip et al. (2011) Ministry of Fisheries (2011)
Kahawai	30 – 40	New Zealand and southern Australia	Duffy and Patherick (1999) Francis et al. (2002)
Yellowbelly flounder	1 – 50	New Zealand	Ayling and Cox (1982) Glova and Sagar (2000)
Sand flounder	22 – 100	New Zealand	Ayling and Cox (1982) Glova and Sagar (2000)
Sole	1 – 100	New Zealand	Ayling and Cox (1982)
Blue moki	10 – 120	New Zealand and southern Australia	Smith et al. (2001)
Spotty	10 – 145	New Zealand	Anderson and Millar (2004)
Red gurnard	1 – 200	New Zealand and Australia	Francis et al. (2002)
Snapper	3 – 200	New Zealand, Australia, Japan	Francis et al. (2002) Stewart (2008)
Jack mackerel	20 – 300	Ecuador, Peru, Chile and New Zealand	Francis et al. (2002) Bertrand et al. (2004)
Blue cod	5 – 360	New Zealand	Francis et al. (2002) Carbines (2003)
Tarakihi	40 – 400	New Zealand, southern Australia and south America	Jordan (2001) Threscher et al. (2007)
Blue warehou	100 – 600	New Zealand and Australia	Francis et al. (2002) Robinson et al. (2008)
Hapuku	50 – 850	Southern Oceans, New Zealand and Australia, off Chile	Francis et al. (2002) Wakefield et al. (2010)
Hoki	10 – 900	New Zealand, Australia and Chile	Connell et al. (2010) Olavarria et al. (2006)
Giant stargazer	12 – 1000	New Zealand	Smith et al. (2006)
Red cod	26 – 1000	New Zealand and Australia	Beentjes and Renwick (2001)
Silver roughy	100 – 1175	Northeast Atlantic, Mediterranean Sea, Indian and South Pacific Oceans	Francis et al. (2002) Madurell and Carter (2005)
Orange roughy	450 – 1800	South Pacific, Indian and Northeast Atlantic Ocean	Branch (2001)

5.4 Results

A 477-bp nucleotide fragment was obtained for all 127 individuals analyzed (Table 5.1). Sequences were deposited in GenBank under Accession Numbers: JX049165-JX049193. There were 177 variable sites among all the samples, but intra-specific variation was low. Aside of few heterozygote positions in seven species (Table 5.3), there was not nucleotide variation within each species, except by the orange roughy at site 190. From the total number of orange roughy samples, at site 190 two individuals had cytosine, 11 had thymine (homozygotes) and six were heterozygotes (see Table 5.3). The translation of the nucleotide sequences into amino acids resulted in a unique amino acid sequence for each species, with a total length of 158 amino acids. An alignment with the bovine amino acidic sequence as a reference to identified potential sites for spectral tuning, showed that the amino acid sequences obtained started at site 161 and ended at site 318, from the α-helix IV to the luminal loop after α-helix VII. In the total length of the amino acid sequences, 60 sites were variable (Table 5.4). Among these variable sites, four are known to be involved in shift the λ_{max} value of RH1 pigments. The potential sites for spectral tuning were P194R, H195A, A292S and A299S (the letters represent the code of the amino acid in the bovine/teleost species sequences and the number indicates the site according to the site number of the bovine RH1 pigment). The critical amino acid changes P194R and H195A were determined for all the teleost species. The change A292S occurred only in spotty, silver roughy and orange roughy. The replacement A299S was identified in nine species: butterfish, kahawai, yellowbelly flounder, sand flounder, sole, blue moki, spotty, snapper and blue cod (Table 5.4).

Table 5.3 Heterozygote positions in the 477-bp nucleotide fragment identified in 14 individuals of seven species. The number of individuals with heterozygote position per species is indicated by (*n*). Scientific names of the species can be found in Table 5.1

Species (*n*)	Positions	Bases
Yellowbelly flounder (1)	166	A/G
Sand flounder (2)	130 - 307	C/T - G/T
Jack mackerel (1)	37	C/T
Blue cod (2)	97 - 208	C/T - C/T
Hoki (1)	121	C/T
Red cod (1)	205	C/T
Orange roughy (6)	190	C/T

Table 5.4 Variable amino acid sites among the 19 teleost species analysed using the bovine sequence as a reference. The site numbers are those of the bovine RH1 pigment. Highlighted sites indicate changes that are known to cause a shift in the λ_{max} value of the RH1 pigments. Scientific names of the species can be found in Table 5.1

Species	162	163	165	167	168	169	173	186	189	194	195	196	197	198	205	209	210	213	214	216	217	218	219	220	225	227	228	229	236	241
Bovine	V	M	L	C	A	A	V	S	I	P	H	E	E	T	I	V	V	I	I	L	I	V	I	F	Q	V	F	T	Q	A
Butterfish	L	M	L	C	S	V	V	S	V	R	A	E	G	F	I	T	C	L	I	L	T	I	V	F	R	L	C	A	A	E
Kahawai	V	M	S	C	A	V	V	S	I	R	A	E	G	F	I	V	C	L	I	L	L	V	V	F	R	L	C	A	A	E
Yellowbelly flounder	F	G	C	C	A	V	L	S	V	R	A	E	G	F	I	T	C	C	I	L	C	I	V	G	R	L	C	A	A	E
Sand flounder	F	G	S	C	A	V	F	S	V	R	A	E	G	F	I	T	C	C	I	L	C	I	V	A	R	L	C	A	A	E
Sole	F	G	C	C	A	V	V	S	V	R	A	E	G	F	V	V	C	C	I	L	I	I	V	G	R	L	C	A	A	E
Blue moki	L	M	S	C	A	V	V	S	V	R	A	E	G	F	V	V	C	L	T	L	T	I	V	F	R	L	C	A	A	E
Spotty	F	M	S	C	A	V	V	S	V	R	A	E	G	F	V	T	C	L	I	M	A	I	V	F	R	L	C	A	A	E
Red gurnard	T	M	L	C	S	V	V	S	V	R	A	E	G	F	I	T	C	M	T	L	T	I	I	F	R	L	C	A	A	E
Snapper	T	M	M	C	A	A	V	S	I	R	A	E	G	F	V	T	C	L	T	L	T	I	V	F	R	L	C	A	A	E
Jack mackerel	F	M	S	C	A	V	V	S	I	R	A	E	G	F	L	S	C	C	I	L	V	I	V	F	R	L	C	A	A	E
Blue cod	L	M	N	C	A	V	V	S	V	R	A	E	G	F	I	C	C	M	T	L	T	V	V	F	R	L	C	A	A	E
Tarakihi	L	M	C	C	A	V	V	S	V	R	A	E	G	F	V	I	C	S	I	L	T	I	V	F	R	L	C	A	A	E
Blue warehou	L	M	S	C	A	V	V	S	V	R	A	E	G	F	V	C	C	C	I	L	I	V	I	F	R	L	C	A	A	E
Hapuku	L	M	S	C	A	V	V	S	V	R	A	E	G	F	I	I	C	S	I	L	T	V	V	F	R	L	C	A	A	E
Hoki	I	M	N	S	A	A	V	S	I	R	A	A	G	Y	V	T	C	C	V	L	V	V	V	F	R	L	C	A	A	E
Giant stargazer	L	M	C	C	A	V	V	S	V	R	A	E	G	F	V	T	C	L	I	L	T	I	V	F	R	L	C	A	A	E
Red cod	V	M	N	C	A	F	V	S	V	R	A	P	G	I	I	V	C	T	V	L	T	V	I	F	R	L	C	A	A	E
Silver roughy	L	M	C	C	S	V	V	A	I	R	A	P	G	V	I	V	C	S	I	L	T	I	I	F	R	L	C	A	A	E
Orange roughy	L	M	L	C	S	V	F	S	I	R	A	P	G	V	V	V	C	S	I	L	T	I	I	F	R	L	C	A	A	E

A=alanine, C=cysteine, D=aspartic acid, E=glutamic acid, F=phenylalanine, G=glycine, H=histidine, I=isoleucine, K=lysine, L=leucine, M=methionine, N=asparagine, P=proline, Q=glutamine, R=arginine, S=serine, T=threonine, V=valine, W=tryptophan, Y=tyrosine

Table 5.4 Continued

Species	242	245	248	251	255	256	259	260	263	266	270	273	274	277	278	281	282	286	290	292	297	298	299	300	304	308	309	311	315	318
Bovine	T	K	K	T	I	I	I	A	I	L	G	F	Y	T	H	S	D	I	I	A	T	S	A	V	V	M	M	K	N	V
Butterfish	T	R	R	T	V	I	V	G	V	C	G	W	W	T	H	S	E	V	L	A	S	S	S	I	M	C	M	K	H	I
Kahawai	T	R	R	T	V	I	I	A	V	V	S	W	W	T	H	S	D	V	L	A	S	S	S	I	M	C	M	K	Q	I
Yellowbelly flounder	T	R	R	T	V	I	I	A	V	V	S	W	Y	I	N	S	D	L	I	A	S	S	S	I	L	L	M	K	H	I
Sand flounder	T	R	R	T	V	I	I	A	V	V	S	W	Y	I	N	S	D	L	I	A	S	S	S	I	L	L	M	K	H	I
Sole	T	R	R	T	V	I	I	A	V	V	S	W	Y	I	N	S	D	L	I	A	S	S	S	I	L	F	M	K	H	I
Blue moki	T	R	R	T	V	L	I	A	V	C	G	W	Y	T	H	S	E	V	F	A	S	S	S	I	M	C	M	K	H	I
Spotty	T	R	R	T	V	I	I	A	V	V	G	W	W	T	H	S	E	V	I	S	S	S	S	I	M	C	M	K	N	I
Red gurnard	T	R	R	S	V	I	I	A	V	V	S	W	Y	L	N	S	E	V	I	A	S	S	A	V	M	C	M	K	T	I
Snapper	T	R	R	T	V	L	I	A	V	I	S	W	W	T	H	S	E	V	I	A	S	S	S	I	M	C	M	K	H	I
Jack mackerel	T	R	R	T	V	I	V	A	V	C	G	W	F	T	H	S	E	V	I	A	S	S	A	I	M	C	M	K	H	I
Blue cod	T	R	R	T	V	L	I	A	V	C	G	W	W	T	H	S	E	V	I	A	S	S	S	I	M	C	M	K	H	I
Tarakihi	T	R	R	T	V	I	I	G	V	C	G	W	F	T	H	S	E	V	F	A	S	S	A	I	M	C	M	K	H	I
Blue warehou	T	R	R	T	V	I	I	G	V	V	S	W	F	T	H	S	E	V	V	A	S	S	A	I	M	C	M	K	N	I
Hapuku	T	R	R	S	V	L	V	A	V	L	G	W	W	T	H	S	E	V	V	A	S	S	A	I	M	C	M	K	H	I
Hoki	T	R	R	T	I	I	I	G	I	C	G	W	F	T	H	I	T	L	V	A	A	A	A	V	M	C	L	K	H	I
Giant stargazer	T	R	R	T	V	L	I	A	V	L	G	W	W	T	N	S	E	V	I	A	S	S	A	I	M	C	M	K	N	I
Red cod	T	R	R	T	I	I	V	G	V	C	G	W	F	T	H	S	T	V	I	A	S	A	A	I	M	C	L	K	H	I
Silver roughy	S	R	R	T	V	L	I	A	I	L	S	W	Y	T	H	S	E	V	L	S	S	S	A	I	M	C	M	K	N	I
Orange roughy	S	R	R	S	V	L	I	A	I	L	G	W	Y	T	H	S	E	V	I	S	S	S	A	I	L	F	M	R	N	I

A=alanine, C=cysteine, D=aspartic acid, E=glutamic acid, F=phenylalanine, G=glycine, H=histidine, I=isoleucine, K=lysine, L=leucine, M=methionine, N=asparagine, P=proline, Q=glutamine, R=arginine, S=serine, T=threonine, V=valine, W=tryptophan, Y=tyrosine

The Bayesian phylogeny of the rhodopsin sequences of 19 teleost species using the bovine sequence as outgroup resulted in four clades, here after referred as Clade A – D (Fig. 5.1). There was a weak relationship between clades composition and the maximum depths reported for the species (see Table 5.2 for depth range of each species). The sequences of butterfish, hapuku and giant stargazer were not part of any clade and their divergence from the rest of the species was supported by high posterior probabilities (PP) values (PP = 0.87 – 0.99). Clades B, C and D were well supported (PP = 0.78 – 1.0); clade A had a lower support (PP = 0.65). Clade A was composed by epipelagic species that habitat at a maximum depth of 40 – 200 m. Clade B was formed by species occupying epipelagic and mesopelagic environments, with a maximum depth of 300 – 1,000 m. Clade C was formed by only two species, the epipelagic species blue moki (maximum depth 120 m) and by Blue cod that is able to occupy epi and mesopelagic environments (maximum depth 360 m). Finally, clade D was composed by the two species able to occur in bathypelagic environments, the silver roughy (maximum depth 1,175 m) and the orange roughy (maximum depth 1,800 m). The species within clades B, C and D presented a unique combination at the critical sites 292 and 299. All the species in clade B had 292A/299A, the two species in clade C had 292A/299S, and the roughies, the only species in clade D, had the combination 292S/299A, which was not found in any other species. Instead, clade A was composed by species presenting the combinations 292A/299S, 292A/299A and 292S/299S (Fig. 5.1).

On the other hand, the grouping of the species according to the combination of the critical sites 292 and 299 shows a better relationship between the maximum depth of the species and the amino acids at these two sites (Table 5.5). However, the relationship is not clear for the species that have a maximum depth of 145-360 m (spotty, snapper, red gurnard, jack mackerel and blue cod).

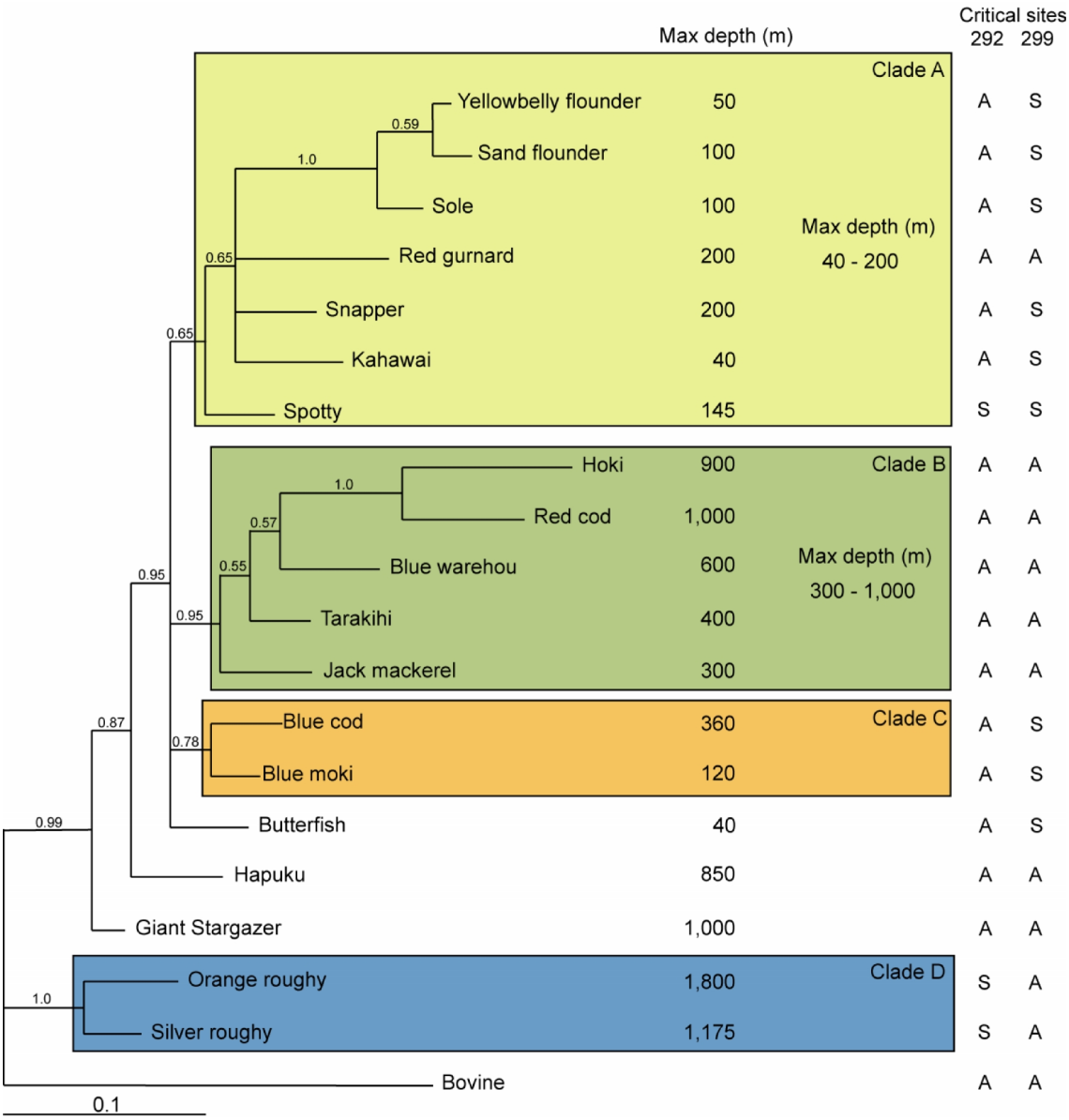


Fig. 5.1 Bayesian phylogram of 19 teleost species using amino acidic data and the bovine sequence as outgroup (accession number: NP_001014890). The numbers above branches correspond to posterior probabilities (PP). The maximum (Max) depth range of each species is found in Table 5.2. The amino acids at the critical sites 292 and 299 are indicated for each species in the right side of the phylogram.

Table 5.5 Grouping of the 19 teleost species according to the combination at the critical sites 292 and 299. Scientific names of the species can be found in Table 5.1

Critical sites	Species	Max depth (m)
292A/299S	Butterfish	40
	Kahawai	40
	Yellowbelly flounder	50
	Sand flounder	100
	Sole	100
	Blue moki	120
	Snapper	200
	Blue cod	360
292S/299S	Spotty	145
292A/299A	Red Gurnard	200
	Jack mackerel	300
	Tarakihi	400
	Blue warehou	600
	Hapuku	850
	Hoki	900
	Giant stargazer	1,000
	Red cod	1,000
292S/299A	Silver roughy	1,175
	Orange roughy	1,800

5.5 Discussion

As hypothesized, I detected amino acid replacement in the rhodopsin sequences of 19 marine teleost that are known to be involved in shift the λ_{max} of RH1 pigments towards the blue light. Hunt et al. (2001), Yokoyama and Takenaka (2004) and Yokoyama (2008) summarized critical amino acid changes in marine fishes. I also detected conserved and functionally important amino acids identified by previous studies (data not shown): K296, C187 and W265 (reviewed by Hope et al. 1997 and by Hunt et al. 2001).

Hunt et al. (2001) determined that a specific λ_{max} value is not always explained by a particular set of amino acid substitutions; rather, the same λ_{max} value results from different combinations of amino acid at specific sites. In the present study, all the species had the substitution P194R, H195A; however, the combination of the replacements P194R, H195A and A292S was only found in three species: spotty, silver roughy and orange rough (but the spotty differed from the roughies in the critical amino acid site 299, which is discussed further below). The combination P194R, H195A, A292S decrease the λ_{max} value of the RH1 pigment by 14 – 20 nm (Yokoyama 2008); and for a conger species this combination resulted in a λ_{max} value of 486 nm (Yokoyama 2008). The same decrease in the λ_{max} value could be achieved by the combination D83N/A292S and by a unique replacement at site 122 (E122Q) (Yokoyama 2008). Because the sequences that I obtained started at site 161, it could not been ruled out that the same functional change is presented by some of the other species studied. The replacement A292S was already detected in silver roughy by Hope et al. (1997). Others marine fishes living at depths of more than 200 m had the substitution A292S, such as the coelacanth, *Latimeria chalumnae* (Yokoyama et al. 1999). Also, Hunt et al. (2001) detected this replacement in 18 deep-sea teleost from six different orders: Beryciformes, Ophidiiformes, Gadiformes, Aulopiformes, Stomiiformes and Osmeriformes. The same replacement has been also detected in shallower species. Yokoyama and Takenaka (2004) found the A292S change in three species of squirrelfishes habiting depths of less than 70 m, and it was also detected in the bottlenose dolphin, *Tursiops truncatus* (Fasick and Robinson 1998). Mutation experiments in the bovine rod pigment performed by Fasick and Robinson (1998) indicated that the change A292S alone is capable of shift the λ_{max} value by 10 nm towards the blue. Another site involved in shortwave shifts is the site 299 (Fasick and Robinson 1998; Hunt et al. 2001). Hunt et al. (2001) noted that

the combination 292S/299A in the silver roughy was related to a λ_{max} value of 479 nm, but a larger λ_{max} value of 485 nm was detected in *Anoplogaster cornuta*, which has the combination 292S/299S. In this study, I found that orange roughy has the same 292S/299A combination than silver roughy; instead, the spotty has 292S/299S. Considering the findings of Hunt et al. (2001), it is possible that the amino acids found at the critical sites 292 and 299 result in a shorter λ_{max} value of the RH1 pigments in the orange roughy in comparison with the spotty; supporting the hypothesis that orange roughy would present a set of amino acid changes that is not found in the shallower species, related to an adaptation to the bathypelagic light environment. Nonetheless, and as stated before, other un-detected critical amino acid sites may influence the λ_{max} value of the rhodopsin pigment in these species.

Interestingly, I found that all the epipelagic species and the epi-mesopelgic species blue cod, had the combination 292A/299S. The exceptions were the red gurnard (292A/299A), and, as stated, the spotty (292S/299S). The combination 292A/299S plus the site 83D was detected in the rhodopsin sequence of the sheep and the freshwater eel; with a λ_{max} value of 502 nm in the later species (see Table 2 in Fasick and Robinson 1998). Since the epipelagic species under study inhabit at minimum depth of 0 – 30 m (see Table 5.2), where green light (wavelength of ~ 500 nm) is available, it is likely that the combination 292A/299S detected here in most of the epipelagic species is related with an adaptation to longer wavelengths of light in comparison with the deeper species.

I found a weak relationship between the phylogeny of the partial rhodopsin sequences and the maximum depth of the species, giving a low support for the third hypothesis. The composition of the clades was not completely explained by depth. The species blue moki and blue cod formed a separated clade even though they occupy the same depths that the species in clades A and B, respectively. Similarly, butterfish, hapuku, and giant stargazer did not grouped with other species according to their depth range. It is possible that a better defined phylogeny would be achieved by using the complete rhodopsin sequence of the species included in this study. On the other hand, it is likely that not all the amino acid sites in the sequences under study are correlated with an adaptation to light conditions. Some sites must be neutral to selection and inherited by ancestry. Therefore, phylogenetic relationships of rhodopsin sequences would reflect selectively neutral and non-neutral changes. On the other hand, the pooling of the species according to the combination at the critical sites 292 and 299 showed a better, but still not complete, relationship with the maximum depth of the species.

To the best of my knowledge, this is the first study in determine partial rhodopsin sequences in all the species under study, except by the hoki (Prado et al., unpublished data, GenBank number: FR832604) and the silver roughy (Hope et al. 1997; Chen et al. 2003). Further studies about the molecular basis of rod pigments in these species should attempt to obtain a larger or complete rhodopsin sequence to identify if there are other replacements at critical sites that have been shown to be involved in spectral tuning in marine fishes (e.g. sites 83, 122, 124, 132). These kinds of studies will also allow to investigate if the combination at different critical sites can be completely related with depth distribution.

To better understand how these and potentially other critical amino acid replacements are involved in the visual adaptations of these species, it is also necessary to determine the λ_{max} value of their rod pigments.

5.6 Conclusion

In summary, I identified four critical amino acid changes in partial rhodopsin sequences of 19 teleost species that have been involved in the spectral tuning of rod pigments. Orange roughy presented the same amino acid combination in two sites already reported for silver roughy, which was not found in any of the other species. This likely reflects an adaptation to the dim-light available in the bathypelagic environment. I found that the phylogeny of the partial rhodopsin sequences of these species was weakly related to depth distribution.

Chapter 6

General Discussion

6.1 Genetic diversity in orange roughy

The DNA sequence and microsatellite DNA analyses presented in Chapters 2 and 3, respectively, showed a high genetic diversity in orange roughy as indicated by the values of the haplotype diversity of the COI and Cyt *b* sequences and by the expected heterozygosity of the microsatellite data. This pattern has been reported before for orange roughy using DNA sequences (Baker et al. 1995) and microsatellite loci (White et al. 2009a; Carlsson et al. 2011). High levels of genetic diversity for both types of molecular markers are common among marine fishes including deep-sea fishes (Stepien et al. 2000; Roques et al. 2002; von der Heyden et al. 2007; Nielsen et al. 2010; White et al. 2010; Friess and Sedberry 2011; White et al. 2011), and the most common explanation for this pattern has been related to large historical effective population sizes (McCusker and Bentzen 2010). As discussed in Chapter 3, these estimates probably do not reflect current population sizes and do not rule out the possibility the fisheries have reduced the population sizes of exploited marine fishes. This would be particularly difficult to detect with molecular markers in *K*-selected species, as orange roughy, because they have late maturity and long-life span and, therefore, only a few generations and overlapped generations may have elapsed since the beginning of commercial exploitation (Baker et al. 1995; Carlsson et al. 2011; Chapman et al. 2011).

6.2 Population structure in orange roughy

The levels of migration (gene flow) are often suggested to be higher in the marine realm than in terrestrial and freshwater habitats because of the lack of obvious barriers in the sea and the high potential for dispersal of marine species. Genetic panmixia has been found in marine species at a range of geographic scales. For example, in the gastropod *Crepipatella fecunda* (Guzmán et al. 2011), the crustacean *Panulirus argus* (Naro-

Maciel et al. 2011), the mammal *Cephalorhynchus heavisidii* (van Vuuren et al. 2002), the fish *Caffrogobius caffer* (Neethling et al. 2008), and also in deep-sea fish species (e.g. *Pseudopentaceros wheeleri*, Martin et al. 1992; *Beryx splendens*, Hoarau and Borsa 2000; *Antimora rostrata*, White et al. 2011) among others. However, there are barriers for dispersal in the oceans such as oceanographic discontinuities, bathymetric features, and behavioural characteristics (e.g. homing), that effectively limit the levels of gene flow among populations; even for marine fishes with high potential for passive (larval transport) and/or active (swimming adults) dispersal (e.g. *Serranus cabrilla*, Schunter et al. 2011; *Brosme brosme*, Knutsen et al. 2009; *Scomberomorus commerson*, Fauvelot and Borsa 2011). The life-history of the species plays a key role in determining the levels of differentiation among populations; however, simple assumptions as a direct correlation between pelagic larval duration (PLD) and levels of connectivity have been largely challenged. For example, Riginos et al. (2011) reviewed the effects of geography and life history traits on genetic differentiation in benthic marine fishes and found that PLD does not often explain genetic population structure. They found that transitions between biogeographic regions and egg type significantly affect genetic population structure in benthic marine fishes. It seems that there are no general rules regarding levels of connectivity in marine species and, on the contrary, this may be highly depend on the species being considered and on the type of molecular marker used. For example, using mtDNA markers, Sedberry et al. (1996) found genetic homogeneity among samples of the deep-sea wreckfish *Polyprion americanus* collected in Brazil, Australia and New Zealand. However, using microsatellite DNA markers, Ball et al. (2000) found two genetic groups among samples of *P. americanus*, one from Brazil and the other one in Australia and New Zealand.

In this thesis, using two types of neutral molecular markers, I studied the population genetic structure of orange roughy, one of the most heavily exploited deep-sea fishes in the world (Pitcher 2010). Using DNA sequence data (Chapter 2), I found that orange roughy exhibit high-historical levels of connectivity at a global scale. Low but significant differentiation was only detected between sites in the Northeast Atlantic Ocean and all the sites in the Southern hemisphere and between the Chatham Rise and Puysegur off New Zealand. On the other hand, at an ecological/more recent time scale given by the higher mutation rate of the microsatellite DNA markers (Chapter 3), low but significant levels of differentiation were also detected within the Southern hemisphere. The pattern of differentiation between the Northeast Atlantic and the

Southern hemisphere revealed by the DNA sequence analyses was supported by the highest values of differentiation between sites of the two hemispheres with the microsatellite DNA data. The clear genetic differentiation between New Zealand and the Northeast Atlantic Ocean samples found in this study differs from the findings of Smith (1986), who reported genetic homogeneity between samples of these two regions using allozyme loci. This is probably explained by the higher resolution power of mtDNA sequences and microsatellite DNA loci compared to allozymes. On the other hand, using microsatellite DNA loci, Oke et al. (2002) reported a lack of differentiation between New Zealand/Australia and the Northeast Atlantic Ocean samples, which also differs from the results of the present study. This discrepancy may be due to the fact that of the six loci used by Oke et al. (2002) three had low heterozygosities and were not used in this study. Instead, all the nine loci used here had high heterozygosities, likely resulting in a higher resolution power of the present data. Regarding the prevalent lack of differentiation between New Zealand and Australia reported here, similar results have been found with allozymes (Elliott and Ward 1992), but disagree with the results of restriction enzyme analysis of mtDNA (Smolenski et al. 1993) and with parasite analyses (Lester et al. 1988). At a fine-scale around New Zealand, a panmictic pattern was revealed with both mtDNA sequences and microsatellite DNA loci. Significant differentiation was detected in only a few comparisons mostly involving the Chatham Rise and/or Puysegur. In contrast, previous studies using RFLPs, allozymes, otolith shape, fish size, and restriction digest of the whole mitochondrial genome have reported significant genetic structure around New Zealand (Smith et al. 1996; Smith and Benson 1997; Smith et al. 2002a). However, mitochondrial DNA polymorphism of the control region and two microsatellite loci revealed lack of differentiation among New Zealand sites (Smith et al. 2002a). As stated before, contrasting results at a fine-scale have been also reported within the Northeast Atlantic Ocean using microsatellite DNA loci (White et al. 2009a; Carlsson et al. 2011). The marked contradictions among population differentiation studies in orange roughy are most likely explained by different signals revealed by the different molecular markers/techniques that have been used. Moreover, each study has used a particular set of sampling sites and they have also differed in the number of individuals sampled in each location. On the other hand, techniques that are highly influenced by recent environmental conditions such as parasite, otolith and morphometrics analyses have showed population differentiation in orange roughy at regional and fine-scales (e.g. Lester et al. 1988; Edmonds et al. 1991; Elliott et al.

1995). However, the genome is not immediately affected by environmental conditions, since a number of generations are necessary to accumulate genetic differences that can be detected with molecular markers. The main advantage of the present study in comparison with previous studies is the high geographical coverage of the sampling squeme and the use of two neutral molecular markers, which revealed signals at different time scales. While the present data is not conclusive about levels of genetic differentiation at a fine-scale (i.e. around New Zealand) and across the Tasman Sea (between New Zealand and Australia), the main conclusion of this study is that orange roughy exhibits a significant genetic structure at a global scale.

The lack of differentiation (mtDNA) and low differentiation (microsatellite DNA) found across the Southern hemisphere (i.e. New Zealand, Australia, Chile, and Namibia) is likely the result of active adult dispersal under a stepping-stone model combined with long-life span and multiple spawning events (White et al. 2009a). However, the clear differentiation of the Northeast Atlantic from the Southern hemisphere and the consistent differentiation between the Chatham Rise and Puysegur off New Zealand with both markers, suggest that there might be oceanographic conditions that limit the dispersal of orange roughy at large and short spatial scales. As discussed in Chapter 2, an abrupt water-mass boundary at 41ºN may be related with the differentiation of the Northeast Atlantic sites from all the other sites. As suggested by Smith et al. (1996), the differentiation of orange roughy samples from the Chatham Rise and Puysegur may be related with a major hydrological barrier between these sites. The Subtropical Convergence zone along the Chatham Rise marks the transition between the Subtropical Water (STW) and the Subantarctic Water (SAW). The STW has its origin in the central Pacific Ocean and is characterized by high temperature and salinity. In contrast, the SAW is cooler and less saline (Heath 1985). Another main water mass around New Zealand is called Antarctic Intermediate Water (AAIW) which sinks at the Convergence zone and travel northwards. The salinity minimum of the AAIW occurs at 700 m south of the Convergence zone and 1,000 m north of the Convergence (Heath 1985). There are pronounced changes in temperature at the surface and mid-depth waters at the Convergence zone, but the influence of these water masses over the temperature at depths inhabited by orange roughy is less documented (Smith et al. 1996). However, differences in temperature have been registered at 500 m depth (see Hadfield et al. 2007), showing a temperature of 8ºC at the Convergence zone, 9-10ºC north of the Convergence, and 6-7ºC to the south. Thus, it is possible that different

water-masses limit the dispersal of orange roughy between the Chatham Rise and Puysegur, and may also explain the low but significant differentiation between few sites in Northern New Zealand and Chatham Rise and/or Puysegur.

Regarding the three types of genetic structure defined by Laikre et al. (2005) that were highlighted in the general introduction of this thesis, the present study suggests that a global scale orange roughy is within category 2: genetic composition change over space (IBD pattern). At a finer scale (around New Zealand), orange roughy may be better represented by category 3: there is extensive gene flow. However, it is likely that oceanographic discontinuities and migratory behaviour (probably not all individuals join the annual migrations to spawning sites, Bell et al. 1992; Zeldis et al. 1997), limits the dispersal of orange roughy preventing the formation of a single panmictic unit.

6.3 Implications for fisheries management in New Zealand

Similar to other deep-sea fisheries, orange roughy fisheries have shown the typical pattern of "boom-and-bust" (reviewed by Pitcher et al. 2010), and most fisheries have been closed or quotas largely reduced (see Clark 2009; Niklitscheck et al. 2010; Foley et al. 2011). However, large-scale fisheries still operate off New Zealand (Clark 2009; New Zealand Ministry of Fisheries 2011) and an effective management is needed to prevent overfishing of stocks. Determining levels of connectivity among populations or stocks (stock structure) of exploited species is crucial for developing sustainable management and conservation plans (Begg et al. 1999; Grant and Waples 2000; Laikre et al. 2005). It has been suggested that the better approach to determine the stock structure of marine resources is to follow and holistic approach (Begg and Waldman 1999; Cadrin et al. 2005). The genetic data generated by this study should be considered in assessments studies as part of a multidisciplinary approach to determine the levels of connectivity among stocks in this species. The main results to consider are: 1) the high levels of genetic diversity in orange roughy found with both molecular markers indicate large past population sizes and do not reflect current population sizes that could have been impacted by fisheries activities; 2) there may be some levels of interchange by adult dispersal between New Zealand and Australian populations; however, levels of differentiation were detected in few comparisons; 3) significant differentiation was

detected between the Chatham Rise and Puysegur with both markers and between few Northern sites and Chatham Rise and/or Puysegur with the microsatellite DNA marker. Smith et al. (1996) and Smith et al. (1997) also found differentiation between the Chatham Rise and Puysegur. Considering all these studies, there is support from genetic data for the current management of these sites as two different stocks; and 4) temporal variation in orange roughy populations cannot be ruled out by the present data due to the high variability of the loci coupled with a low sample size for the temporal analyses and, therefore, this should be assessed in the future (about temporal variation see Smith and Benson 1997; Smolenski et al. 1993).

6.4 Demographic history of orange roughy

Different combinations of small and large levels of haplotype and nucleotide diversities have been used to define groups of species in relation to their demographic history (Grant and Bowen 1998). As reviewed by Grant and Waples (2000) (see Fig. 6.1 reconstructed following Grant and Waples 2000), most marine fishes are within category 2; these species have rapidly recovered from severe population reductions. Some species have not yet recovered from bottleneck events and are within category 1. Others, those in the category 3, have had large and stable population sizes for long periods of time. None of the marine species considered by Grant and Waples (2000) or by some other studies (see legend in Fig 6.1) are in category 4, which is most common in inshore or freshwater species (Grant and Waples 2000).

In figure 6.1, orange roughy appears in the category 1 and 2 considering the values reported by Smolenski et al. (1993) using restriction enzyme analysis of mtDNA and by Baker et al. (1995) using Cyt *b* sequences, respectively (dot numbers 48 and 49 in Fig. 6.1). The demographic analyses conducted in Chapter 2 with COI sequences support that orange roughy is within the category 2 (dot number 51 in Fig. 6.1): there is a pattern of high haplotype diversity and low nucleotide diversity that can be related with a drastic reduction in population size in the past followed by sudden expansion. The analyses presented in Chapter 2 inferred two expansion events during the Pleistocene period, likely related with glaciations events. Taking into account the values of haplotype and nucleotide diversities for the Cyt *b* sequences shown in Chapter 2, orange

roughy falls into category 3 (dot number 50 in Fig. 6.1). The discrepancy between the COI and Cyt *b* signals may be because the Cyt *b* is generally more variable than the COI gene. However, using Cyt *b* sequences Baker et al. (1995) also found that orange roughy is within category 2. On the other hand, the average nucleotide diversity in the Cyt *b* sequences was 0.86%, which is very close to the limit of 0.8% between low and high nucleotide diversity (see Fig. 6.1) as defined by Grant and Bowen (1998). Furthermore, the demographic analyses conducted with the COI sequences (mismatch distribution, Bayesian skyline analyses and the start-like shape of the haplotype genealogy) indicated the occurrence of expansion events. Thus, it is more likely that orange roughy is within category 2 defined by Grant and Bowen (1998).

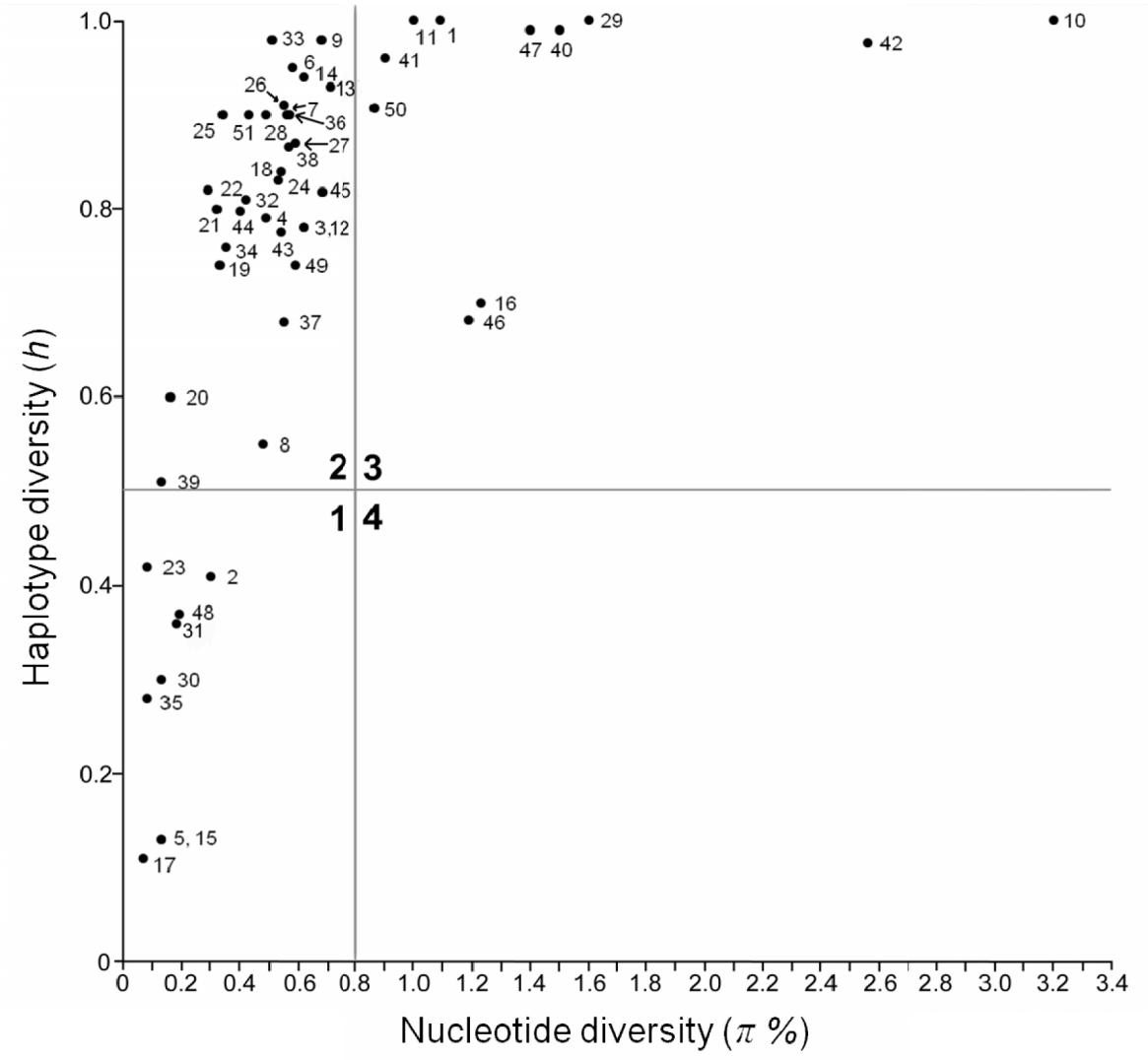


Fig. 6.1 Relationship between haplotype diversity (h) and nucleotide diversity (π) for marine fishes. Reconstructed following Grant and Waples (2000). Values are from Grant and Bowen (1998) unless stated. Numbers represent species. 1-22: shallow-sea species (i.e. max. depth ≤ 200 m), 23-51: deep-sea species (i.e. max. depth > 200 m). 1, redlip blenny; 2, beaugregory damselfish; 3, slippery dick; 4, sergeant major; 5, weakfish; 6, red drum (Gulf of Mexico); 7, red drum (Atlantic); 8, bluehead; 9, goldspot goby; 10, menhaden (Atlantic); 11, menhaden (Gulf of Mexico); 12, french grunt; 13, stickleback; 14, longjaw squirrelfish; 15, red snapper; 16, bluefish (Atlantic); 17, bluefish (Australia); 18, blue marlin (Atlantic-Indo-Pacific); 19, blue marlin (Atlantic); 20, blue marlin (Pacific); 21, sailfish; 22, white/striped marlin; 23, red grouper; 24, spanish sardine; 25, greater amberjack; 26, atlantic herring; 27, haddock; 28, pacific herring; 29, broadbanded thornyhead (Stepien et al. 2000); 30, atlantic cod (N Atlantic); 31, atlantic cod (NW Atlantic); 32, capelin (NW Atlantic); 33, capelin (NE Atlantic); 34, shortfin mako; 35, hoki; 36, cape hake; 37, deepwater hake; 38, cape hake (von der Heyden et al. 2007); 39, deepwater hake (von der Heyden et al. 2007); 40, shortspine thornyhead (Stepien et al. 2000); 41, longspine thornyhead (Stepien et al. 2000); 42, bluemouth (control region, Aboim et al. 2005); 43, bluemouth (cyt *b*, Aboim et al. 2005); 44, alfonsino (Friess and Sedberry 2011); 45, alaskan pacific halibut (Nielsen et al. 2010); 46, spikey oreo (Elliott et al. 1998); 47, blackfin flounder (Xiao et al. 2010); 48, orange roughy (Smolenski et al. 1993); 49, orange roughy (Baker et al. 1995); 50, orange roughy (cyt *b*, present study, Chapter 2); 51, orange roughy (COI, present study, Chapter 2)

6.5 Genetic divergence and phylogenetic relationships of *Hoplostethus* spp.

The analyses conducted using COI sequences of six *Hoplostethus* species in Chapter 4, showed the typical pattern found in the barcode DNA region, there is a marked difference between intra and inter-species divergence (Hebert et al. 2003).

The analyses of the nucleotide data clearly showed that *H. atlanticus* is a separate taxon from the clade formed by *H. latus, H. crassispinus, H. japonicus,* and *H. mediterraneus.* The phylogenetic relationship between *H. atlanticus* and *H. gigas* was not resolved at the nucleotide level. However, the amino acid data differentiated *H. atlanticus* from all the other species including *H. gigas*. This was evident by the presence of three non-synonymous substitutions in *H. atlanticus.* Even more, two of these substitutions were among different physicochemical amino acids. These results are correlated with morphological differences. *Hoplostethus atlanticus* have different morphological characteristics respect to *H. gigas, H. crassispinus H. japonicus,* and *H. mediterraneus* Kotlyar (1986).

Considering the species used here, the analyses indicated that the genus *Hoplostethus* is monophyletic and derived (Maximum Likelihood and Bayesian analyses) respect to the genera *Aulotrachichthys*, *Gephyroberyx* and *Paratrachichthys* within the family Trachichthyidae. Further studies including all the *Hoplostethus* species and representatives of all the genera within Trachichthyidae would be needed to propose a complete molecular phylogeny for the genus *Hoplostethus,* and for clearly place the phylogenetic position of this genus within the family.

6.6 Visual adaptations of orange roughy to the bathypelagic light environment

It is well known that certain amino acid replacements (critical sites) in the opsin protein of vertebrates' visual pigments shift the maximal absorbance (λ_{max}) of the pigments to adapt to different light environments (reviewed by Yokoyama 2008). The diversity of marine fish species inhabiting a wide range of depths and photic conditions (e.g. turbidity, color of the water and brightness of the downwelling light) provide an ideal model to study the evolution of visual systems (Bowmaker 1995; Yokoyama 2000). The visual pigments of many fish species are shifted towards the blue light when compared with terrestrial species (e.g. the bovine *Bos taurus*) (reviewed by Yokoyama 2008).

In Chapter 5, using partial rhodopsin sequences of 19 marine fish species inhabiting different depths (1 – 1,800 m), I identified 60 variable amino acid sites; four of them are known to be involved in spectral tuning in marine fish species: P194R, H195A, A292S and A299S (Hunt et al. 2001; Yokoyama and Takenaka 2004; Yokoyama 2008). The comparisons of the amino acid sequences of the species under study, revealed that orange roughy have a particular set of amino acids at two critical sites (292S/299A), which has been previously reported for silver roughy and was related with a λ_{max} value of 479 nm (Hunt et al. 2001). This combination was not found in any of the other species and, therefore, may represent an adaptation to the light available in bathypelagic environments (i.e. blue bioluminescence).

The study of rhodopsin gene sequences conducted here included very divergent species (belonging to16 families). The species used were available since they are common in New Zealand (although the samples of *Trachurus murphyi* were obtained from Chile). The diversity of species used allowed the identification of potential sites for spectral tuning for species covering a wide range of depth distribution, and thus, different light environments. Nonetheless, the use of rhodopsin gene sequences to identify critical amino acid sites has an enormous potential to study intra-specific adaptive evolution in many and diverse organisms (Yokoyama 2000). For example, a recent study reported a correlation between critical sites in the rhodopsin gene and the photic environments of different populations of the sand goby, *Pomatoschistus minutus* (Larmuseau et al. 2009b). Similarly, the influence of natural selection in visual systems has been related with diversification of marine fishes flocks in sand goby and seven-spined gobies species (Teleostei: Gobiidae) (Larmuseau et al. 2010; Larmuseau et al. 2011). Future studies aiming to study functional variation in the rhodopsin gene at intra and inter-species levels would be highly valuable to continue the research about the influence of natural selection (operating over visual systems) in the evolution and diversification of fishes. These kinds of studies are part of three steps needed to determine the molecular basis of adaptive changes in visual systems. The two other steps involve cloning and characterization of the retinal opsin genes and determination of the actual effects of critical amino acid sites on shifts in the λ_{max} of visual pigments using *in vitro* assay (Yokoyama 2000).

6.7 Concluding remarks and future research

This thesis covered several different aspects of genetic research on the deep-sea fish orange roughy. The main goal was to add knowledge into the already extensive research field of population connectivity in this heavily exploited fish. This is the first study to use large sample sizes, two types of neutral molecular markers and samples from New Zealand, Australia, Namibia, Chile, and the Northeast Atlantic Ocean; including multiple locations around New Zealand within the EEZ and in the high seas. Future studies should also include markers under selection to evaluate if adaptive variation plays a role in the levels of connectivity among orange roughy populations. The rapid advance in molecular technology (i.e. genome-wide studies) combined with continuos reductions in costs could facilitate the research into adaptive vs. neutral divergence in non-model species and will likely influence fisheries sciences (Hauser and Seeb 2008).

I also aimed to study the intra/inter genetic divergence and phylogenetic relationships of orange roughy respect to other congeners using a portion of the DNA barcode region. The lack of access to samples of other congeners limited the proposition of a molecular phylogeny for the genus *Hoplostethus*, and, therefore, constitutes a future area of research.

A third topic of study was about the molecular basis for visual adaptations of orange roughy to the bathypelagic light environment. The inclusion of other fish species inhabiting different depths allowed to gain insights into a particular feature not found in any of the other species, except by the congener silver roughy, which also occur in bathypelagic environments. Further studies about this aspect should aim to obtain the complete rhodopsin gene sequence in orange roughy to identify other potential tuning sites.

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Appendix A

COI haplotype frequencies in each sampling site. GenBank Accession Numbers are indicated for each haplotype

Accession number	ORH1	Rit	Chat	Puy	Cha	LHR	Lou	NTas	Alb	Nam	JF	Por	Sed
JN580075	0	0	0	0	0	1	0	0	2	0	0	0	0
JN580076	9	8	10	12	7	8	7	8	8	12	16	7	0
JN580077	7	7	4	10	5	4	6	8	9	10	13	18	12
JN580078	1	2	7	4	1	5	5	0	7	5	3	2	12
JN580079	0	0	0	0	0	0	0	0	1	3	0	0	0
JN580080	0	1	0	0	1	0	0	0	1	0	0	1	2
JN580081	0	0	1	0	0	0	0	0	2	0	0	0	0
JN580082	2	0	0	0	1	0	1	0	1	1	1	0	0
JN580083	0	0	0	0	0	0	0	0	1	0	0	0	0
JN580084	1	0	3	0	2	1	0	0	1	0	0	2	0
JN580085	0	0	0	0	0	0	0	0	1	0	0	0	0
JN580086	0	1	2	0	1	2	4	0	1	2	0	0	0
JN580087	0	0	1	0	1	1	0	0	1	0	0	0	0
JN580088	0	0	0	0	0	0	0	0	1	0	0	0	0
JN580089	2	2	2	4	0	2	1	2	1	1	0	0	0
JN580090	0	1	0	0	0	0	0	0	1	0	0	0	0
JN580091	0	0	0	0	0	0	0	0	1	0	3	0	0
JN580092	0	0	0	0	0	0	0	0	1	0	2	0	0
JN580093	2	1	1	1	1	0	0	0	1	0	0	1	1
JN580094	0	0	0	1	0	2	0	0	1	0	0	0	0
JN580095	0	0	0	0	0	0	1	0	1	0	0	0	0
JN580096	0	0	1	0	0	1	0	0	1	0	0	0	0
JN580097	1	0	0	1	0	1	0	0	1	0	0	0	0
JN580098	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580099	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580100	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580101	0	1	1	1	1	1	0	0	0	0	0	0	0
JN580102	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580103	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580104	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580105	1	0	1	0	0	1	0	1	0	0	0	0	0
JN580106	0	0	1	0	0	0	0	0	0	1	0	0	0
JN580107	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580108	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580109	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580110	0	1	1	2	0	1	0	0	0	0	0	0	0
JN580111	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580112	0	0	1	0	0	0	0	0	0	0	0	0	0
JN580113	0	0	1	0	0	1	0	0	0	0	0	0	0
JN580114	0	0	0	0	1	0	0	0	0	0	0	0	0

Appendix A continued

Accession number	ORH1	Rit	Chat	Puy	Cha	LHR	Lou	NTas	Alb	Nam	JF	Por	Sed
JN580115	0	1	0	1	1	0	0	0	0	2	0	0	0
JN580116	0	0	0	0	1	0	0	0	0	0	0	0	0
JN580117	0	0	0	0	1	0	0	0	0	0	0	0	0
JN580118	0	0	0	0	1	0	0	0	0	0	0	0	0
JN580119	1	2	0	0	1	1	1	0	0	0	0	7	2
JN580120	0	0	0	0	1	0	0	0	0	0	0	0	0
JN580121	0	0	0	0	1	0	0	0	0	0	0	0	0
JN580122	0	0	0	0	1	0	0	0	0	0	0	0	0
JN580123	0	0	0	0	0	0	0	0	0	1	2	0	0
JN580124	0	0	0	0	0	0	0	0	0	0	1	0	0
JN580125	0	2	0	0	0	1	0	0	0	0	1	0	0
JN580126	0	0	0	0	0	0	1	0	0	0	3	0	0
JN580127	0	0	0	0	0	0	0	1	0	0	3	0	0
JN580128	0	0	0	0	0	0	0	0	0	0	1	0	0
JN580129	0	0	0	0	0	0	0	0	0	0	2	0	0
JN580130	0	0	0	0	0	0	1	0	0	0	1	0	0
JN580131	2	1	0	0	0	0	0	1	0	0	0	0	0
JN580132	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580133	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580134	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580135	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580136	0	0	0	0	0	0	0	0	0	0	0	2	2
JN580137	0	0	0	0	0	0	0	0	0	0	0	2	2
JN580138	0	0	0	0	0	0	0	0	0	0	0	6	2
JN580139	0	0	0	0	0	0	0	0	0	0	0	2	3
JN580140	1	0	0	0	0	0	1	1	0	0	0	0	0
JN580141	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580142	0	0	0	0	0	1	0	0	0	0	0	0	0
JN580143	1	0	0	1	0	1	1	0	0	0	0	0	0
JN580144	0	0	0	0	0	1	0	0	0	0	0	0	0
JN580145	0	0	0	0	0	1	0	0	0	0	0	0	0
JN580146	0	0	0	0	0	1	0	0	0	0	0	0	0
JN580147	0	0	0	0	0	0	1	0	0	0	0	0	0
JN580148	0	0	0	0	0	0	1	0	0	0	0	0	0
JN580149	0	0	0	0	0	0	1	0	0	0	0	0	0
JN580150	0	0	0	0	0	0	1	0	0	0	0	0	0
JN580151	0	0	0	0	0	0	1	0	0	0	0	0	0
JN580152	0	0	0	0	0	0	1	0	0	0	0	0	0
JN580153	0	0	0	0	0	0	1	0	0	0	0	0	0
JN580154	0	2	0	1	0	0	1	0	0	0	0	0	0

Appendix A continued

Accession number	ORH1	Rit	Chat	Puy	Cha	LHR	Lou	NTas	Alb	Nam	JF	Por	Sed
JN580155	0	0	0	0	0	0	1	0	0	0	0	0	0
JN580156	1	0	0	0	0	0	1	0	0	1	0	0	0
JN580157	0	0	0	0	0	0	0	0	0	0	0	0	1
JN580158	0	0	0	0	0	0	0	0	0	1	0	0	0
JN580159	0	0	0	0	0	0	0	0	0	2	0	0	0
JN580160	0	0	0	0	0	0	0	0	0	1	0	0	0
JN580161	0	0	0	0	0	0	0	0	0	1	0	0	0
JN580162	0	0	0	0	0	0	0	0	0	1	0	0	0
JN580163	0	0	0	0	0	0	0	1	0	0	0	0	0
JN580164	0	0	0	0	0	0	0	1	0	0	0	0	0
JN580165	0	0	0	0	0	0	0	1	0	0	0	0	0
JN580166	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580167	0	0	0	0	0	0	0	0	0	0	0	1	0
JN580168	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580169	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580170	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580171	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580172	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580173	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580174	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580175	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580176	0	0	0	1	0	0	0	0	0	0	0	0	0
JN580177	0	1	0	0	0	0	0	0	0	0	0	0	0
JN580178	0	1	0	0	0	0	0	0	0	0	0	0	0
JN580179	0	1	0	0	0	0	0	0	0	0	0	0	0
JN580180	0	1	0	0	0	0	0	0	0	0	0	0	0
JN580181	0	1	0	0	0	0	0	0	0	0	0	0	0
JN580182	0	1	0	0	0	0	0	0	0	0	0	0	0
JN580183	0	1	0	0	0	0	0	0	0	0	0	0	0
JN580184	1	0	0	0	0	0	0	0	0	0	0	0	0
JN580185	0	0	0	0	0	0	0	0	0	0	0	0	1
JN580186	0	0	0	0	0	0	0	0	0	0	0	0	1
JN580187	0	0	0	0	0	0	0	0	0	0	0	0	1
JN580188	0	0	0	0	0	1	0	0	0	0	0	0	0

Appendix B

Cyt *b* haplotype frequencies in each area. GenBank Accession Numbers are indicated for each haplotype

Accession number	New Zealand	Australia	Namibia	Chile	NE Atlantic
JN580189	0	2	0	0	0
JN580190	0	1	0	0	0
JN580191	1	3	5	2	8
JN580192	0	2	0	2	0
JN580193	8	2	5	4	2
JN580194	0	1	1	1	0
JN580195	0	1	0	0	0
JN580196	0	1	0	0	0
JN580197	1	0	0	0	0
JN580198	3	0	1	1	0
JN580199	1	0	0	0	0
JN580200	0	0	0	1	0
JN580201	0	0	0	3	0
JN580202	0	0	0	1	0
JN580203	0	0	0	1	0
JN580204	0	1	0	1	0
JN580205	0	0	0	1	0
JN580206	0	0	1	1	0
JN580207	0	0	0	0	2
JN580208	1	0	0	0	3
JN580209	0	0	0	0	1
JN580210	1	0	0	0	0
JN580211	0	0	2	0	0
JN580212	0	0	1	0	0
JN580213	0	0	1	0	0
JN580214	0	0	1	0	0
JN580215	0	1	0	0	0
JN580216	0	1	0	0	0
JN580217	0	1	0	0	0
JN580218	0	1	0	0	0
JN580219	0	1	0	0	0
JN580220	0	0	0	0	2
JN580221	1	0	0	0	0
JN580222	0	0	0	0	1
JN580223	0	0	0	0	1
JN580224	1	0	0	0	0
JN580225	1	0	0	0	0

Appendix C

Genotypes of each individual in GENEPOP format for the spatial analyses. Sampling sites codes as in Table 3.1.

Orange roughy
Hat2a
HopAt2
Hat3
Hat9a
Hat7
Hat45
HopAt4
HopAt5
HopAt11
Pop
Kai1 , 187207 183201 130172 140156 000000 000000 000000 000000 000000
Kai2 , 187203 193209 130152 142152 208210 144144 160160 160192 146148
Kai3 , 207207 201225 130152 142144 238238 136144 158160 160160 144146
Kai4 , 187203 203217 150168 150158 222238 142144 156160 160192 144148
Kai5 , 191211 189201 130130 150154 210262 142144 154162 160160 146146
Kai6 , 195203 211211 130142 146148 238268 144144 154156 184188 132140
Kai7 , 000000 197211 130142 144152 238248 134144 000000 178192 000000
Kai8 , 191207 203207 000000 000000 222242 144146 154160 160184 140144
Kai9 , 191203 197211 130132 140150 210242 144144 154154 188198 000000
Kai10 , 187195 189215 130142 142154 200210 142146 154172 188196 140146
Kai11 , 195223 189207 160182 142142 228228 142144 156158 180186 148156
Kai12 , 207207 189209 164168 142154 236238 144144 154184 184192 138144
Kai13 , 207211 207231 130154 144152 236238 146152 152160 160186 140144
Kai14 , 187191 201205 168178 146150 228254 144146 156164 160188 136144
Kai15 , 199211 197201 146194 142144 228246 144146 156170 160192 138142
Kai16 , 187187 191227 158164 146154 232262 146152 148168 168192 134146
Kai17 , 187191 207209 164172 140154 210258 144144 152158 160160 000000
Kai18 , 191207 197203 142154 142146 238238 144144 154156 180188 000000
Kai19 , 207211 195197 148164 150152 210210 144146 156182 160188 000000
Kai20 , 191191 193203 168168 144150 238248 126144 158182 160188 132146
Kai21 , 191199 185207 132142 152154 236238 144144 160162 160188 136140
Kai22 , 187195 193201 130160 148154 226240 134136 156162 160194 134146
Kai23 , 199199 175221 160166 146152 232244 000000 000000 160160 144144
Kai24 , 199199 193197 142166 152154 246246 144144 152164 160188 134138
Kai25 , 191191 209225 000000 000000 242282 140144 164168 186192 144152
Kai26 , 000000 205205 140170 142144 226236 134146 154154 160188 138140
Kai27 , 199199 000000 130148 142146 220250 144144 156188 182184 144146
Kai28 , 199203 199203 132148 138148 234246 146146 156164 160188 146150
Kai29 , 191195 189197 130164 150162 234242 140144 154158 000000 136144
Kai30 , 195203 205215 130132 144150 210220 144146 160184 160184 134146
Kai31 , 191203 193211 130142 152154 208238 144144 158164 160160 144146
Kai32 , 199227 197199 142166 152154 236260 144144 158158 190190 140150
Kai33 , 191227 199203 130160 144144 210234 144144 158158 160180 134138
Kai34 , 191191 193211 142152 132140 210228 134150 156178 160180 138146
Kai35 , 191211 195219 142152 144150 246290 146146 154180 188192 136150
Kai36 , 191227 000000 142164 140144 210228 144144 148160 160188 132144

Kai37 , 195199 195197 130148 142154 240262 144146 148156 160188 128146
Pop
Tau1 , 191207 177193 164180 144152 210210 144146 156160 160188 146160
Tau2 , 187187 189193 132182 152152 210274 000000 152152 160160 134160
Tau3 , 187187 185201 148162 148152 212260 000000 158160 188192 140152
Tau4 , 187199 181211 164172 144150 210226 142144 154158 190196 142142
Tau5 , 199207 201211 130130 142152 224256 144144 154156 180192 140144
Tau6 , 199207 000000 158166 144148 212224 144144 154158 160192 146146
Tau7 , 199207 199199 130168 140142 210238 144144 156164 160180 138144
Tau8 , 195207 189189 130166 144148 226238 144146 156160 180180 146146
Tau9 , 187203 205205 130156 126150 000000 144146 160160 160196 000000
Tau10 , 187191 193197 156160 144154 210234 144144 152180 160192 000000
Tau11 , 187191 201213 130160 146158 236268 144144 154158 160180 144144
Tau12 , 187219 201201 138164 142152 218236 134146 156160 184198 140140
Tau13 , 195195 187205 142176 142152 208274 144146 154160 160192 146146
Tau14 , 187207 193195 130130 142148 236244 000000 000000 000000 140146
Tau15 , 195227 191239 130146 142148 234236 142144 154162 180184 134148
Tau16 , 187203 199211 174188 144158 228234 144150 158158 188190 000000
Tau17 , 187219 181201 130142 144148 210236 142144 162182 160192 000000
Tau18 , 191191 183209 130140 134142 000000 144144 164172 192198 144144
Tau19 , 191199 189213 140148 142154 236236 134140 156160 160190 134142
Tau20 , 187195 201213 130154 144148 228228 144144 148162 160160 150150
Tau21 , 187187 211223 164168 150154 228244 140152 158160 160160 142150
Tau22 , 207207 199205 130130 154154 210242 144146 162188 160160 134150
Tau23 , 187195 195199 158182 140152 230246 134144 154156 160188 144152
Tau24 , 000000 193199 130172 134158 234238 144144 156164 184188 000000
Pop
Exp1 , 195199 187193 162178 142154 226232 144144 148176 180180 138140
Exp2 , 199211 197201 130136 142160 200244 144146 156160 160192 138140
Exp3 , 187191 199211 168172 152162 226238 144156 156182 186188 146160
Exp4 , 195199 181201 130130 144150 240244 142142 160164 160160 134138
Exp5 , 191199 201203 130130 142152 248254 152158 154154 160160 128140
Exp6 , 191199 175187 142148 140150 234258 144144 158160 160160 140140
Exp7 , 191207 195195 130154 154154 226250 144154 148154 160188 000000
Exp8 , 187191 187205 130176 146156 000000 142146 154162 160188 144146
Exp9 , 191227 203215 130174 142146 236254 144144 158158 160192 136146
Exp10 , 187191 189201 164170 148148 234248 134144 154158 160188 140154
Exp11 , 191211 187197 130160 148156 222266 144154 152158 180188 128144
Exp12 , 187191 185203 160162 142144 234240 144144 000000 160184 146150
Exp13 , 000000 201211 158168 132148 238238 144144 154158 160190 146154
Exp14 , 187191 197203 162164 140142 210226 144144 148160 188192 138140
Exp15 , 199203 197201 142158 000000 220240 134134 158158 160184 140144
Exp16 , 187187 185211 142142 132150 234240 144146 000000 000000 144144
Exp17 , 207211 000000 168176 140154 250262 144144 000000 192192 138144
Exp18 , 187187 199205 138174 136142 228252 144152 148156 160198 144144
Exp19 , 187207 000000 000000 150154 000000 134142 156158 160192 138140
Exp20 , 199207 000000 148174 146156 000000 140144 156156 196196 138170
Exp21 , 187191 195199 134142 156170 222230 146152 146156 188192 154154
Exp22 , 195195 195205 130142 138142 212238 144144 000000 160178 130144
Exp23 , 187207 189209 130168 146156 240240 144146 156184 160160 148156
Exp24 , 191203 205207 158176 142150 234234 144144 156160 160160 152158
Pop
Ton1 , 191207 187197 152174 140168 210244 144154 160168 160160 146148
Ton2 , 187199 189215 130160 140152 226234 142144 000000 160160 136146
Ton3 , 207223 195205 138148 144144 210226 134140 154156 160192 144152

Ton4 , 191195 207207 130162 144154 206254 144152 156160 160192 136146
Ton5 , 187195 197205 144148 150156 218230 134140 154158 160188 146148
Ton6 , 191219 195195 148158 144154 212214 144146 160164 160192 144146
Ton7 , 187191 195213 148166 146148 222234 144146 164184 000000 140148
Ton8 , 000000 177207 142164 142152 226250 144146 148154 178180 132144
Ton9 , 191211 203209 138148 142144 224260 134144 148154 186200 138144
Ton10 , 187211 193195 000000 000000 000000 144146 000000 000000 152166
Ton11 , 000000 193197 130144 150154 214228 144152 148152 160188 144146
Ton12 , 187207 183211 138152 138148 230238 152152 152156 184192 144146
Ton13 , 191191 199217 130172 148148 226238 146152 152160 160188 146146
Ton14 , 203207 199207 130130 146150 228232 144146 000000 176192 138146
Ton15 , 187191 183193 148164 142148 000000 134144 154154 182192 136146
Ton16 , 207223 187203 130172 142144 210212 144156 000000 160192 146146
Ton17 , 199199 000000 000000 000000 210226 144150 170182 180188 140140
Ton18 , 187207 000000 130168 136148 226228 144146 154160 160192 144152
Ton19 , 187223 193199 130158 142150 228230 144146 158158 160180 140144
Ton20 , 199207 195207 130168 138142 234242 144144 156160 160160 144144
Ton21 , 191199 199211 162172 144152 000000 134150 154154 188192 140146
Ton22 , 187191 185215 130152 142152 228246 144144 158158 160188 144146
Ton23 , 191199 197205 130142 136146 234234 134146 154154 160160 144150
Ton24 , 191199 179201 130168 148150 236242 144144 154164 160192 138148
Pop
Yas1 , 191199 193223 130142 140144 236246 138146 166182 160160 140140
Yas2 , 187187 195197 152152 142150 222246 144146 156164 160192 146146
Yas3 , 187203 175213 130170 142148 200200 142144 154190 160198 144150
Yas4 , 191199 000000 130168 138152 246276 144150 148152 192192 140144
Yas5 , 199207 191201 142168 144150 218228 144144 158158 160160 000000
Yas6 , 187187 000000 130148 148158 242252 142144 152156 180188 132144
Yas7 , 195199 199201 130162 144150 228238 134144 156188 160192 144146
Yas8 , 191195 201205 130170 144148 236236 140144 152160 160180 128146
Yas9 , 191199 197211 130152 144150 238258 144158 160160 160160 148148
Yas10 , 000000 209211 158182 140146 210218 144144 148158 160192 146146
Yas11 , 187211 197201 130170 150156 210210 134144 152154 160190 138146
Yas12 , 187199 197207 130162 138154 246264 134144 164174 178194 140150
Yas13 , 199207 195203 148158 142148 238254 144144 148148 188192 138138
Yas14 , 187207 000000 164168 136148 224230 144144 160160 160160 128144
Yas15 , 191207 177203 146168 142142 210234 144144 152170 160188 144144
Yas16 , 187195 197215 158200 152156 232236 144146 156160 160188 132132
Yas17 , 191199 183195 142190 146152 208210 144152 152152 180196 136138
Yas18 , 187207 207209 130166 140142 224224 144146 156156 184192 140144
Yas19 , 191207 209229 130158 148158 210210 144150 156170 180180 140146
Yas20 , 187211 195195 160180 154158 210236 140144 160162 188192 130144
Yas21 , 191191 187201 130190 144154 212234 144152 158158 160192 144146
Yas22 , 191207 187205 148186 142142 238238 144144 152164 160188 134144
Yas23 , 187191 000000 148168 144150 210242 146146 156160 160186 128150
Yas24 , 187211 191195 152172 134150 226236 146146 158164 160184 138146
Pop
Bir1 , 191199 195209 142182 152154 242250 144146 154160 192194 144144
Bir2 , 191195 193203 142168 150152 234264 144144 150162 188192 146146
Bir3 , 191203 193231 158172 142144 224234 144146 152180 192198 150150
Bir4 , 207211 193207 130150 128142 236244 134146 154154 160192 134140
Bir5 , 207231 187213 162184 150152 210234 144152 148160 160160 140146
Bir6 , 187195 191223 130180 142152 230238 144144 156160 160188 140156
Bir7 , 199211 205229 130130 154156 226246 134142 148156 160180 144144
Bir8 , 195195 183195 148166 150150 236260 144144 154164 160160 144148

Bir9 , 191191 203203 130190 144152 210252 134142 156182 192194 138144
Bir10 , 191195 189197 130156 144148 222234 144154 156156 160160 146146
Bir11 , 199227 193199 158160 140142 228244 144144 154160 160188 146148
Bir12 , 207207 177193 158162 142154 236242 144146 152186 180188 144144
Bir13 , 187199 197201 142166 146148 208224 146164 156168 160160 000000
Bir14 , 187207 195201 160196 142152 254266 144144 164186 160190 000000
Bir15 , 187199 187193 130142 150154 000000 134144 158192 160198 000000
Bir16 , 191195 195209 132162 138140 210224 146152 158158 160194 140146
Bir17 , 191199 191205 148158 144154 238286 144146 158158 160180 142162
Bir18 , 187187 195205 130168 142150 214242 144144 154154 160192 000000
Bir19 , 000000 205223 148152 142152 232232 144146 182182 184192 136140
Bir20 , 187199 187195 162172 138156 230234 144144 156166 188192 000000
Bir21 , 203211 189213 130148 146150 236242 144144 160164 188190 000000
Bir22 , 199207 000000 130172 152156 234238 144146 000000 160192 000000
Bir23 , 000000 181197 142170 128162 222250 142146 160160 160160 000000
Bir24 , 199203 197213 148152 142152 222230 146146 148160 160176 134142
Bir25 , 195199 191205 162164 142154 230240 146146 152168 160186 000000
Bir26 , 207211 191199 152158 146150 230238 144144 000000 186200 144146
Bir27 , 187191 195197 130142 146146 256258 144152 158176 160182 144144
Bir28 , 191199 215231 130130 140148 226230 144144 148152 180188 140144
Bir29 , 187191 187205 000000 000000 000000 000000 156164 184188 134152
Bir30 , 191207 197203 142172 140156 000000 146154 184194 160182 128140
Bir31 , 207215 189195 130142 146160 000000 144144 154158 160184 000000
Bir32 , 199207 199205 130142 152152 232236 144146 154160 160192 134146
Bir33 , 199203 000000 130142 142152 218230 144152 152158 160188 140144
Bir34 , 191211 199203 154166 150158 236236 144144 152158 172180 128140
Bir35 , 187187 207207 148158 136142 228228 144144 000000 160192 000000
Bir36 , 191195 197199 148168 142148 220242 142144 156164 190194 134140
Pop
Bou1 , 187199 193209 130154 152154 234270 144150 158172 160180 140140
Bou2 , 187187 205215 158160 142160 230264 144144 172176 160180 128146
Bou3 , 199199 207213 130188 148152 236240 140150 160176 160190 138146
Bou4 , 207207 197201 160168 142156 230238 144146 154188 000000 138144
Bou5 , 187191 177195 158164 144148 210234 144144 156182 160184 144158
Bou6 , 187191 195203 158164 150152 236240 144146 156156 160188 144148
Bou7 , 199203 205211 158164 140144 236238 144144 154184 160160 130136
Bou8 , 199203 193197 130130 142146 216244 134146 154162 160160 140144
Bou9 , 191207 185185 130140 146150 210226 144146 158170 160184 130140
Bou10 , 199211 199201 140168 000000 210230 146158 152156 160188 144146
Bou11 , 191195 191197 000000 000000 230244 146146 154158 184190 138140
Bou12 , 191207 187209 130158 152156 232234 144144 154184 160194 140144
Bou13 , 203219 203205 152158 148156 238262 142152 156160 160160 138154
Bou14 , 191195 203215 130134 128148 212236 142144 156160 160160 140146
Bou15 , 203207 193217 130186 144150 234234 134146 156158 192198 138146
Bou16 , 191195 199205 168186 140142 244244 144144 158158 160188 132134
Bou17 , 000000 213215 130166 142148 226254 152152 148154 190196 144144
Bou18 , 191191 203211 130168 144152 212236 132144 148158 180192 144144
Bou19 , 187195 193201 142142 148154 212226 146158 154156 184186 134152
Bou20 , 207207 185187 158168 144152 236238 144144 160160 184188 140166
Bou21 , 203207 199209 138172 142148 226238 144146 156160 160192 134164
Bou22 , 195195 195203 130164 138144 000000 142146 154156 160190 138140
Pop
Mil1 , 203223 197199 000000 000000 000000 144152 152160 196198 138156
Mil2 , 187195 000000 164168 150152 228240 144150 148158 160188 138144
Mil3 , 191203 197207 162172 148154 228234 142144 152192 160188 136148

Mil4 , 191191 187187 160172 152154 236254 144144 156170 160160 146146
Mil5 , 199203 193205 142158 140154 222232 138144 156156 160182 136150
Mil6 , 191191 191203 130182 150154 226248 144152 154156 160188 138138
Mil7 , 199219 189215 162162 142144 246288 144146 158158 192196 140146
Mil8 , 187191 197215 142142 138154 238242 142144 156162 188192 146166
Mil9 , 191199 217223 130130 144152 210228 144144 158158 188192 132140
Mil10 , 191203 199201 130142 152152 210222 144158 158186 160192 138146
Mil11 , 191199 000000 158162 136142 234238 144144 156158 190192 148154
Mil12 , 187191 187197 148148 148152 228228 144146 174180 160192 134140
Mil13 , 203207 215225 132162 142154 254260 144144 152160 180188 140150
Mil14 , 187207 199207 130172 144158 206228 144152 160160 186192 140146
Mil15 , 187199 193197 138198 150156 210228 134136 154160 172188 138146
Mil16 , 187195 187199 142148 132154 234246 134144 154156 190192 140146
Mil17 , 187199 189201 130130 148160 234234 144144 152158 160184 128144
Mil18 , 195199 211217 154172 144154 234234 144144 154168 160192 140140
Mil19 , 199211 193193 130166 142148 242248 144146 156156 160192 144164
Mil20 , 207211 187201 130136 136150 228244 144146 156162 160188 140140
Mil21 , 191199 181205 130166 144150 208210 144144 156166 160160 146148
Mil22 , 191211 189225 130148 144150 236238 144144 154170 190196 136138
Mil23 , 203207 191199 130168 142158 238244 144152 158160 160184 150160
Mil24 , 191191 193203 130180 142142 228236 140154 152156 180188 136144
Mil25 , 187199 193195 186194 148168 222234 146152 154154 186192 134150
Mil26 , 187195 203211 142168 140150 238244 136142 160188 160190 144148
Mil27 , 191199 199209 146176 142146 218228 144144 154158 186196 128138
Mil28 , 187191 183205 130142 136158 226256 144144 154158 192192 138146
Mil29 , 187219 187199 158176 142150 246248 144152 160192 190192 140146
Mil30 , 191195 197205 170174 140142 216238 134146 148184 160160 134138
Mil31 , 191191 203207 000000 156156 236288 142144 158158 178180 140144
Mil32 , 207215 197201 152160 142142 222246 144144 154172 160178 144146
Mil33 , 191195 000000 130130 148152 236254 144144 154154 188192 144152
Mil34 , 187207 207223 000000 000000 212212 144150 156158 160160 138144
Mil35 , 199207 209211 130130 140154 252258 144144 134164 160200 138148
Mil36 , 199215 199211 130158 152168 224236 142150 154156 184190 146166
Pop
Nuk1 , 000000 189189 130176 144168 232256 150152 158160 180192 144146
Nuk2 , 195207 193199 142154 140152 234264 144152 152162 180186 132144
Nuk3 , 207207 191195 130152 142144 210226 142158 158166 160160 144144
Nuk4 , 187207 203203 130164 144152 238244 144144 156156 160160 148154
Nuk5 , 191207 183205 142186 148156 224248 144150 158164 160192 144148
Nuk6 , 195207 205211 152158 138154 246282 144152 146152 160160 146158
Nuk7 , 187199 197211 146160 144156 226276 144154 152152 160160 128158
Nuk8 , 195207 203213 130148 144154 228234 142144 156156 184192 140144
Nuk9 , 199207 199209 166168 146158 236250 144144 186194 160186 140144
Nuk10 , 199207 193193 000000 144150 210232 144146 148148 160188 144146
Nuk11 , 187195 000000 130162 146152 222230 146150 158158 160160 144148
Nuk12 , 195195 187193 148180 148150 220240 146156 158158 160196 138148
Nuk13 , 191207 189195 176180 150156 210226 144144 148158 180196 134140
Nuk14 , 187223 199215 138192 142144 210230 142142 154158 160198 140144
Nuk15 , 191199 195207 142166 142152 228286 144144 160160 160188 134150
Nuk16 , 191207 189189 146154 142150 228234 144146 154156 160192 136136
Nuk17 , 191207 000000 146176 142144 210254 144144 160162 160160 144146
Nuk18 , 187191 211215 130142 148152 226258 134146 160194 160196 138150
Nuk19 , 207211 197225 130164 146148 218228 134154 154156 160180 138148
Nuk20 , 199227 201205 168176 152152 228244 144144 134154 160192 140146
Nuk21 , 191191 205211 130148 152152 238262 134142 158162 160196 126144

Nuk22 , 187207 195203 130188 146148 232248 144144 148148 160160 148154
Pop
Col1 , 187191 193203 130186 140158 000000 126144 152156 000000 146156
Col2 , 187207 179193 130130 154160 238246 144150 164166 180196 138146
Col3 , 187199 195195 130148 152156 228234 144144 160186 192198 146146
Col4 , 187199 197207 142168 150150 220262 144146 154156 160188 134140
Col5 , 187207 197197 130168 142152 212232 144144 000000 192196 144146
Col6 , 187211 203229 130160 146152 224244 144152 148164 160192 134140
Col7 , 191199 207207 130176 146158 232234 146146 150172 160192 138138
Col8 , 187199 201215 162182 144150 238260 144144 156160 160192 140152
Col9 , 187187 181205 164164 140144 000000 144146 156162 160160 144154
Col10 , 191199 211211 146190 142146 212236 134144 160164 160176 000000
Col11 , 203223 193215 130180 144152 210238 000000 000000 000000 000000
Col12 , 187207 203203 130142 148154 228232 000000 158160 188194 000000
Col13 , 191199 000000 160164 140148 232234 140140 144152 000000 140146
Col14 , 187191 193195 140148 132154 000000 000000 154162 160196 146166
Col15 , 187199 193197 142158 144152 000000 144146 154156 160190 000000
Col16 , 187191 000000 130150 148152 230238 142144 176190 188198 148154
Col17 , 191191 207207 130130 144144 226252 146152 154160 160160 000000
Col18 , 191199 195205 142160 146150 234246 142144 156156 180192 000000
Col19 , 000000 195201 130154 150154 210250 144146 154172 190192 000000
Col20 , 187199 195223 132148 142146 000000 144144 154156 180190 000000
Col21 , 187191 185199 138182 144150 240242 142152 156170 160184 000000
Col22 , 191191 197199 152152 142156 214218 144144 154184 180196 146152
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Por12 , 199211 185207 132150 142142 228230 144152 160182 176188 146150
Por13 , 187207 189193 148184 142144 214238 142144 148158 188190 144146
Por14 , 195207 195203 132184 154156 240252 142146 156156 160180 144144
Por15 , 203203 187189 130176 144146 210226 146152 162164 160160 138146
Por16 , 191195 207207 130172 152154 232236 146146 158160 180192 144152
Por17 , 199207 189207 148176 144144 230240 142144 156162 160160 138146
Por18 , 199199 000000 142172 144152 244244 144156 162162 160180 134146
Por19 , 191203 203205 176184 152156 228246 144146 160160 182192 138146
Por20 , 199207 181195 130166 144150 234242 140144 000000 160192 138146
Por21 , 203211 187193 130130 152162 210244 134144 156162 160192 144144
Por22 , 187211 193197 130152 142152 238244 144146 000000 160160 150156
Por23 , 000000 193193 130142 000000 000000 142144 000000 180192 144144
Pop
Sed1 , 199199 193213 130176 148152 210226 152152 154158 160180 138152
Sed2 , 191199 193193 130176 144158 000000 144144 154160 160180 138152
Sed3 , 207207 207209 130148 142162 218236 142144 156158 160200 144160
Sed4 , 187195 193195 132148 140162 210234 146152 148156 190194 134144
Sed5 , 187195 000000 130176 144150 210232 146154 172180 178186 130138
Sed6 , 187187 199205 130148 154156 210228 152156 158160 176180 130152
Sed7 , 191211 189201 130180 144146 208250 142144 000000 160186 134144
Sed8 , 199199 183207 130132 132144 210266 144144 158158 160192 146146
Sed9 , 187207 217217 130168 154158 226244 144146 158164 160180 144144
Sed10 , 199199 225229 130148 156162 222240 142142 156158 160192 144146
Sed11 , 187199 199213 148168 142142 210230 144152 154160 160192 152152
Sed12 , 191203 199209 148166 128154 240264 142142 154154 186194 138138
Sed13 , 195195 209215 148172 144146 236244 144150 158180 160188 134154
Sed14 , 199203 199209 130158 144150 210244 144152 154154 192192 144154
Sed15 , 187187 187207 130184 144148 238240 146152 156162 000000 144152
Sed16 , 187199 225229 130148 142144 226244 144146 156168 160184 136144
Sed17 , 187203 225229 164184 148156 244246 144146 162162 160192 136152
Sed18 , 187187 183187 130162 142144 226236 144146 156158 160160 146150
Sed19 , 199199 183229 132154 140152 238238 144144 158158 160180 146160
Sed20 , 187207 203229 132172 144146 238246 144146 148154 160180 144144
Sed21 , 191203 203205 148176 146148 228228 144146 148156 160196 146152
Sed22 , 199203 193199 158166 144152 226236 144154 164164 160160 144146
Sed23 , 187187 213217 130140 146154 236238 144144 158160 160192 134148
Sed24 , 187199 183207 148166 142156 240244 142144 160160 160160 144150
Sed25 , 195199 189217 158176 132132 232238 142156 154158 160192 134152
Sed26 , 207211 000000 152162 144156 236244 144146 156158 160192 136150

Appendix D

Genotypes of each individual in GENEPOP format for eight sampling sites from the ORH 1 area sampled in 2010. Sampling sites codes as in Table 3.1.

Orange roughy
Hat2a
HopAt2
Hat3
Hat9a
Hat7
Hat45
HopAt4
HopAt5
HopAt11
Pop
Kai_2.1 , 187191 211213 130168 138142 226248 142144 152154 188188 136138
Kai_2.2 , 191191 193195 142194 132158 226264 144146 156188 160186 144152
Kai_2.3 , 203203 205209 130166 138144 212238 144152 154162 190194 138152
Kai_2.4 , 195199 197197 130162 142142 232276 142144 160160 160192 144156
Kai_2.5 , 203211 179183 158170 148152 238242 144144 158160 160192 000000
Kai_2.6 , 191195 199205 168180 152168 228236 144152 152152 160180 130146
Kai_2.7 , 195207 191193 130148 142150 220226 144146 158190 160196 132144
Kai_2.8 , 187203 207215 130146 128152 238238 144146 158180 188196 128146
Kai_2.9 , 191203 197201 158174 140148 210234 144144 152158 188192 150156
Kai_2.10 , 207207 000000 130180 142144 212236 144146 152154 160192 134146
Kai_2.11 , 187191 193229 130166 142154 236240 144144 000000 160160 138144
Kai_2.12 , 191203 193209 142158 150150 222268 142146 158158 160180 138140
Kai_2.13 , 191211 183193 130142 140148 244248 146152 152164 190196 140150
Kai_2.14 , 195199 187207 130130 148162 238248 134152 154160 188192 146146
Kai_2.15 , 195195 195215 132160 150150 232238 144150 000000 160160 140146
Kai_2.16 , 207207 201211 136158 144152 234236 144144 160164 160192 146156
Kai_2.17 , 191199 203223 130188 138162 210210 144146 158194 180190 146148
Kai_2.18 , 191207 205209 176176 144148 228236 144152 156160 180192 136160
Kai_2.19 , 199203 189207 130160 134142 228240 144144 156160 160160 134138
Kai_2.20 , 191199 199205 146176 142146 256258 134146 154158 160160 146156
Kai_2.21 , 191223 195199 130154 142144 238246 144146 158158 160160 154160
Kai_2.22 , 203211 187187 154164 142154 222254 142144 154158 188190 144150
Kai_2.23 , 191195 193205 130158 146152 214260 146158 154156 160190 146150
Kai_2.24 , 000000 000000 000000 000000 000000 000000 000000 000000 134140
Pop
Tau_2.1 , 187199 000000 142160 150152 210240 144146 152162 160188 146148
Tau_2.2 , 187207 185203 130142 140152 228234 144144 148160 180194 136140
Tau_2.3 , 191199 209211 130152 154160 238244 144144 158162 160160 128146
Tau_2.4 , 187207 185199 150176 148154 234246 134142 154158 160194 140144
Tau_2.5 , 187187 189195 130176 152160 222248 144146 156160 160180 138140
Tau_2.6 , 195199 183197 130142 144148 224226 144144 156160 196196 144146
Tau_2.7 , 203211 203223 170192 128160 240248 134144 158162 160192 144152
Tau_2.8 , 191203 191195 000000 000000 000000 142144 154160 186190 144144
Tau_2.9 , 191195 195205 188188 144154 240250 144144 152156 000000 140146
Tau_2.10 , 191199 191205 158164 000000 228256 144146 156158 160188 144146
Tau_2.11 , 191199 205209 130130 142162 236236 142152 158160 160180 144150
Tau_2.12 , 207207 191207 130186 152162 222226 144144 162164 160190 144144
Tau_2.13 , 187199 189203 154174 152158 230238 144146 156158 192196 134144

Tau_2.14 , 187199 197203 142142 140146 236288 144146 154160 180190 138138
Tau_2.15 , 191199 189211 130154 142142 234242 142158 148160 160184 136146
Tau_2.16 , 191195 195199 162162 142142 210244 134144 160168 160198 134136
Tau_2.17 , 187203 177215 164172 150152 228236 142146 158160 160188 136144
Tau_2.18 , 187191 199207 144154 148152 234246 146152 154154 160160 146148
Tau_2.19 , 187191 199211 130168 148152 238248 142144 000000 160196 144144
Tau_2.20 , 207211 185211 132170 150154 230230 144152 186188 160192 136144
Tau_2.21 , 187187 177197 130168 142150 210236 144144 160184 160192 140144
Tau_2.22 , 203203 207213 130172 144152 228234 134144 160188 180192 140144
Tau_2.23 , 199203 199201 130162 146150 226252 134146 156156 160184 136140
Tau_2.24 , 191207 187211 142144 144146 222226 144144 156162 186194 128144
Pop
Exp_2.1 , 187203 185189 142188 140154 228240 144158 156160 160190 146152
Exp_2.2 , 187207 207213 164174 142156 208224 144158 156158 160160 140140
Exp_2.3 , 187187 195209 130158 154162 210210 144146 154160 196200 134134
Exp_2.4 , 187207 201221 130130 140152 244248 144146 154162 160180 134144
Exp_2.5 , 187187 000000 130144 148148 226240 144144 156160 188192 140146
Exp_2.6 , 191199 209211 140154 140154 208240 134146 154158 188192 136138
Exp_2.7 , 191207 207215 150160 154162 240246 144146 154176 180192 128132
Exp_2.8 , 199199 179209 130174 148154 210238 000000 000000 000000 150164
Exp_2.9 , 207207 000000 152168 144150 216228 140144 158168 160194 144146
Exp_2.10 , 195199 201217 130142 152154 210236 142152 160168 188192 144148
Exp_2.11 , 187187 191193 130138 150150 240242 144154 160184 188192 136140
Exp_2.12 , 191199 199199 130158 148150 210248 134146 154162 160192 140146
Exp_2.13 , 191203 189193 130160 142152 226226 144144 152156 160186 138148
Exp_2.14 , 203207 189197 164174 150150 222246 144144 162174 160160 146148
Exp_2.15 , 191191 195195 142148 140144 210222 142154 154188 160160 128138
Exp_2.16 , 000000 185209 142168 138152 000000 144144 154158 160184 140144
Exp_2.17 , 187199 193201 142176 142152 228234 144146 160190 160188 140150
Exp_2.18 , 000000 000000 170190 142152 230264 144144 160160 160160 000000
Exp_2.19 , 191195 179207 142142 142144 252256 144144 156172 180192 144152
Exp_2.20 , 191191 201207 130144 144152 234236 144146 156186 184192 136148
Exp_2.21 , 187191 181193 130142 142144 210226 144146 154192 184194 140146
Exp_2.22 , 191203 193203 130142 142142 000000 144144 154168 160160 144144
Exp_2.23 , 000000 000000 000000 000000 000000 144144 162194 160184 000000
Exp_2.24 , 187199 195213 130166 138150 236242 144144 152158 180192 138144
Pop
Ton_2.1 , 187191 203215 142170 142168 226246 142144 156188 160160 136140
Ton_2.2 , 187191 195209 130154 144158 242248 146154 152180 160160 146148
Ton_2.3 , 191195 199205 142162 140166 214216 144146 156188 180192 138156
Ton_2.4 , 191199 199203 154172 146152 222244 146146 154158 180188 144146
Ton_2.5 , 191195 199221 130130 140142 226256 144144 154164 184190 136146
Ton_2.6 , 211211 193211 158164 128154 210246 144146 158170 160186 132138
Ton_2.7 , 187195 199213 130142 144150 226234 144144 158170 160188 134144
Ton_2.8 , 207211 197211 146176 142144 210238 142144 152154 160192 140144
Ton_2.9 , 199207 213215 168180 140152 212276 134146 158168 160184 140144
Ton_2.10 , 187207 195195 130148 142150 234242 142144 148154 184188 144156
Ton_2.11 , 187191 183203 130148 140146 208234 142144 158158 160196 140146
Ton_2.12 , 187187 197205 130158 148148 238248 144144 154174 174188 130140
Ton_2.13 , 207223 193211 174190 148154 238246 134158 152158 160160 132134
Ton_2.14 , 191191 203207 148148 132156 228238 140146 154158 160188 140146
Ton_2.15 , 187191 205213 168174 152154 240248 144146 154158 160188 138144
Ton_2.16 , 187187 199219 130130 138154 210210 134144 152158 188194 144144
Ton_2.17 , 191191 189203 130130 146148 236242 144146 154160 160160 146154
Ton_2.18 , 187203 181185 172176 148154 226250 146152 158166 190192 140140

Ton_2.19 , 195207 197203 152176 142154 246268 146154 156158 160184 146146
Ton_2.20 , 203203 209211 136160 148164 212240 146146 152154 160188 136136
Ton_2.21 , 191207 197205 130150 144146 234234 146152 158180 188194 140150
Ton_2.22 , 187187 191211 148150 142142 244246 144144 152160 160192 144146
Ton_2.23 , 191195 207209 000000 144152 238238 144146 000000 186188 138156
Ton_2.24 , 195207 195205 136142 144150 238246 144164 158160 160194 138146
Pop
Bir_2.1 , 187207 187193 130142 142144 226226 144146 152154 192194 144146
Bir_2.2 , 187207 193199 130168 142148 210212 144154 158158 178192 144144
Bir_2.3 , 199219 189193 130132 132154 228254 144146 148154 188192 144144
Bir_2.4 , 191227 199205 130164 142148 244244 144146 152158 160192 134146
Bir_2.5 , 187187 195195 130162 142148 226234 144144 156158 160180 144148
Bir_2.6 , 195195 199207 130182 148152 236244 146146 148154 160190 146146
Bir_2.7 , 195195 195197 130168 152154 210226 134144 158158 190200 146146
Bir_2.8 , 199207 189203 130138 150152 240240 142144 154184 160196 146150
Bir_2.9 , 187207 000000 158158 136146 216242 144144 152160 160200 132136
Bir_2.10 , 187195 209211 130160 142148 242242 144146 158162 180198 144150
Bir_2.11 , 191195 193223 152158 138148 232240 142144 154160 160192 140146
Bir_2.12 , 191211 197197 168176 148154 232234 144144 152174 188192 146146
Bir_2.13 , 207207 193203 158168 138148 210226 134144 154154 160192 150152
Bir_2.14 , 187211 189193 130152 142150 224234 144144 154170 192194 138138
Bir_2.15 , 187187 179189 148176 140148 214240 144146 154158 160160 140144
Bir_2.16 , 183187 183201 142152 152156 000000 146146 160164 180180 144150
Bir_2.17 , 191207 185203 148182 142148 222236 146154 154158 160196 148152
Bir_2.18 , 191207 185199 130174 154164 218240 140144 154166 160190 140144
Bir_2.19 , 187191 207209 138138 144150 246250 144144 152154 160192 144144
Bir_2.20 , 187191 193207 130130 148150 248248 144144 154160 160184 144150
Bir_2.21 , 191199 193197 130130 144146 236248 142142 158158 190192 144150
Bir_2.22 , 187211 179209 152176 148152 230234 146154 158158 160180 128144
Bir_2.23 , 199203 201203 130148 140152 236238 152156 166190 160188 150150
Bir_2.24 , 195195 179205 160182 138146 222234 144144 154160 168188 138144
Pop
Bou_2.1 , 187191 000000 136166 142150 232238 134144 156188 160192 136146
Bou_2.2 , 187207 187207 130174 154154 210236 138140 152190 160160 140146
Bou_2.3 , 195195 187195 142148 140142 226254 142142 148160 160180 126152
Bou_2.4 , 191207 207207 142168 140144 234234 134144 158186 160160 140146
Bou_2.5 , 187199 199207 130190 148154 240250 142144 152176 180192 144146
Bou_2.6 , 191191 211239 130130 146150 216226 144144 156160 160188 144144
Bou_2.7 , 187187 197209 130158 152162 222232 144146 160172 160180 140174
Bou_2.8 , 191203 181185 000000 000000 000000 144146 148156 188196 128136
Bou_2.9 , 199199 000000 130142 142150 232232 140144 160168 160160 140144
Bou_2.10 , 187187 205211 130168 142150 238250 144146 156160 192196 140148
Bou_2.11 , 191199 189193 148180 150152 234240 144146 154160 160160 128144
Bou_2.12 , 000000 000000 130164 150156 228228 144144 160190 160174 000000
Bou_2.13 , 199207 000000 130160 150152 226256 144144 148162 160188 134146
Bou_2.14 , 191207 000000 158162 140154 230260 142144 160160 160192 144146
Bou_2.15 , 199199 215225 138158 148154 232244 136144 156160 160160 138140
Bou_2.16 , 187207 201217 154168 146154 246248 144146 160160 188192 144150
Bou_2.17 , 187207 199209 160168 148162 236242 140144 152156 160160 146146
Bou_2.18 , 191203 193201 146166 132156 226244 138146 160160 160194 132134
Bou_2.19 , 187215 207207 130154 136152 210246 144144 152182 160192 146146
Bou_2.20 , 203207 181203 130142 148150 224234 144144 152186 190192 134138
Bou_2.21 , 187199 187191 130168 136162 212230 142146 158164 160160 144144
Bou_2.22 , 187195 199213 160164 142152 236242 144152 158192 192192 130138
Bou_2.23 , 199203 181195 164168 146156 234244 144144 158158 188190 150156

Bou_2.24 , 187207 179209 130154 140152 234238 144144 152158 160180 132138
Pop
Mil_2.1 , 187199 000000 154164 132144 234236 134136 156186 188192 136136
Mil_2.2 , 191199 000000 130174 140152 222234 144144 162168 160190 136146
Mil_2.3 , 187207 185203 130160 140148 234240 144144 152154 160188 140144
Mil_2.4 , 000000 000000 146168 156158 210242 144152 160170 160180 146148
Mil_2.5 , 191207 197207 130174 142144 238240 144150 148154 160188 138148
Mil_2.6 , 187207 193223 130164 142144 218236 140146 156170 160192 140146
Mil_2.7 , 191211 201205 168168 152152 220230 144146 156162 160190 144144
Mil_2.8 , 187199 195195 154164 132144 234236 134136 156186 188192 136136
Mil_2.9 , 207211 000000 148164 142142 236242 146150 156172 188200 136150
Mil_2.10 , 187211 191211 168172 144150 210240 126146 154158 184188 136150
Mil_2.11 , 187207 185203 130160 140148 234240 144144 152154 160188 140144
Mil_2.12 , 000000 000000 150178 148160 228236 132154 154156 192196 000000
Mil_2.13 , 000000 000000 130192 142156 222236 000000 160160 160160 140150
Mil_2.14 , 000000 000000 130160 144154 210238 136144 154156 192194 128150
Mil_2.15 , 191191 000000 160178 142144 210264 144146 152154 180188 140144
Mil_2.16 , 000000 197199 142168 142148 234254 142152 150158 160184 144150
Mil_2.17 , 187191 000000 130154 140148 218234 142146 158160 160160 132140
Mil_2.18 , 191195 189231 130166 152156 212236 126136 152158 160176 144144
Mil_2.19 , 191207 195197 162164 142142 234246 144152 148160 160188 144170
Mil_2.20 , 199207 000000 168174 138142 244244 140144 154156 160190 138156
Mil_2.21 , 000000 203205 130188 142142 226238 144144 148156 160160 146146
Mil_2.22 , 191207 191201 000000 150162 218236 134146 156192 160184 144144
Mil_2.23 , 187199 193209 130160 144144 252252 136136 154170 190192 144166
Mil_2.24 , 191191 165195 160178 142144 210264 144146 154154 180188 140144
Pop
Col_2.1 , 191207 000000 142146 140152 210210 144146 158162 180192 132146
Col_2.2 , 191191 211213 130142 140146 234244 144154 152154 192192 128140
Col_2.3 , 191195 000000 154174 142148 210238 134146 152158 160180 134144
Col_2.4 , 203211 189193 164166 142158 210218 144144 158182 160196 144144
Col_2.5 , 203207 213215 168182 144144 226256 144150 152156 180180 134140
Col_2.6 , 191199 199201 130162 140140 232232 144146 158174 000000 140140
Col_2.7 , 195199 179195 148158 134152 240248 144146 152158 160168 144144
Col_2.8 , 207207 189213 132168 142166 242256 144144 152156 160178 136150
Col_2.9 , 191207 193205 130130 142168 226230 146150 152158 160188 150156
Col_2.10 , 191231 193195 000000 000000 000000 134144 154154 190192 140158
Col_2.11 , 195199 175203 130154 000000 210228 140144 154158 160160 140146
Col_2.12 , 000000 187205 130164 144150 230238 144150 156172 160160 138140
Col_2.13 , 187199 199209 130130 148164 216222 144144 154158 160196 144148
Col_2.14 , 187211 193205 142142 140152 222240 144144 156156 160190 140146
Col_2.15 , 195207 191205 144174 136144 210236 134146 154158 180192 128136
Col_2.16 , 195195 189207 130168 146156 238242 146146 158160 160176 140152
Col_2.17 , 199207 185189 136172 152152 208210 144144 000000 186192 138146
Col_2.18 , 191211 191201 130144 144152 222230 144144 148164 160180 144166
Col_2.19 , 191199 189215 148160 144148 222238 144152 152154 160184 138156
Col_2.20 , 195215 187201 130194 142150 222248 144146 152154 160180 140146
Col_2.21 , 187211 201203 160182 142146 224230 144144 000000 160160 138144
Col_2.22 , 191191 193201 142152 144152 218218 134146 156170 188194 144152
Col_2.23 , 187207 203209 130168 140152 210216 134158 154158 000000 144144
Col_2.24 , 191191 191195 144148 152154 228236 144146 154156 160160 128138

Appendix E

Summary of genetic statistics for each locus for 22 sampling sites considering the samples from both seasons (Total n = 812). Total number of alleles (A), allelic richness (A_r), expected heterozygosity (H_E) and the inbreeding coefficient (F_{IS}). $P > 0.002$ for all F_{IS} values (Bonferroni correction $\alpha = 0.00025$). Codes as in Table 3.1

	Hat2a				*HopAt2*				*Hat3*			
Sites	A	A_r	H_E	F_{IS}	A	A_r	H_E	F_{IS}	A	A_r	H_E	F_{IS}
Kai	9	8.1	0.85	0.111	27	17.1	0.95	0.016	25	15.7	0.90	-0.037
Tau	9	7.6	0.83	0.051	21	16.8	0.95	0.034	27	17.7	0.90	0.054
Exp	8	7.1	0.82	0.052	21	16.8	0.95	0.022	24	15.4	0.89	0.002
Ton	9	8.0	0.83	0.032	23	16.3	0.94	0.007	22	15.7	0.89	0.006
Yas	7	6.8	0.82	-0.064	19	18.1	0.94	-0.007	18	15.6	0.91	-0.055
Bir	12	8.6	0.86	0.055	23	16.2	0.94	0.011	24	14.8	0.89	-0.006
Bou	9	7.2	0.84	0.138	22	17.5	0.95	0.029	22	14.3	0.89	-0.052
Mil	10	8.2	0.83	-0.068	22	16.9	0.95	0.012	28	16.5	0.90	0.051
Nuk	8	7.6	0.82	-0.053	17	16.2	0.94	0.154	18	16.9	0.93	-0.077
Col	10	7.9	0.83	0.025	21	16.2	0.94	0.084	26	16.5	0.89	0.049
Mer	7	6.9	0.83	-0.045	20	17.5	0.95	0.031	18	15.9	0.88	-0.144
Cla	8	7.8	0.86	0.036	18	16.5	0.94	0.072	22	18.7	0.93	-0.033
Rit	8	7.5	0.83	0.095	20	17.3	0.94	0.021	15	13.7	0.88	0.022
Chat	8	7.6	0.80	0.154	18	16.5	0.94	0.031	15	14.1	0.92	-0.041
Puy	9	7.9	0.84	0.088	21	17.6	0.95	0.036	19	15.0	0.89	-0.022
Cha	9	8.1	0.83	-0.008	21	18.2	0.95	0.164	15	13.0	0.85	-0.025
NTas	6	5.9	0.77	0.117	14	14.0	0.91	0.088	16	15.2	0.94	-0.016
Alb	7	6.9	0.81	-0.128	21	18.4	0.95	0.081	18	16.2	0.92	-0.045
Nam	10	7.7	0.83	-0.023	22	17.1	0.95	0.042	10	9.4	0.88	0.102
JF	9	8.1	0.84	0.051	20	15.1	0.92	-0.089	13	11.1	0.91	0.020
Por	8	7.9	0.86	0.228	14	13.7	0.91	0.014	13	12.0	0.90	0.063
Sed	7	6.9	0.80	0.237	16	14.9	0.94	0.029	15	13.0	0.88	-0.142
Total/Ave.	13	7.8	0.83	0.049	34	16.9	0.94	0.040	36	16.7	0.90	-0.015

Appendix E continued

	Hat9a				*Hat7*				*Hat45*			
Sites	A	A_r	H_E	F_{IS}	A	A_r	H_E	F_{IS}	A	A_r	H_E	F_{IS}
Kai	16	11.6	0.90	-0.017	29	17.9	0.94	0.061	10	6.9	0.66	0.001
Tau	14	11.4	0.90	-0.019	24	16.9	0.94	0.054	8	6.3	0.64	-0.040
Exp	15	12.1	0.91	0.041	28	18.8	0.95	0.071	9	7.4	0.63	0.092
Ton	17	12.5	0.91	-0.005	27	17.8	0.94	0.036	11	8.1	0.75	-0.053
Yas	13	12.3	0.91	-0.006	21	17.9	0.93	0.200	9	8.3	0.66	-0.014
Bir	17	12.0	0.90	-0.053	30	18.9	0.95	0.105	9	6.4	0.65	0.112
Bou	15	11.9	0.91	-0.073	24	16.8	0.94	0.059	11	8.3	0.70	0.075
Mil	16	12.1	0.90	0.058	28	17.4	0.94	0.045	13	9.2	0.72	0.038
Nuk	12	11.2	0.89	-0.017	25	21.9	0.96	-0.041	9	8.6	0.75	0.150
Col	17	12.2	0.90	0.037	26	19.1	0.95	0.027	10	7.7	0.69	0.033
Mer	14	12.6	0.90	-0.014	20	18.5	0.95	0.052	11	9.6	0.68	0.147
Cla	13	11.6	0.87	0.048	22	18.8	0.95	0.171	9	8.3	0.78	0.148
Rit	14	12.8	0.91	-0.052	20	18.2	0.94	-0.010	8	7.4	0.69	0.161
Chat	13	12.2	0.91	0.005	21	19.4	0.95	0.052	9	8.1	0.62	0.063
Puy	11	9.6	0.85	0.018	25	19.3	0.96	0.019	10	7.6	0.63	0.092
Cha	16	14.2	0.91	0.046	23	20.2	0.96	-0.001	7	6.2	0.67	0.003
NTas	8	7.9	0.89	0.101	15	14.4	0.92	-0.033	10	9.5	0.81	0.057
Alb	13	11.9	0.89	-0.075	19	16.4	0.93	0.054	9	7.8	0.68	0.177
Nam	11	9.2	0.87	0.064	24	16.8	0.93	0.118	10	8.1	0.69	0.047
JF	15	12.1	0.91	0.002	20	15.8	0.94	0.159	11	9.5	0.82	0.024
Por	11	10.7	0.90	0.050	17	15.9	0.94	0.094	7	6.7	0.75	0.107
Sed	13	12.3	0.91	-0.020	17	14.6	0.92	0.005	7	6.5	0.75	0.031
Total/Ave.	22	11.9	0.90	0.005	41	18.0	0.94	0.059	18	8.1	0.70	0.066

Appendix E continued

Sites	*HopAt4*				*HopAt5*				*HopAt11*			
	A	A_r	H_E	F_{IS}	A	A_r	H_E	F_{IS}	A	A_r	H_E	F_{IS}
Kai	18	11.8	0.88	0.055	13	9.1	0.78	-0.013	16	12.1	0.89	-0.061
Tau	15	10.7	0.87	-0.019	10	9.0	0.80	-0.030	12	10.6	0.86	0.165
Exp	20	12.9	0.89	0.007	12	8.8	0.77	0.067	18	12.8	0.89	0.057
Ton	15	11.2	0.87	0.006	14	9.4	0.78	-0.066	14	10.5	0.85	0.023
Yas	14	12.4	0.90	0.216	11	9.4	0.78	0.035	11	10.3	0.87	0.204
Bir	21	12.8	0.89	0.113	16	10.6	0.82	-0.078	14	10.4	0.85	0.181
Bou	18	12.8	0.89	0.046	11	8.5	0.74	0.004	19	12.8	0.89	0.048
Mil	20	12.1	0.88	0.054	15	9.8	0.82	-0.055	17	11.4	0.89	0.066
Nuk	13	12.1	0.90	0.297	8	7.6	0.66	-0.038	13	12.3	0.88	-0.028
Col	20	12.9	0.89	-0.049	13	9.8	0.81	-0.011	15	12.3	0.89	0.059
Mer	14	13.4	0.90	-0.057	12	10.8	0.83	-0.002	11	9.9	0.87	-0.061
Cla	12	11.4	0.90	0.129	9	8.8	0.82	-0.163	13	11.6	0.87	-0.009
Rit	14	12.9	0.90	-0.067	10	9.3	0.84	-0.038	12	10.9	0.84	-0.039
Chat	14	12.9	0.89	-0.020	9	8.2	0.78	-0.064	10	9.5	0.88	-0.047
Puy	18	14.1	0.90	0.015	13	10.2	0.70	-0.102	15	12.6	0.90	0.021
Cha	17	14.9	0.91	-0.106	13	11.9	0.86	-0.116	13	11.5	0.87	0.033
NTas	11	10.6	0.88	0.091	9	8.8	0.81	0.006	14	12.7	0.86	-0.107
Alb	13	11.8	0.91	-0.013	10	9.4	0.77	-0.033	14	12.4	0.88	0.003
Nam	16	12.7	0.90	0.037	12	9.1	0.76	-0.093	17	11.6	0.89	0.016
JF	17	11.7	0.87	0.014	13	8.6	0.68	0.069	14	11.3	0.89	0.102
Por	7	6.9	0.84	0.255	10	9.0	0.77	0.006	10	9.1	0.83	0.000
Sed	10	9.3	0.85	0.159	12	9.9	0.75	-0.125	11	10.4	0.88	0.080
Total/Ave.	28	12.2	0.89	0.053	17	9.3	0.78	-0.038	23	11.6	0.87	0.032

Appendix F

Varela AI, Ritchie PA, Smith PJ (2012) Low levels of global genetic differentiation and population expansion in the deep-sea teleost *Hoplostethus atlanticus* revealed by mitochondrial DNA sequences. Marine Biology 159:1049-1060

Varela AI, Ritchie PA, Smith PJ (2013) Global genetic population structure in the commercially exploited deep-sea teleost orange roughy (*Hoplostethus atlanticus*) based on microsatellite DNA analyses. Fisheries Research 140:83-90

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ORIGINAL PAPER

Low levels of global genetic differentiation and population expansion in the deep-sea teleost *Hoplostethus atlanticus* revealed by mitochondrial DNA sequences

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Abstract The orange roughy *Hoplostethus atlanticus* is a well-known commercial species with a global distribution. There is no consensus about levels of connectivity among populations despite a range of techniques having been applied. We used cytochrome *c* oxidase subunit I (COI) and cytochrome *b* sequences to study genetic connectivity at a global scale. Pairwise Φ_{ST} analyses revealed a lack of significant differentiation among samples from New Zealand, Australia, Namibia, and Chile. However, low but significant differentiation ($\Phi_{ST} = 0.02$–0.13, $P < 0.05$) was found between two Northeast Atlantic sites and all the other sites with COI. AMOVA and the haplotype genealogy confirmed these results. The prevalent lack of genetic differentiation is probably due to active adult dispersal under the stepping-stone model. Demographic analyses suggested the occurrence of two expansion events during the Pleistocene period.

Introduction

Deep-sea fishes (i.e. >200 m) live in the largest habitat on Earth. Many deep-sea fishes are widely distributed and have a circumglobal distribution, for example the alfonsino *Beryx decadactylus*. Some bathyal fishes inhabit continental slopes, the slopes of oceanic islands, and seamounts that are separated by extensive areas of deep ocean. The dispersal of deep-sea fishes may be less constrained by their habitat than that of coastal fishes. However, while there are few obvious barriers in the deep sea, migration of individuals could be limited by ocean currents and bathymetric features, as reported for the demersal fish *Brosme brosme* (Knutsen et al. 2009). There are several examples of genetic differentiation among deep-sea fish populations at different geographical scales, including the spikey oreo *Neocyttus rhomboidalis* (Elliott et al. 1998), the bluemouth *Helicolenus dactylopterus* (Aboim et al. 2005), the Patagonian toothfish *Dissostichus eleginoides* (Smith and McVeagh 2000; Rogers et al. 2006), and the Cape hake *Merluccius paradoxus* (von der Heyden et al. 2007). However, there are also species that exhibit panmixia at regional, oceanic, and inter-oceanic scales such as the armorhead *Pseudopentaceros wheeleri*, the wreckfish *Polyprion americanus*, the alfonsino *Beryx splendens*, and the oreos *Allocytus niger* and *Pseudocyttus maculatus* (Martin et al. 1992; Sedberry et al. 1996; Hoarau and Borsa 2000; Smith et al. 2002a).

The orange roughy, *H. atlanticus,* has a cosmopolitan distribution (Paulin 1979). It occurs at depths of 450–1,800 m and is locally abundant off the coasts of New Zealand, Australia, Namibia, and in the Northeast Atlantic Ocean (Horn et al. 1998; Branch 2001) and off Chile (Labbé and Arana 2001). Orange roughy aggregate near prominent topographic features such as seamounts, plateaus, and canyons, especially during spawning and feeding periods (Clark et al. 2000). It is thought that adults migrate hundreds of kilometers to spawn, as mature fish are widespread, but spawning occurs in just a few specific areas (Francis and Clark 1998). Orange roughy fisheries

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Global genetic population structure in the commercially exploited deep-sea teleost orange roughy (*Hoplostethus atlanticus*) based on microsatellite DNA analyses

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ABSTRACT

The widely distributed teleost orange roughy, *Hoplostethus atlanticus*, has been one of the main species targeted in deep-sea fisheries. While morphometric, parasite and otolith analyses have generally found differentiation among populations within ocean basins, genetic techniques have shown contradictory results at different geographical scales. Here, we used nine microsatellite DNA loci to study genetic diversity and differentiation in orange roughy at a global scale using samples from New Zealand, Australia, Namibia, Chile, and the Northeast Atlantic Ocean. Additionally, temporal genetic variation was assessed for eight sites in Northern New Zealand sampled in two different years. The expected heterozygosity was high in all the sites, suggesting high levels of genetic diversity in orange roughy. Overall, we detected low but significant differentiation at the global scale: across the Southern hemisphere and between the Southern hemisphere regions and the Northeast Atlantic Ocean. However, genetic homogeneity was found between New Zealand and Australia. The present data does not showed temporal variation in orange roughy from Northern New Zealand. A pattern of isolation by distance at the global scale suggests stepping-stone migration, which is likely the result of active adult dispersal. This study revealed levels of genetic differentiation at the global scale that were undetected with mitochondrial DNA sequences analyses.

1. Introduction

The marine environment was once considered a largely "open" system with few boundaries to dispersal of marine organisms. However, increasing research has revealed that many marine species are not panmictic. Despite the potential for widespread dispersal of swimming adults and/or pelagic early life stages, many studies have reported restricted gene flow at different geographic scales in marine fishes. Oceanographic and topographic features, coupled with behavioural characteristics provide barriers for dispersal in marine species. For example, Fauvelot and Borsa (2011) found high levels of genetic divergence in the widely-distributed Spanish mackerel *Scomberomorus commerson* and suggested that the high migrating ability of this fish may be associated with philopatric behaviour rather than promoting wide-scale dispersal. In contrast, there are examples of species with genetically homogeneous populations over wide geographical scales; Wu et al. (2010) found that the yellowfin tuna *Thunnus albacares* exhibits no genetic differentiation between populations from the Western Pacific and Western Indian Oceans.

Genetic studies are particularly useful for deep-sea species for which tag-recapture techniques are difficult to apply. Population genetics studies have been conducted on deep-sea fishes such as the wreckfish *Polyprion americanus* (Ball et al., 2000), the tusk *Brosme brosme* (Knutsen et al., 2009) and the alfonsino *Beryx decadactylus* (Friess and Sedberry, 2011), among others. While dispersal by larval stages and swimming adults was related to the finding of a single genetic stock in *B. decadactylus* in the North Atlantic Ocean (Friess and Sedberry, 2011), genetic differentiation among North Atlantic samples of *B. brosme* was related to bathymetric barriers (Knutsen et al., 2009). At a wider scale including samples from the North and South Atlantic Ocean, the Mediterranean Sea, and the South Pacific Ocean, Ball et al. (2000) found three genetic stocks in the wreckfish *P. americanus*.

Many deep-sea fishes are *K*-selected: they are long-lived, have late maturation and low fecundity. The increased fishing pressure on deep-sea species over the last three decades (see Pitcher

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