Connectivity and exploitation of *Acanthurus triostegus* and *Acanthurus leucosternon* in the Indian Ocean: Application of genetics and single stock assessment to aid coral reef management

A dissertation submitted by

Levy Michael Otwoma

to the Faculty 2 (Biology and Chemistry)

University of Bremen,

in partial fulfillment of the requirements for the degree of

Doctor of Natural Sciences (Dr. rer. nat),

September 2018, Bremen

This thesis was conducted between April 2015 and September 2018 at the Leibniz Centre for Tropical Marine Research (ZMT) in Bremen, Germany

Examination committee

1. Reviewer: Prof. Dr. Martin Zimmer

   University of Bremen, Germany

   Leibniz Centre for Tropical Marine Research (ZMT), Bremen

2. Reviewer: PD. Dr. Hauke Reuter

   University of Bremen, Germany

   Leibniz Centre for Tropical Marine Research (ZMT), Bremen

Additional examiners

1. Examiner: Prof. Dr. Wilhelm Hagen

   University of Bremen, Germany

2. Examiner: Dr. Achim Meyer

   Leibniz Centre for Tropical Marine Research (ZMT)

Attending students

1. Rebecca Borges

   Ph.D. student at the Leibniz Centre for Tropical Marine Research

   University of Bremen, Germany

2. Anna Feuring

   Masters Student (Marine Biology), University of Bremen

Abstract

Extrinsic/abiotic and intrinsic/biotic factors can influence the connectivity and exploitation of reef fish. Coral reef fish from the genus *Acanthurus* have evolved different life history characteristics that can affect their connectivity and exploitation. The aim of this thesis is to explore the population genetic structure and growth parameters of *Acanthurus triostegus* and *Acanthurus leucosternon* in the Indian Ocean, to determine the influence of biotic and abiotic factors on the connectivity and exploitation of coral reef species. First, a 491bp fragment of cytochrome b and microsatellite loci was used to show that the long pelagic larval duration of acanthurids can confer widespread genetic connectivity to *A. leucosternon* in the Eastern Africa region. Although the global AMOVA (Analysis of Molecular Variance) involving all *A. leucosternon* Eastern African population is significant, the hierarchical AMOVA and STRUCTURE does not show any genetic breaks consistent with known Eastern African oceanographic and biogeographical barriers to dispersal. Second, a mitochondrial DNA fragment spanning the ATPase8 and ATPase6 gene regions is used to demonstrate that the genetic differentiation of *A. triostegus* is correlated with geographic distance throughout the Indo-Pacific. In addition, this study shows that populations of *A. triostegus* are significantly differentiated in the Indian Ocean (Western Indian Ocean and East Indian Ocean), but not in the Pacific Ocean (West, Central, and East Pacific).

Third, using syntopic sampling of the spawning aggregating *A. triostegus* and monogamous pairing *A. leucosternon* this study determined the influence of mating behaviour on the connectivity of these two *Acanthurus* species. Contrary to expectations, DAPC (discriminant analysis of principal components), hierarchical AMOVA, and pairwise comparisons showed that the divergent mating behaviour does not lead to differences in the connectivity patterns of *A. leucosternon* and *A. triostegus*, but the two species experienced differences in their demographic history. A detailed analysis in BEAST (Bayesian Evolutionary analysis Sampling Trees) showed

that *A. leucosternon* which is often restricted to coral reef habitats had a faster and more recent demographic expansion than the habitat generalist *A. triostegus*. Finally, the growth parameters and mortality of *A. triostegus* and *A. leucosternon* were estimated, to determine whether differences in mating behaviour can lead to differences in exploitation rate. Consistent with expectations, the length-based stock assessment showed that the *A. triostegus*, the species that often forms spawning aggregation has a higher exploitation rate than the monogamous pairing *A. leucosternon*, supporting previous studies indicating that spawning aggregation may increase the susceptibility of coral reef fish to fishing.

Zusammenfassung

Extrinsische/abiotische und intrinsische biotische Faktoren können die Konnektivität und Nutzung von Rifffischen beeinflussen. Die Konnektivität und Nutzung von Doktorfischen der Gattung *Acanthurus* wird durch verschiedene biologische Merkmale und ihren Lebenszyclus bestimmt. Ziel dieser Arbeit ist es die Populationsstruktur und Wachstumsparameter von *Acanthurus triostegus* und *Acanthurus leucosternon* im Indischen Ozean zu ermitteln, um den jeweiligen Einfluß der biotischen und abiotischen Faktoren auf Korallenriff Organismen festzustellen.

Zunächst wurde mit einem 491bp großen Fragment der Cytochrome b Oxidase und genomischen Mikrosatelliten gezeigt, dass die lange pelagische Larvalphase *A. leucosternon* eine weitreichende genetische Konnektivität in der Ostafrikanischen Region verleiht. Obwohl die globale Analyse der molekularen Varianz (AMOVA) in der Ostafrikanischen Population von *A. leucosternon* signifikant ist, konnte mit der hierarchischen AMOVA und dem Programm STRUCTURE keine genetische Trennlinie gefunden werden, die mit bekannten ozeanographischen oder biogeographischen Verbreitungsgrenzen im Einklang steht.

Zweitens zeigt das mitochondrielle Fragment der Gene ATPase8 und ATPase6, dass die genetische Differenzierung bei *A. triostegus* mit der geographischen Distanz im Indopazifik korreliert. Zusätzlich wird gezeigt dass *A. triostegus* im Indischen Ozean (westlichen und östlichen Indischen Ozean) signifikant differenziert ist, im Gegensatz zu der Population im Pazifischen Ozean (West-, Zentral- und Ost-Pazifik).

Drittends wurde durch ein syntopes Sampeln des in Gruppen laichenden *A. triostegus* und des paarlaichenden *A. leucosternon* der Einfluß des unterschiedlichen Paarungsverhaltens auf die Konnektivität dieser beiden *Acanthurus* Arten untersucht. Entgegen der Erwartungen konnte weder die DAPC (discriminant analysis of principal components), hierarchische AMOVA oder

paarweise Vergleiche Unterschiede der genetischen Konnektivität bei *A. triostegus* und *A. leucosternon* aufzeigen, aber die beiden Arten weisen Unterschiede in ihrer demographischen Entwicklung auf. Eine genaue Analyse in BEAST (Bayesian Evolutionary Analysis Sampling Trees) zeigte, dass *A. leucosternon*, dessen Vorkommen auf Korallenriffe beschränkt ist, eine jüngere und schnellere demographische Expansion aufweist als der Generalist A. *triostegus*.

Zum Abschluss wurden Wachstumsparameter und Mortalität von *A. triostegus* und *A. leucosternon* bestimmt um festzustellen, ob die Unterschiede im Paarungsverhalten zu Unterschieden in Nutzungsraten führen. In Übereinstimmung mit den Erwartungen zeigte *A. triostegus* bei der längenbasierten Bestandsabschätzung eine stärkere Befischung als der paarbildende *A. leucosternon*. Dieses Ergebnis steht im Einklang mit früheren Studien die darauf hindeuten, dass in Gruppen laichende Korallenfische einer stärkeren Nutzung ausgesetzt sind.

Contents

Table of figures

List of tables

1. CHAPTER - General introduction

1.1. Connectivity of reef species

Connectivity in marine ecology refers to the extent to which populations in different parts of a marine species' range are linked by exchange of larvae, recruits, juveniles or adults (Palumbi, 2003; Sale *et al.*, 2005). Regularly, this term is also used as an umbrella to show variations in the level of linkages in marine organisms from no connectivity (where all populations are self-recruiting = closed populations) to high connectivity (where most of the recruitment occurs through exchange among populations = open populations). Knowledge of connectivity among marine organisms is vital because it has important implications to the natural processes that determine the growth and persistence of populations (Mora and Sale, 2002; Warner and Cowen, 2002; Sale *et al.*, 2005; Jones *et al.*, 2009). In marine systems, however, the extent to which offspring disperse from natal locations or where juveniles recruiting at a particular reef come from remains largely unknown (Jones *et al.*, 2009; Pinsky *et al.*, 2017), because the dispersing propagules are minute and difficult to track (Thorrold *et al.*, 2002; Thorrold *et al.*, 2006).

For most benthic organisms such as reef species, dispersal and larval exchanges among disparate populations occurs mainly during the pelagic larval stage (Cowen *et al.*, 2000; Cowen *et al.*, 2006). Traditionally, it was assumed that ocean currents are the sole driver of larval dispersal in the marine environment, with the implications that dispersal is extensive and that marine populations are homogeneous over ecological scales (Roberts, 1997). However, this appears to contradict the high biodiversity and species abundance found in coral reefs, which requires isolation for an extended period of time, be it physical or behavioural (Gerlach *et al.*, 2007; Gaither *et al.*, 2015). Recent empirical studies also indicate that self-recruitment (the amount of offspring that are derived from parents in the same location) and larval retention in marine species is higher than was previously thought (Almany *et al.*, 2007; Gerlach *et al.*, 2007). Coral

reef species, in particular, have larvae that are efficient swimmers both in terms of speed and endurance (Leis and Carson-Ewart, 1997; Stobutzki and Bellwood, 1997), which can enable them to determine their dispersal distance in relation to ocean currents (Fisher *et al.*, 2005; Fisher and Hogan, 2007). These new revelations have been enabled by the methodological and technological advancements in the field of population genetics, tagging, biophysical modeling, larval ecology, elementary chemistry, and adult ecology (Jones *et al.*, 2009).

1.2. Estimators of connectivity in reef species

Given the importance of connectivity in coral reef ecosystems, a variety of approaches have been developed to identify the source and destination of reef species larvae. These approaches track the pelagic larvae either directly or indirectly and generally fall into ten broad categories: (i) larval tagging, (ii) population genetics, (iii) physical and biophysical dispersal models, (iv) parentage analysis, (v) larval behaviour, (vi) phylogeography, (vii) elemental chemistry, (viii) recruitment or adult ecology, (ix) post-recruitment studies, and (x) spatial population models (Jones *et al.*, 2009; Leis *et al.*, 2011). Although these ten methods can be used interchangeably, they rarely measure the same thing and have intrinsic uncertainties, which depend on the analytical procedures, type of markers, and statistical methodology employed (Leis *et al.*, 2011; Nolasco *et al.*, 2018). For example, the otolith tagging method does provide unequivocal estimates of larval dispersal, but its application has been limited to small spatial scales because it requires large sample sizes to determine the dispersal between distant sites. As a result, the output of the otolith tagging method may often underestimate the actual mean dispersal distance of coral reef species (Leis *et al.*, 2011; Green *et al.*, 2015). Physical and biophysical modeling, on the other hand, have a great capacity to estimate connectivity of reef species over broad spatial and temporal scales, but often assume an average natural mortality for the dispersing propagules (Leis *et al.*, 2011), which may compromise the results of this approach.

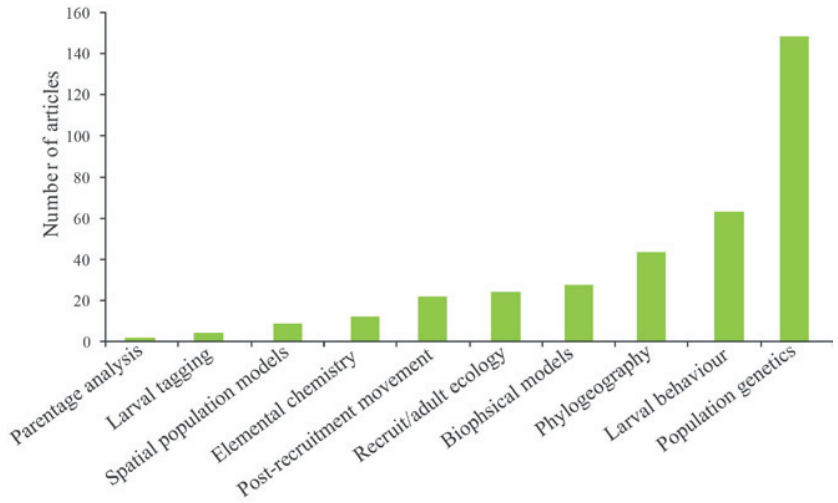


Figure 1.1 Graph showing the frequency of connectivity studies modified from Jones *et al*., (2009).

Currently, the population genetic approach is the most frequently used to infer connectivity and dispersal in reef species (Jones *et al.*, 2009; Lowe and Allendorf, 2010) (Figure 1.1), because it has the potential to measure connectivity at both evolutionary and ecological time scales (Leis *et al.*, 2011). This approach uses genetic data to illustrate spatial differentiation in reef species as a result of genetic drift. Because the influence of genetic drift varies inversely with effective population size, the efficiency of this method to discern reef populations depends on the natural abundance of reef species being studied. For example, reef species with large natural abundance can have weak genetic differentiation even in the absence of gene flow, because the magnitude of genetic drift is very low (Palumbi, 2003; Marandel *et al.*, 2017).

Genetic differentiation in reef species can be characterized by haplotypes variants (mitochondrial DNA: mtDNA), protein variants (allozymes), simple sequence repeat variants (microsatellites), or single nucleotide polymorphisms (SNPs) (Hellberg *et al.*, 2002; Jones *et al.*, 2009; Leis *et al.*, 2011). Because the genetic structure of plants and animals is subject to change over time, the resolution of the molecular marker used should match the time scale of interest (Féral, 2002). For example, allozymes markers have slow mutation rates that tend to reduce the proportion of total

variation in the DNA sequence, leading to overestimation of the level of genetic connectivity among reef populations being studied (Leis *et al.*, 2011). On the other hand, the high mutation rate of mitochondrial DNA (1-10 faster mutation rate than typical nuclear DNA) can saturate the haplotype differences between individuals, leading to the conclusion that connectivity is higher among reef population than is actually the case (Hellberg *et al.*, 2002).

In the absence of gene flow, genetic drift can lead to non-adaptive divergence between populations, because of the loss or fixation of certain alleles. At this point, the fixation index (F_{ST} or Φ_{ST}) that measures genetic differentiation will be equal to 1, indicating that there is an absolute genetic partition between the different populations (Leis *et al.*, 2011). This indicates that the fixation index is inversely related to the degree of resemblance among individuals within populations, but directly related to the variance of allele or haplotype frequency among populations. Therefore, if allele or haplotype frequencies within each population are similar, F_{ST} or Φ_{ST} will be small, while large differences in allele or haplotype frequencies between populations will yield a higher F_{ST} (Holsinger and Weir, 2009; Leis *et al.*, 2011).

1.3. Population genetics of reef species

Over the last decade, genetic tools have been widely used to study marine connectivity (Selkoe *et al.*, 2016), because they offer insights into the scale of dispersal in species that cannot be distinguished by means of other natural or artificial tags (Knowlton, 1992; Berumen *et al.*, 2010). This approach has also enabled testing of hypotheses that specifically ask questions about spatial patterns of larval exchange or drivers of larval exchange in marine species (Selkoe *et al.*, 2016). The ISI (International Scientific Indexing) search conducted by Selkoe et al. (2016), indicated that most of the population genetics studies (68 %) have been conducted in the temperate region, while tropical (26%) and polar (6%) studies are still rare (Figure 1.2). Selkoe and colleagues also

found that the vast majority of the tropical population genetic studies were concentrated on coral reef species, with most focusing on subtidal (65%) as compared to the intertidal zone (15%). The findings of these previous studies have shown that many reef species have unique population genetic structure, possibly due to their ecological, environmental or distributional differences (Leis *et al.*, 2011).

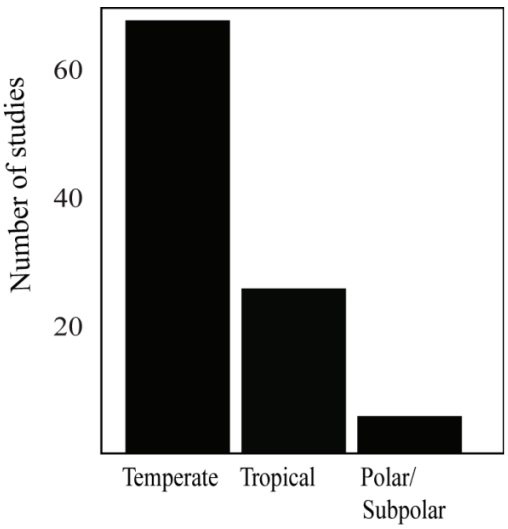


Figure 1.2 Global representation of seascape genetics adopted from Selkoe *et al*., (2016).

For example, across the Indo-Pacific invertebrate reef species such as the blue starfish *Linckia laevigata* (Crandall *et al.*, 2008b; Kochzius *et al.*, 2009; Crandall *et al.*, 2014; Alcazar and Kochzius, 2015; Otwoma and Kochzius, 2016), crown-of-thorns *Acanthaster planci* (Benzie, 1999), mantis shrimp *Haptosquilla pulchella* (Barber *et al.*, 2002), and giant clams *Tridacna* species (Hui *et al.*, 2016) exhibit genetic divergence between the Indian and Pacific populations, supporting the vicariance between the Indian and Pacific Oceans during the Pleistocene low sea level stands. In contrast, sea urchin of the genus *Diadema*, *Tripneustus*, and *Eucidaris* do not exhibit this genetic break between the Indian and Pacific population (Lessios *et al.*, 1999; Lessios *et al.*, 2001; Lessios *et al.*, 2003). Similar discordance in population structure across the Indo-Pacific have also been indicated by vertebrate species, with species such as honeycomb grouper *Epinephelus merra*, humbug damselfish *Dascyllus abudafur*/*Dascyllus aruanus* (Borsa *et al.*,

2016), and peacock grouper *Cephalopholis argus* (Gaither *et al.*, 2011a) exhibiting genetic break between their Indian and Pacific populations, while lutjanids (*Lutjanus kasmira* and *Lutjanus fulvus*) and Naso species (*Naso vlamingii* and *Naso brevirostris*) are homogeneous across the two ocean basins (Horne *et al.*, 2008; Gaither *et al.*, 2010). This discordance in population structure of invertebrate and vertebrate reef species support the assertion that marine species respond uniquely to the dynamic marine environment (Crandall *et al.*, 2008a; DiBattista *et al.*, 2012).

Regardless of the pattern of structure, fish and invertebrate reef species show similar trends with regard to pelagic larval duration (PLD) and dispersal distance. An intuitive assumption across reef species studies is that long pelagic larval duration confers high genetic connectivity among species (Portnoy *et al.*, 2012). Although the vast majority of studies support this assertion, a growing number of studies have found that individual PLDs are not correlated with net dispersal distance (Selkoe and Toonen, 2011). This discrepancy suggests that PLD is not the sole determinant of dispersal in reef species and factors such as ocean currents, historical effects, and life history can lead to variation in genetic patterns (Selkoe *et al.*, 2014). Nevertheless, the discrepancy between PLD and genetic connectivity was shown to be more pervasive in invertebrate species compared to fishes, because generally fish species have behaviors that promote dispersal (Eble *et al.*, 2011a; Poortvliet *et al.*, 2013; Selkoe *et al.*, 2014).

1.4. Factors affecting the genetic connectivity of reef species

As discussed above, the disagreement between gene flow and PLD indicate that connectivity in reef species is not only affected by the dispersal potential, but also by other intrinsic/biotic (local adaptation, larval behaviour, location of fertilization, egg mass production, mating behaviour, and mode of larval development) and extrinsic/abiotic (oceanographic conditions, historical processes, geographical distance, topographic features, and coastal pollution) factors (Imron *et*

al., 2007; Timm and Kochzius, 2008; Yasuda *et al.*, 2009; Puritz and Toonen, 2011; Riginos *et al.*, 2011; Crandall *et al.*, 2014; Liggins *et al.*, 2016; Otwoma and Kochzius, 2016).

1.4.1. Extrinsic factors

Coral reef species display some of the extreme genetic structure in the marine environment, with their populations being either completely closed (all recruits from within) or completely open (all recruits from other populations) (Jones *et al.*, 2009). Ocean currents are among the most pervasive hydrographic features that play a significant role in shaping the genetic structure of reef species (Barber *et al.*, 2006; SILVA *et al.*, 2010a; White *et al.*, 2010; Nakajima *et al.*, 2014). Currents may be circuitous, forming eddies, fonts, or gyres which can prevent larval mixing even in a population located at two adjacent sites. For example, in the Coral Triangle, the Halmahera eddy has been shown to prevent the mixing of stomatopods populations in the Celebes, Maluku and Banda Seas, which has led to the formation of diverged lineages in *Haptosquilla pulchella*, *Haptosquilla gylptocercus* and *Gonodactyellus viridis* (Barber *et al.*, 2006). Alternatively, ocean currents can act as dispersal corridors for the dispersing propagules, potentially linking widespread sedentary coral reef populations (Mitarai *et al.*, 2009).

However, the influence of ocean currents on the connectivity of reef species is usually coupled with the effects of other extrinsic factors such as historical processes, geographic distance, habitat availability, coastal pollution, and topographic features. For example, Plane and Fauvelot (2002) found that the exchange of migrants in a reef fish was much favoured between neighbouring populations, while long-distance dispersal was more sporadic, suggesting that efficiency of ocean currents in shaping the genetic structure of reef species may be constrained by geographical distance (Planes *et al.*, 2009; Saenz-Agudelo *et al.*, 2011; Almany *et al.*, 2013). The general pattern of increasing genetic differentiation with the increase in geographical distance is called isolation-by-distance (IBD) (Planes and Fauvelot, 2002; Palumbi, 2003; Puebla *et al.*, 2009;

Riginos *et al.*, 2011). IBD can result in stepping stone dispersal mechanisms, which often leads to low genetic differentiation, but do not necessarily imply long-distance dispersal (Puebla *et al.*, 2012). Overall, the influence of geographical distance on reef species dispersal is more stable than ocean currents and its correlation with genetic distance usually indicates that populations have reached equilibrium between gene flow and drift (Hutchison and Templeton, 1999; Riginos and Liggins, 2013).

1.4.2. Intrinsic factors

The variation in the temporal and spatial genetic structure of reef species can also depend on local adaptation, larval behavior, location of fertilization, egg mass deposition, mating behavior, and mode of larval development. Although the influence of extrinsic factors may override the effect of intrinsic factors (Marko, 2004; Liggins *et al.*, 2016), previous studies indicate that slight differences in the life history strategies of reef species can lead to strikingly different variation in their structuring patterns (Ayre and Hughes, 2000). For example, the populations of the brooding corals *Seriatopora hystrix* and *Stylophora pistilata* exhibit higher genetic differentiation than the broadcast spawning *Pocillopora darmiconis* in the Great Barrier Reef. Ayre and Hughes, (2000) attribute these differences to the longer larval pre-competency period in the broadcast spawners as compared to the brooding species.

For reef fish species, the diverse reproductive mating behaviors have also been shown to determine the extent of larval dispersal and connectivity among populations (Antoro *et al.*, 2006; Jackson *et al.*, 2014). For instance, spawning aggregation events that are coupled with short-term oceanographic conditions can enhance larval retention, leading to strong genetic differentiation among populations (Antoro *et al.*, 2006; Jackson *et al.*, 2014). This would suggest that species spawning in a spatially diffuse manner in reefs may be expected to have more connected populations than their counterparts forming spawning aggregations (Portnoy *et al.*, 2012).

Although this prediction has been substantiated in two *Epinephelus* species (Antoro *et al.*, 2006; Jackson *et al.*, 2014), findings on red hind, *Epinephelus guttatus* and Coney, *Cephalopholis fulva* indicate that the influence of mating system can sometimes be diminished by other biotic or abiotic factors (Portnoy *et al.*, 2012).

Finally, the egg mass deposition and mode of larval development can determine the genetic structure of reef populations (Riginos *et al.*, 2011; Riginos *et al.*, 2014). In particular, species with benthic egg development tend to show weaker connectivity than their counterparts having pelagic egg development. This is because the mean dispersal distance for species with benthic eggs tends to be less than that of species with pelagic eggs (Riginos *et al.*, 2011; Riginos *et al.*, 2014). Overall, the possibility that the genetic structure of reef species can be influenced by more than one abiotic/extrinsic or biotic/intrinsic factors, suggest that it is critical to disentangle the contribution of extrinsic and intrinsic factors in structuring genetic variation of reef taxa (Papadopoulou and Knowles, 2016; DiBattista *et al.*, 2017).

1.5. Study genus: *Acanthurus*

Acanthurus is the most conspicuous and dominant genus of the family Acanthuridae, containing 40 species. Most of these species occupy the reef habitats of the Indo-Pacific, but four species are restricted in the Atlantic Ocean (Randall, 1956; Bellwood *et al.*, 2014; Marshell and Mumby, 2015). They have multi-denticulated teeth specialized for cropping the fast-growing epithelial algal community (Bellwood *et al.*, 2014). Through their feeding *Acanthurus* species not only limit the establishment of algal communities that impede coral recruitment but also provide a link for energy flow to higher trophic levels in the reefs (Crossman *et al.*, 2005; Marshell and Mumby, 2015). Their territorial behavior can also influence benthic communities and enhance within-territory coral diversity by providing protection against predators (Crossman *et al.*, 2005; Comeros-Raynal *et al.*, 2012).

Acanthurus are broadcast spawners that reproduce throughout the year, but spawning has been shown to peak around February and March in some species (Robertson *et al.*, 1979; Craig, 1998). Mating in this genus involves either the formation of spawning pairs or resident spawning aggregation, with the release of pelagic fertilized eggs that are approximately 0.7mm (Robertson *et al.*, 1979). The pelagic larval duration (PLD) of *Acanthurus* ranges from 44 to 83 days (Thresher, 1984; McCormick, 1999; Fisher *et al.*, 2005) and can confer widespread genetic connectivity among populations. However, after hatching the *Acanthurus* larvae becomes acronurus that exhibits swimming speeds that can reach up to 65.3 cm/s and can enhance larval retention (Figure 1.3), when the average swimming speed of the larvae exceeds the mean ocean current velocity (Leis and Carson-Ewart, 1997; Stobutzki and Bellwood, 1997; Fisher and Hogan, 2007). Therefore, species in the genus are excellent models to study the influence of biotic/intrinsic and abiotic/extrinsic factors on larval dispersal and connectivity of reef taxa.

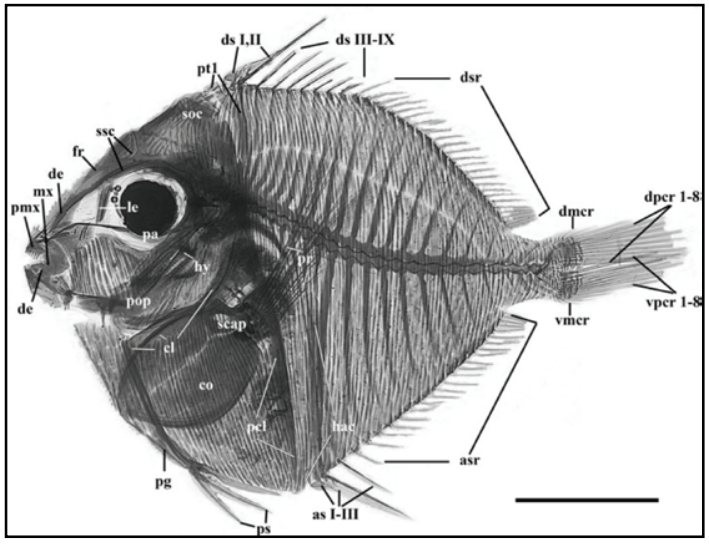


Figure 1.3 Image of acronurus stage of unidentified *Acanthurus* species.
de: dermethmoid, ds: dorsal-fine spine, dsr: dorsal-fin soft ray, pr: pectoral-fin rays, ps: pelvic-fin spine, pcl: postcleithrum, pg: pelvic girdle, vmcr: ventral marginal caudal ray, vpcr: ventral principal caudal ray, ssc: supraorbital sensory canal, ppsr: proximal portion of soft ray, soc: supraoccipital, pop: preopercle, hac: haemaxanal, mx: maxilla, as: anal-fin spine. This figure is adopted from Tyler and Micklich, (2011).

1.6. Exploitation status of *Acanthurus*

In many parts of the world, *Acanthurus* species are prized components of coral reef fisheries and ornamental trade (Craig *et al.*, 1997). But the harvesting of these species typically occurs in poor developing countries such as in the Caribbean, Philippines, East Africa, and Malaysia where fishing pressure has continuously increased (Craig *et al.*, 1997; Comeros-Raynal *et al.*, 2012; Okemwa *et al.*, 2016). There is already a concern that *Acanthurus* species may be driven to extinction as their exploitation increase and habitat disappears due to climate change (Comeros-Raynal *et al.*, 2012). In East Africa, for example, Under Visual Census (UVC) surveys indicate that the abundance of some of the *Acanthurus* species is significantly lower than records made thirty years ago (Samoilys *et al.*, 2017). The overall decline in abundance can be risky in marine fish species that usually have effective population size (Ne) that is several orders of magnitude smaller than the census population size (N) (Hauser *et al.*, 2002; Turner *et al.*, 2002; Hutchinson *et al.*, 2003). Nevertheless, a large data gap exists in the growth, mortality, and exploitation status of Indo-Pacific *Acanthurus* species, where they are widespread and abundant. So far, estimates of growth and mortality exist for only nineteen out of the forty *Acanthurus* species (Choat and Robertson, 2002), indicating that almost more than half of the species of this genus have not yet been evaluated.

1.7. Study region background: Indian Ocean

The Indian Ocean is comprised of coral reefs that exist in a wide range of environments, from fringing and patch reefs that grow in highly unstable environments to oceanic atolls found in the calm clear waters (Sheppard, 2000). However, extensive and abundant reefs occur mostly along the equatorial band of the Indian Ocean that stretches almost three-quarters of the earth's circumference, while in the north part of the Indian Ocean, reefs are limited by the lack of hard

substrate, massive fresh water, and sedimentary inputs (Sheppard, 2000). According to Spalding et al. (2007), the Indian Ocean can be categorized into seven biogeographic provinces that include Western Indian Ocean, Somalia/Arabian, West and South Indian Shelf, Central Indian Ocean Islands, Bay of Bengal, Andaman, and Northwest Australia shelf. These biogeographic provinces coincide with geologic and oceanographic boundaries, which led to distinct reef faunas (Obura, 2012; Borsa *et al.*, 2016). Nevertheless, the heterogeneity of this region is constrained by ocean currents that transport larvae of endemic species across boundaries of different biogeographic provinces (Schott and McCreary Jr, 2001). These currents include those flowing throughout the year (South Equatorial Current, East African Coast Current, Northeast and Southeast Madagascar Current, and Leeuwin Current) or changing with the monsoon seasons (Somali Current and South Equatorial Counter Current) (Schott and McCreary Jr, 2001) (Figure 1.4).

Several studies have examined the connectivity and exploitation of reef fishes in the Indian Ocean, showing different trends in different species (Horne *et al.*, 2008; Gaither *et al.*, 2010; Grandcourt *et al.*, 2010; McClanahan and Hicks, 2011; Hicks and McClanahan, 2012; Borsa *et al.*, 2016; Rehren *et al.*, 2018). However, compared to other parts of the Indian Ocean, the population genetics and exploitation of the Western Indian Ocean reef fish fauna has received relatively little attention (Ridgway and Sampayo, 2005; Gaither *et al.*, 2010; Visram *et al.*, 2010), despite this region experiencing rapid environmental degradation. Successful management of impacts facing reef fish fauna in the Western Indian Ocean and other parts of the Indian Ocean requires a better understanding of factors that shape their connectivity and exploitation.

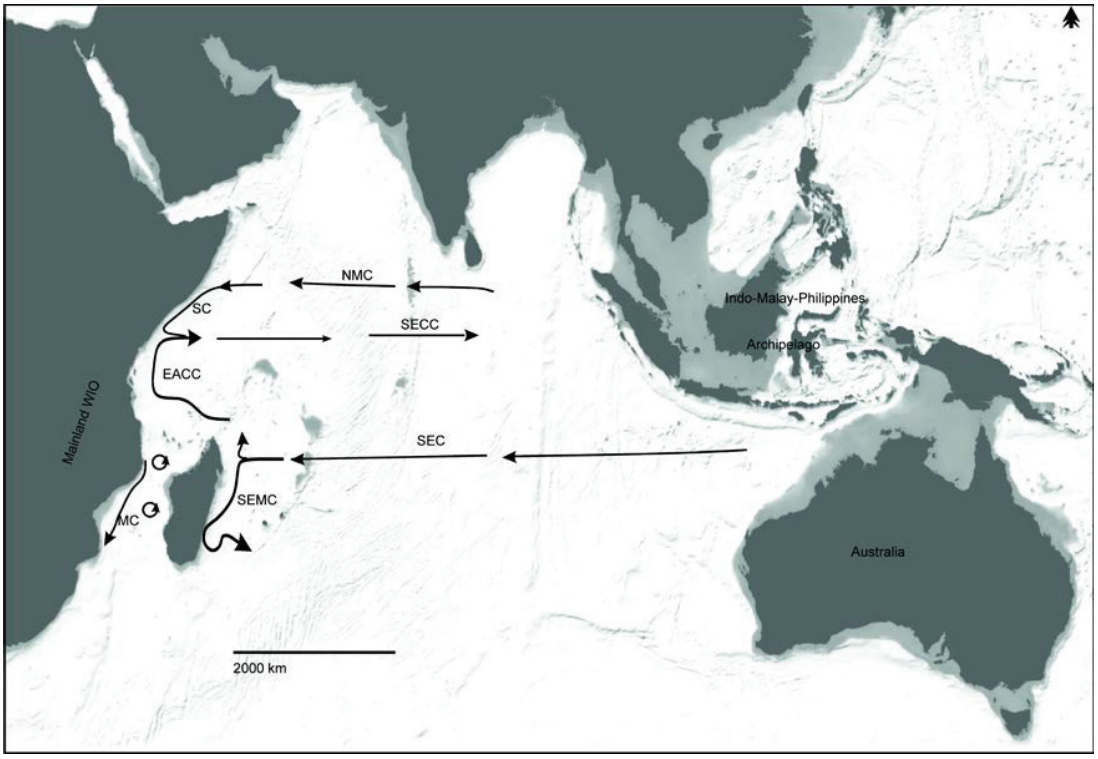


Figure 1.4 Map of the Indian Ocean with the main surface currents during the north monsoon period. SC: Somali Current, NMC: North Monsoon current, EACC: East African Coastal Current, MC: Mozambique Current, SEC: South Equatorial Current, SECC: South Equatorial Counter Current. Light grey colour indicate areas the Pleistocene sea-level low stands

1.8. Aims and thesis structure

In this dissertation, I use molecular (mtDNA and microsatellites) and single stock assessment techniques to assess the influence of biotic and abiotic factors on the connectivity and exploitation of *Acanthurus leucosternon* and *A. triostegus* in the Indian Ocean. These two *Acanthurus* species were selected because they differ in aspects of their reproductive behaviour that can be predicted to affect both connectivity and exploitation rate. *A. triostegus* forms resident spawning aggregations and spawn year-round in equatorial waters. During midday to dusk, fish migrate in dense streams to aggregation sites reaching tens of thousands in numbers, to spawn (Domeier and Colin, 1997). On the other hand, *A. leucosternon* spawn in a more spatially diffuse mode, with a single male and female pairing in their home territories. These two species, therefore, offer an excellent opportunity to test the influence of biotic and abiotic factors on the

connectivity and exploitation of *Acanthurus* species. For example, whether aggregate spawning populations (*A. triostegus*) are less connected than non-aggregate spawners (*A. leucosternon*), assuming that the site fidelity associated with spawning aggregation does enhance larval retention. In addition, a higher fishing mortality and exploitation rate would be expected in aggregate spawners compared to non-aggregate spawners, if fishing is efficient at capturing conspecifics individuals when they are gathered together. The specific objectives of the study were addressed through four research questions as follows:

1) What is the connectivity of *Acanthurus* species in the Western Indian Ocean? Do the connectivity patterns coincide with the known biogeographic and oceanographic boundaries of the Western Indian Ocean?
2) What are the connectivity patterns between the populations of *Acanthurus* species in Western Indian Ocean and their counterparts in the Indo-Pacific (the eastern Indian Ocean, west Pacific, central Pacific, and east Pacific)?
3) Does mating behaviour (biotic factors) affect the genetic connectivity of *Acanthurus* species?
4) Do differences in species-specific traits (mating behaviour) lead to differences in the exploitation rate among *Acanthurus* species?

These questions are addressed through four research-based chapters (chapters two, three, four, and five) that are either published or in preparation and represent different topics with specific objectives, introduction, methods, results, and discussion. The current chapter (chapter one) provides an overview of the thesis research topics and approaches that were used. In chapter two, I use mitochondrial DNA and microsatellite markers to examine the genetic connectivity and structure of *A. leucosternon* in the Western Indian Ocean, testing the hypothesis that its long pelagic larval duration of acanthurids can confer widespread genetic homogeneity. This chapter

has been published in the journal Hydrobiologia "L.M Otwoma, H. Reuter, J. Timm, A. Meyer (2018) Genetic connectivity in herbivorous coral reef fish (*Acanthurus leucosternon*) in the East African region. 806:237-250. doi: 10.1007/s10750-017-3363-4". In chapter three, the genetic population structure and connectivity of convict surgeonfish *A. triostegus* is investigated in five Indo-Pacific biogeographic regions (Western Indian Ocean, eastern Indian Ocean, western Pacific, central Pacific, and eastern Pacific), using mitochondrial DNA spanning the ATPase8 and ATPase6 gene regions. This chapter test the roles contemporary and historical barriers play in shaping the genetic structure of *A. triostegus* and has been published in the Journal of Fish Biology "L.M Otwoma, V. Diemel, H. Reuter, M. Kochzius, A. Meyer (2018) Genetic population structure of the convict surgeonfish, *Acanthurus triostegus*: a phylogeographic reassessment across its range. doi: 10.1111/jfb.13686".

In chapter four, I compared the population genetics of *A. leucosternon* and *A. triostegus*, to determine whether the reproductive mating behaviour has an influence on the connectivity patterns of *Acanthurus* species. This chapter is currently in preparation for submission to a journal. Chapter five of this thesis compared the biological (growth) parameters and mortality of *A. leucosternon* and *A. triostegus*, to deduce whether the species-specific traits or differences would lead to different exploitation rate. Finally, in chapter six I summarize the finding of each chapter, indicate the implication for management and provide recommendations and future directions.

2. CHAPTER - Connectivity of *Acanthurus leucosternon*

Genetic connectivity in a herbivorous coral reef fish (*Acanthurus leucosternon* Bennet, 1833) in the Eastern African region

Levy Michael Otwoma[1, 2, 3*], Hauke Reuter[1, 2], Janne Timm[2], Achim Meyer[1]

[1]Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany

[2]University of Bremen, Bremen, Germany

[3]Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, Kenya

A school of *Acanthurus leucosternon* in a Kenyan reef. © Tim McClanahan.

This is the authors' version of the work. Please cite the final version as follows:

Otwoma, L. M., Reuter, H., Timm, J. & Meyer, A. (2018). Genetic connectivity in a herbivorous coral reef fish (*Acanthurus leucosternon* Bennet, 1833) in the Eastern African region. *Hydrobiologia* **806**, 237-250.

CHAPTER 2

Abstract

Knowledge of larval dispersal and connectivity in coral reef species is crucial for understanding population dynamics, resilience, and evolution of species. Here, we use ten microsatellites and one mitochondrial marker (cytochrome *b*) to investigate the genetic population structure, genetic diversity, and historical demography of the powder-blue tang *Acanthurus leucosternon* across more than 1000 km of the scarcely studied Eastern African region. The global AMOVA results based on microsatellites revealed a low but significant F_{ST} value (F_{ST} = 0.00252 p < 0.001; D_{EST} = 0.025 p = 0.0018) for the 336 specimens sampled at ten sample sites, while no significant differentiation could be found in the mitochondrial cytochrome *b* dataset. On the other hand, pairwise F_{ST}, PCOA, and hierarchical analysis failed to identify any genetic breaks among the Eastern African populations, supporting the hypothesis of genetic homogeneity. The observed genetic homogeneity among Eastern African sample sites could be explained by the lengthy post-larval stage of *A. leucosternon*, which can potentiate long-distance dispersal. Tests of neutrality and mismatch distribution signal a population expansion during the mid-Pleistocene period.

Keywords: Western Indian Ocean, Kenya, Tanzania, Mozambique, Seychelles, Surgeonfish

CHAPTER 2

2.1. Introduction

Many reef species are sedentary as juveniles and adults and depend on their planktonic larval stage for dispersal. These sedentary species display highly variable dispersal capacity, from having long-lived larvae that drift for months along ocean currents to short-lived pelagic larvae that have limited dispersal capacity (Hellberg *et al.*, 2002; Thorrold *et al.*, 2006; Jones *et al.*, 2009). Species with long pelagic larval duration (PLD) tend to exhibit more extensive gene flow and have less structured populations than those with short PLDs (Duda Jr and Palumbi, 1999; Faurby and Barber, 2012; DiBattista *et al.*, 2016). For example, the blue starfish (*Linckia laevigata* Linnaeus, 1758) has a long PLD (~22 days) and exhibits a higher gene flow across the Indo-Pacific than the crown-of-thorns starfish (*Acanthaster planci* Linnaeus, 1758), which has a shorter PLD (~14 days) (Benzie, 1999). However, evidence is accumulating in marine organisms that show little congruence between observed genetic structure and PLD even in closely related species with comparable life history characteristics (Barber *et al.*, 2002; Imron *et al.*, 2007; DiBattista *et al.*, 2012), suggesting that various factors other than PLD may also influence gene flow among marine populations e.g. ocean current systems (Yasuda *et al.*, 2009), larval behavior (Bird *et al.*, 2007), topographic features (Ahti *et al.*, 2016), historical processes (Gaither *et al.*, 2010), habitat preference (Rocha *et al.*, 2002), and habitat fragmentation (Pellissier *et al.*, 2014). Notably, the inconsistency between PLD and gene flow is more pronounced in invertebrates (Barber *et al.*, 2002; Imron *et al.*, 2007) than fishes, which indicates that besides having a pelagic larval phase, most fishes have also reproductive and ecological behaviours capable of enhancing long-distance dispersal (Eble *et al.*, 2011a; Selkoe *et al.*, 2014).

In marine organisms, discordant population structures may also arise due to the transient nature of marine barriers. These anomalous barriers cannot provide absolute vicariance between different populations because dispersal across them is usually possible when conditions are favourable (Mirams *et al.*, 2011). Such porous barriers may be found in the Eastern African

region, which can be divided into three eco-regions: the North Monsoon Current, the Seychelles, and the East African Coral Coast (Obura, 2012). These eco-regions have biogeographic and oceanographic boundaries that underlie the restriction of gene flow in the various coral reef and mangrove species (Ragionieri *et al.*, 2010; Visram *et al.*, 2010; Muths *et al.*, 2015). Nevertheless, some studies on taxa that disperse through their planktonic phase fail to document genetic discontinuity between the different Eastern Africa eco-regions (Silva *et al.*, 2010b; Muths *et al.*, 2012; Huyghe and Kochzius, 2017), which indicates permeability of these Eastern African marine barriers to dispersing marine propagules. The sporadic permeability of these barriers may be influenced or enabled by the complex Eastern African current system (Schott and McCreary Jr, 2001; Benny, 2002).

The present-day oceanography in the Eastern African region is dominated by the South Equatorial Current (SEC) that flows westward across the Indian Ocean to the southern coast of Tanzania and northern coast of Mozambique. At the boundary of Mozambique and Tanzania, this current bifurcates to form the permanent northward flowing East African Coast Current (EACC) and complex eddies in the Mozambique Channel. The splitting of the SEC current at the Eastern African coast potentially creates an oceanographic barrier to dispersal between the southern and northern populations. On the other hand, the EACC, traveling up the Eastern African coastline, is strongly influenced by both monsoon winds and the Somali Current. During the northeast monsoon (November to March), the EACC is weakened, causing it to converge with the Somali Current that flows southward. This forms the seasonal eastward flowing South Equatorial Counter Current (SECC) and a strong upwelling wedge in areas North of Kenyan that extends up to the Somali coast. During the southeast monsoon, the Somali Current is weakened and it joins the EACC beyond Malindi in Kenya, where it develops into different gyres and cells that extend to the Horn of Africa (Figure 2.1) (Schott and McCreary Jr, 2001; Benny, 2002).

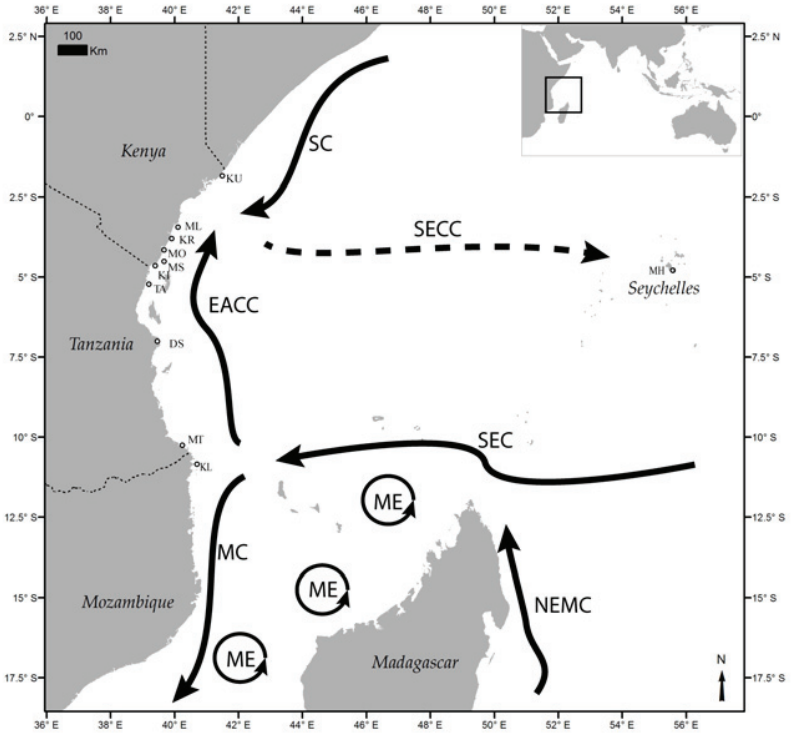


Figure 2.1 Map of the eastern African coast with *A. leucoternon* sample sites (for abbreviations see Table 2.1), main ocean currents (solid lines), and seasonal changing current (dashed lines). EACC, East African Coast Current; SEC, South Equatorial Current; MC, Mozambique Current; SECC, South Equatorial Counter Current; NEMC, North Equatorial Madagascar Current; ME, Mozambique Current Eddies (Schott & McCreary Jr, 2001; Benny, 2002).

The powder-blue tang surgeonfish (*Acanthurus leucosternon* Bennet, 1833) is widely distributed along reef flats in the Indian Ocean; from the Eastern Indian Ocean (EIO) to the Western Indian Ocean (WIO) (Randall, 2002). The largest densities of *A. leucosternon* are observed in the Maldives, but the primary distribution area is at the Eastern African coastline. *Acanthurus leucosternon* is a prized ornamental species that is heavily traded by Kenyan exporters, in addition to being targeted by artisanal fishing (Okemwa *et al.*, 2016). Fishing pressure results in significant density differences (up to 75%) between adjacent protected and unprotected reefs (McClanahan *et al.*, 1999; McClanahan, 2015). *Acanthurus leucosternon* is considered an ecological indicator species because its abundance correlates with healthy coral reefs (McClanahan *et al.*, 1999). Despite a presumably short generation time of only three to four years, a depleted stock needs about 20 years to recover to its previous density after the closure of a fishing area (McClanahan *et al.*, 2007). Like congener species of the genus *Acanthurus*, its

feeding activity not only limits the establishment of algal communities in coral reef ecosystems, but also provide a link for energy flow to higher trophic levels (Crossman *et al.*, 2005; Mumby *et al.*, 2007). *Acanthurus leucosternon* has, like many other reef organisms, a bipartite lifestyle, with sedentary adults and planktonic larval phase. Although the PLD of *A. leucosternon* has not yet been estimated, acanthurids are known for their long PLD of approximately 55 days (Thresher, 1984; McCormick, 1999; Fisher *et al.*, 2005). The potentially high dispersal capacity of *A. leucosternon* offers an excellent opportunity to examine the patterns of connectivity across Eastern African biogeographical and oceanographic barriers.

Despite their contribution of substantial goods and services to coastal economies (Obura *et al*., 2017), the genetic connectivity of coral reef species in Eastern Africa remains amongst the least studied globally (Gaither *et al.*, 2010; Visram *et al.*, 2010; Muths *et al.*, 2015; Otwoma and Kochzius, 2016). These species are usually managed homogeneously (UNEP-WCMC, 2008; Obura *et al*., 2017), without taking into account that different populations may have restricted larval exchanges. However, such a uniform management strategy may lead to significant alteration of the genetic subdivisions, with reduced genetic variation and fitness. Therefore, increasing genetic connectivity studies in this region aim to identify a congruent pattern on how ocean currents and other factors interact to influence larval dispersal, which will be essential in devising effective conservation strategies (Almany *et al.*, 2007; Jones *et al.*, 2009). In this study, we investigate the population genetic structure and connectivity of *A. leuocsternon* in the Eastern African region using microsatellite markers and the mitochondrial cytochrome *b* gene. In addition, we elucidate the genetic diversity and population expansion of *A. leucosternon* in the context of historical processes. The survey of microsatellite genotypes and mitochondrial sequences of *A. leucosternon* intend to answer two questions: (1) are there patterns of genetic population structure among populations of *A. leucosternon* in the Eastern African region? (2) do the structuring patterns coincide with the known Eastern African barriers to dispersal?

2.2. Materials and methods

2.2.1. Sampling and DNA extraction

A total of 336 fin clips were taken from adult *A. leucosternon* at ten sampling locations (n = 16-51) along the Eastern African coastline between August and December 2015 (Table 2.1). The fish were obtained from local fishermen who use spear guns, basket traps, and reef seines. The sampled fin clips were preserved in 100% ethanol and stored at 4 °C prior to DNA extraction. Total genomic DNA was extracted using the standard salting-out protocol (Sunnucks and Hales, 1996).

Table 2.1 Sample information and molecular diversity indices of the microsatellite dataset for *A. leucosternon*.Sample site, location code, number of specimens (n), mean number of alleles (Na), allelic richness (Ar), observed heterozygosity (H_O), expected heterozygosity (H_E), fixation index (F_{IS}), and number of private alleles (PVA). Asterisks indicate significant deviations from the Hardy-Weinberg-Equilibrium (HWE). Sample sites are arranged from north to south

Sample site	Code	*n*	*Na* ± SD	*Ar*	H_o ± SD	H_E ± SD	F_{IS} (10 loci)	F_{IS} (6 loci)	PVA
Kiunga	KU	25	10.33 ± 3.14	9.080	0.819 ± 0.040	0.858 ± 0.038	0.140^{***}	0.046^{ns}	1
Malindi	ML	40	13.50 ± 2.88	10.05	0.857 ± 0.076	0.867 ± 0.031	0.068^{***}	0.010^{ns}	1
Kuruwitu	KR	35	11.67 ± 2.58	9.030	0.856 ± 0.116	0.842 ± 0.040	0.057^{**}	-0.016^{ns}	3
Mombasa	MO	33	13.67 ± 4.23	10.82	0.865 ± 0.080	0.886 ± 0.024	0.079^{***}	0.024^{ns}	0
Msambweni	MS	35	13.33 ± 4.50	10.19	0.839 ± 0.070	0.861 ± 0.040	0.070^{***}	0.025^{ns}	3
Kisite-Mpunguti	KI	51	15.00 ± 4.19	10.27	0.819 ± 0.064	0.859 ± 0.045	0.095^{***}	0.047^{*}	7
Tanga	TA	29	11.50 ± 3.08	9.780	0.805 ± 0.116	0.850 ± 0.050	0.195^{***}	0.054^{*}	3
Dar es Salaam	DS	16	11.17 ± 2.14	10.86	0.864 ± 0.072	0.863 ± 0.034	0.061^{*}	-0.001^{ns}	2
Mtwara	MT	41	14.67 ± 4.13	10.28	0.875 ± 0.052	0.859 ± 0.042	0.053^{**}	-0.019^{ns}	5
Kilindi	KL	31	12.00 ± 2.68	9.530	0.818 ± 0.116	0.846 ± 0.078	0.082^{***}	0.034^{ns}	1

*$0.05 \geq P \geq 0.01$; **$0.01 > P \geq 0.001$; ***$P < 0.001$; ns= not significant

2.2.2. Mitochondrial DNA amplification and sequencing

We amplified the mitochondrial cytochrome *b* gene using polymerase chain reaction (PCR) with the heavy-strand primer 5' GTGACTTGAAAAACCACCGTTG 3' (Song *et al.*, 1998) and the light strand primer 5' AATAGGAAGTATCATTCGGGTTTGATG 3' (Taberlet *et al.*, 1992). The PCR reactions were performed in 20 µl volumes containing 2µl DNA template (50-100 ng), 2 µl PCR buffer B (Roboklon), 13.4 µl H_2O, 400 µm dNTPs, 1 µl BSA (10 mg/ml), 0.4 µl of reverse and forward primer each (10 µM), and a final concentration of 1µM MgCl. The PCRs were conducted with the following temperature profile: 95 °C for 3 minutes, followed by 35

cycles of 30 seconds denaturation at 94 °C, 45 seconds of annealing at 63 °C, and 45 seconds of extension at 72 °C. The final extension was done at 72 °C for 10 minutes (DiBattista *et al.*, 2016). The PCR products were analysed using the Dye Deoxy terminator (Applied Biosystems) and sequenced on an automated sequencer (ABI PRISM 310 and 3100, Applied Biosystems).

For mitochondrial DNA analysis, a total of 48 sequences were subsampled from the 336 individuals. The 48 sequences from Kiunga, Dar es Salaam, and Kilindi were supplemented by 30 published sequences from Mahe, Seychelles (DiBattista *et al*., 2016), altogether representing the three Eastern African eco-regions (the North Monsoon Current, the Seychelles, and the East African Coral Coast (Obura, 2012)) that are separated from each other by oceanographic and/or biogeographic boundaries known to disrupt gene flow in marine organisms (Ragioneri *et al*., 2010; Visram *et al*., 2010; Muths *et al*., 2015).

2.2.3. Microsatellite amplification and genotyping

Individuals were genotyped at 10 published microsatellite loci: Ahy49, Ahy54, Ahy65, Ahy75, Ahy112, Ahy119, Ahy170, Ahy178, Ahy182, and Ahy203 (DiBattista *et al.*, 2011), using an M13-tailed primer PCR protocol (Schuelke, 2000). PCR amplification was conducted in 10 µl reaction volume containing 1 µl DNA template (50-100 ng), 1 µl PCR buffer B (Roboklon), 6.5 µl H_2O, 200 µm dNTPs, 0.5 µl BSA (10mg/ml), 0.2 µl of M13 fluorescent labeled tail primer (10 µM), 0.2 µl of reverse primer (10 µM), 0.2 µl forward primer (2.5µM) with M13 tail, and 500nM of MgCl. The temperature profile consisted of 95 °C for 3 min, followed by 35 cycles of 30 seconds denaturation at 94 °C, 45 seconds of annealing at a locus-specific temperature and 45 seconds of extension at 72 °C. The final extension was done at 72 °C for 7 minutes (DiBattista *et al*., 2011).

The PCR products were labelled with different dye colours and pooled for genotyping along with an AlexaFluoro660 (IBA GmbH) labelled oligo as an internal size standard. Generation of the LIZ size marker followed the protocol described in (DeWoody *et al*., 2004) using pUC19 as a template and resolved with an ABI 3730 genetic analyser (Applied Biosystems), at the Ludwig-Maximilians-Universität München, Germany. The software Geneious version 8.1.6 (Kearse *et al.*, 2012) was used to manually assign allele sizes of the microsatellite loci. In total 336 individuals were genotyped from 10 sample sites (Table 2.1) along the Eastern African mainland coastline, while the published sequences from Mahe, Seychelles were only used in the cytochrome *b* dataset (Table 2.2).

Table 2.2 Mitochondrial cytochrome *b* diversity characteristics of *A. leucosternon* in the Eastern African region. Sample size (n), number of haplotypes (Nhp), haplotype diversity (h), nucleotide diversity (π), time since the recent population expansion (T), random sequence evolution (Tajima's D and FU's F_S), sum of square deviation (SSD), and Harpending's raggedness index (HRI).

Sample site	Code	*n*	*Nhp*	*h*	π	*T* (yrs)	Tajima's *D*	FU's F_S	SSD	HRI	Source
Kiunga	KU	16	12	0.97	0.005	n/a	-1.39^{ns}	-7.15^{***}	0.013^{ns}	0.08^{ns}	Present study
Dar es Salaam	DS	16	12	0.96	0.004	n/a	-1.67^{*}	-8.39^{***}	0.007^{ns}	0.06^{ns}	Present study
Kilindi	KL	16	10	0.87	0.004	n/a	-1.06^{ns}	-5.35^{***}	0.004^{ns}	0.03^{ns}	Present study
Mahe	MH	30	17	0.91	0.005	n/a	-1.62^{*}	-10.8^{***}	0.003^{ns}	0.03^{ns}	DiBattista et al. 2016
All samples		78	35	0.92	0.005	143,000-287,000	-2.04^{**}	-26.81^{***}	0.0004^{ns}	0.03^{ns}	

*$0.05 \geq P \geq 0.01$; **$0.01 > P \geq 0.001$; ***$P < 0.001$; ns= not significant

2.2.4. Data analysis

Mitochondrial DNA

The cytochrome *b* sequences were edited using Geneious version 8.1.6 (Kearse *et al*., 2012) and aligned in BIOEDIT version 7.0.4.1 (Hall, 1999). To ensure that only functional mitochondrial DNA was used and not pseudogenes the sequences were translated into amino acids by the software Squint Alignment Editor version 1.02 (Goode and Rodrigo, 2007). The online services of FABOX (Villesen, 2007) were used to collapse sequences into haplotypes. Haplotype and nucleotide diversity were calculated in Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010).

The null hypothesis of neutral evolution of cytochrome *b* was tested using the Tajima *D*-test (Tajima, 1989) and Fu's *Fs* tests (Fu, 1997). Significant negative Tajima's *D* values indicate population expansion following either selective sweeps, genetic bottleneck event or purifying selection (Tajima, 1989). Besides, population expansion was tested by comparing observed sequence mismatch distributions within sampling sites and those simulated under Rogers's (1995) sudden population expansion model (Schneider and Excoffier 1999) and the goodness-of-fit of observed to simulated distributions was tested using both the sum of square deviation (SSD) and Harpending's raggedness index (HRI) (Rogers, 1995). A multimodal mismatch distribution indicates a population under a demographic equilibrium while unimodal distribution suggests a recent and fast demographic expansion.

The time (T) since the recent population expansion was determined using the formula $T = \tau/2u$ (Rogers and Harpending 1992), where Tau (τ) is the expansion parameter estimate and u equals the mutation rate x generation time x sequence length. The cytochrome *b* divergence rate range of 1% to 2 % per million years in reef fish were used (Bowen *et al.*, 2001; Lessios, 2008; Reece *et al.*, 2010) together with a generation time of 3.4 years (estimated from Eastern African *A. leucosternon* length-frequency data; T.R McClanahan pers. comm.). The parameter Tau (τ) was estimated from Alerquin under a sudden population expansion hypothesis.

We used Arlequin to run an analysis of molecular variance (AMOVA) to estimate the genetic differentiation and pairwise Φ_{ST} values among populations of *A. leucosternon* (Excoffier *et al.*, 1992). A network of haplotypes was constructed with the program TCS version 1.21 (Clement *et al.*, 2000), to infer the evolutionary relationships between populations of *A. leucosternon*, with the proportion of haplotypes found at each sample site being reflected in the pie diagrams.

Microsatellites

Genetic diversity was estimated as the mean number of alleles (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E), and private alleles in the program Arlequin version 3.5.1.3 (Excoffier and Lischer, 2010). The program FSTAT version 2.9.3.2 (Goudet, 1995) was used to estimate the mean allelic richness (*Ar*) and fixation index (F_{IS}). For each locus, an exact test for the departure from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) was estimated using Arlequin. The software MICRO-CHECKER version 2.2.3 (van Oosterhout *et al.*, 2004) was used to screen for possible genotypic errors, large allele dropout, and null alleles. Genotypic errors were further minimized by repeating PCR and fragment analysis in 132 randomly selected individuals (39.3% of all analysed specimens) at all the 10 loci.

Because null alleles have the likelihood of inflating F_{ST} values, the null allele corrected global AMOVA and pairwise F_{ST} values were estimated in the software FreeNA (Chapuis and Estoup, 2007). FreeNA uses the ENA (Excluding Null Alleles) method to efficiently correct for null allele bias and F_{ST} overestimation. Since the estimates of F_{ST} have been observed to decline with increasing microsatellites polymorphism, Jost's D_{EST} was also estimated in this study in GenAlEX version 6.5 (Hedrick, 2005; Jost, 2008; Peakall and Smouse, 2012). The correlation between geographical and genetic distances in the *A. leucosternon* dataset was tested using the Mantel test in GenAlEX by utilising the pairwise F_{ST} and D_{EST} values.

A hierarchical AMOVA was carried out, testing for significant differences among groups of sites in Arlequin with composing groupings based on oceanographic conditions and the geographical locations of sites in the Eastern African region. In addition, a principal coordinate analysis (PCoA) was done in GenAlex, to examine the spatial variation among *A. leucosternon* populations using the unbiased Nei genetic distance. The software STRUCTURE version (Pritchard *et al.*, 2000) was used to define genetic clusters (K) without a priori information on the geographical origin of specimens. To estimate the optimal number of homogeneous genetic units

(K), STRUCTURE was run under the admixture model for K = 1-10, using 10 iterations, a burn-in length of 100,000 and 1,000,000 MCMC (Markov chain Monte Carlo) replications. The most probable value of K was determined using the software STRUCTURE HARVESTER web version 0.6.94 (Earl and vonHoldt, 2012) by plotting log probability L (K) and ΔK (Evanno *et al.*, 2005).

2.3. Results

2.3.1. Genetic diversity

Mitochondrial DNA

A 491 base pair (bp) fragment of cytochrome *b* was obtained after a sequence alignment, which did not contain indels and stop codons. The 78 sequences from Eastern Africa yielded 35 haplotypes of which 26 were unique and nine were shared by 19 to 2 individuals (Figure 2.2). There were 35 polymorphic sites that included 33 transitions and 2 transversions. Overall, haplotype and nucleotide diversity estimates were similar among the Eastern African sampling sites, ranging from 0.87 to 0.97 and from 0.004 to 0.005 respectively (Table 2.2).

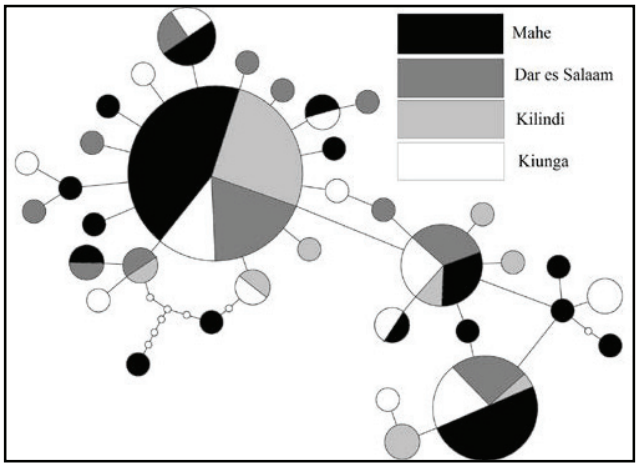


Figure 2.2 Haplotype network from cytochrome b sequences of *A. leucosternon*. Each circle represents a haplotype and its size is proportional to the total frequency. The lines show mutational steps while the smallest circles represent intermediate missing haplotypes.

Microsatellites

Based on within-site comparisons around a quarter of the loci (27 out of 100, $P \leq 0.025$) deviated significantly from the expectations of the HWE; these differences were mostly represented by four markers (Ahy54, Ahy75, Ahy182, and Ahy203). Further analysis with MICROCHECKER indicated that the deviations at these four loci could be due to the presence of null alleles. The re-amplification and re-genotyping results indicated negligible evidence of misamplification and genotyping disagreement, 0.76% of all re-genotyped loci (10 out 1320). Low levels of linkage disequilibrium were also noted after the removal of the four loci not conforming to HWE (14 out of 150 within-site comparisons, $P \leq 0.04$).

Overall populations, mean number of alleles (*Na*) and Allelic richness (*Ar*) varied from 10.33 to 15.00 and 9.03 to 10.86, respectively. The expected heterozygosity values ranged between 0.842 and 0.886, while the observed heterozygosity values ranged between 0.805 and 0.875. Private alleles were detected in all sample sites, with exception of Mombasa, which shared all its alleles with the other sample sites. Populations from two sample sites (Kisite-Mpunguti and Tanga) exhibited significant F_{IS} values even after the exclusion of the four loci (Ahy54, Ahy75, Ahy182, and Ahy203) not in HWE (Table 2.1).

2.3.2. Historical demography

Overall, tests of neutral evolution of the cytochrome *b* marker revealed negative and significant Fu *FS* and Tajima's *D* values, supporting population expansion following selective sweeps, genetic bottleneck or purifying selection (Table 2.2). The analysis of the sequence mismatch distribution revealed that the model of sudden expansion could not be rejected in the Eastern African population, using both SSD and HRI goodness-of-fit (Table 2.2). The range of mutation rates and τ estimated for all sample sites revealed a demographic expansion that began between 143,000 and 287,000 years ago.

2.3.3. Genetic population structure

Mitochondrial DNA

The cytochrome *b* gene AMOVA results showed no genetic differences among Eastern Africa populations, even after inclusion of published sequences from Mahe, Seychelles (Φ_{ST} = -0.021 P = 0.96), with 100% genetic variation being observed within populations. Similarly, pairwise Φ_{ST} estimates were low and not significant, showing genetic homogeneity among all four sampling sites at the mitochondrial marker (Kiunga, Dar es Salaam, Kilindi, and Mahe). The evolutionary relationship of 35 *A. leucosternon* haplotypes found in Eastern Africa is presented in the haplotype network (Figure 2.2), showing a distinct star-like pattern of three common haplotypes surrounded by singletons. The distribution of the shared haplotypes throughout all four sample sites, provide further evidence of a single panmictic population.

Microsatellites

Global F_{ST} (F_{ST} = 0.00252 and F_{ST}ENA = 0.00249) and pairwise F_{ST} (Table 3) estimates from the ENA corrected and uncorrected dataset were not significantly different (t-test: p = 0.45), suggesting that null alleles had little influence on genetic differentiation estimates. The global AMOVA revealed a low but significant F_{ST} value (F_{ST} = 0.00252, p < 0.001, D_{EST} = 0.025 p = 0.0018), with most of the genetic differences being within locations (99%). Similarly, all pairwise F_{ST} and D_{EST} estimates among populations were low and nonsignificant after Bonferroni correction (P < 0.001) (Table 2.3 and Supplementary Table 2.1). The hierarchical AMOVA grouping based on ocean currents and the geographical location of sample sites was not significant, supporting the hypothesis of panmixia in the Eastern African region (data not shown). The Mantel test revealed no significant correlation between geographic distance and pairwise F_{ST} (R^2 = 0.081, p = 0.32) and D_{EST} (R^2 = 0.0006, p = 0.42) estimates.

Table 2.3 Raw and ENA-corrected Pairwise F_{ST} values for populations of *Acanthurus leucosternon* in the Eastern Africa region (for sample site abbreviations see Table 2.1). Raw microsatellite estimates (below the diagonal) and ENA corrected estimates (above the diagonal).

	KU	ML	KR	MO	MS	KI	TA	DS	MT	KL
KU		0.00287^{ns}	0.00826^{ns}	-0.00006^{ns}	0.00605^{ns}	0.00473^{ns}	0.00634^{ns}	0.00366^{ns}	0.00139^{ns}	0.00597^{ns}
ML	0.00349^{ns}		0.00444^{ns}	0.00035^{ns}	0.00073^{ns}	-0.00127^{ns}	0.00281^{ns}	0.00233^{ns}	-0.00085^{ns}	-0.00068^{ns}
KR	0.00775^{ns}	0.00481^{ns}		0.00826^{ns}	0.00239^{ns}	0.00369^{ns}	0.00216^{ns}	0.00569^{ns}	0.00484^{ns}	0.00505^{ns}
MO	0.00033^{ns}	0.00029^{ns}	0.00808^{ns}		0.00231^{ns}	0.00369^{ns}	0.00209^{ns}	0.00729^{ns}	0.00257^{ns}	0.00308^{ns}
MS	0.00632^{ns}	0.00046^{ns}	0.00286^{ns}	0.00191^{ns}		-0.00172^{ns}	-0.00221^{ns}	-0.00021^{ns}	0.00261^{ns}	0.00085^{ns}
KI	0.00495^{ns}	-0.00185^{ns}	0.00381^{ns}	0.00367^{ns}	-0.00237^{ns}		0.00155^{ns}	0.00408^{ns}	0.00254^{ns}	0.00018^{ns}
TA	0.00661^{ns}	0.00409^{ns}	0.00145^{ns}	0.00361^{ns}	-0.00133^{ns}	0.00291^{ns}		0.00224^{ns}	0.00204^{ns}	0.00159^{ns}
DS	0.00267^{ns}	0.00209^{ns}	0.00717^{ns}	0.00776^{ns}	-0.00022^{ns}	0.00332^{ns}	0.00181^{ns}		0.00521^{ns}	0.00686^{ns}
MT	0.00163^{ns}	-0.00091^{ns}	0.00491^{ns}	0.00224^{ns}	0.00247^{ns}	0.00232^{ns}	0.00183^{ns}	0.00496^{ns}		0.00256^{ns}
KL	0.00571^{ns}	-0.00039^{ns}	0.00577^{ns}	0.00271^{ns}	0.00116^{ns}	0.00021^{ns}	0.00128^{ns}	0.00692^{ns}	0.00221^{ns}	

*P < 0.001 (after Bonferroni correction); ns= not significant

The software STRUCTURE HARVESTER identified the optimum ΔK at K = 2 (Supplementary Figure 2.1), with few individuals within sample sites KR, MO, and TA being genetically distinct from the other Eastern African populations (Figure 2.3). On the other hand, the PCoA based on unbiased Nei genetic distances did not reveal any genetic breaks between the geographical locations, but two sample sites (KU and DS) were slightly separated from the other sample sites (Supplementary Figure 2.2).

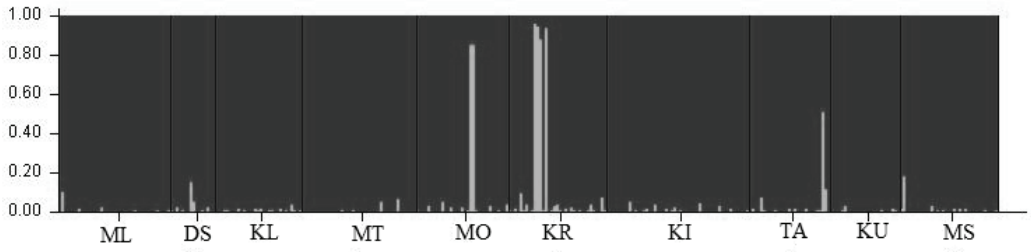


Figure 2.3 Structure analysis performed on *A. leucosternon* populations using 10 microsatellite loci with K = 2. For abbreviations, see Table 2.1.

2.4. Discussion

This is the first study that examines genetic diversity and structure among populations of powder-blue tang surgeonfish along the Eastern African coastline. The findings using microsatellite and

cytochrome *b* markers complement previous studies on coral reef fish species such as *Scarus ghobban* Forsskål, 1775, *Lutjanus kasmira*, and *Amphiprion akallopisos* (Visram et al., 2010; Muths et al., 2012; Huyghe & Kochzius, 2016). As expected, for a species with a lengthy post-larval stage, our results based on microsatellites revealed a weak genetic differentiation (F_{ST} = 0.00252 p < 0.001, D_{EST} = 0.025 p = 0.0018) among populations of *A. leucosternon*. However, pairwise F_{ST}, PCOA, and hierarchical analysis could not identify any genetic breaks among the eastern African populations, suggesting a homogeneous connectivity pattern.

2.4.1. Genetic diversity and historical demography

Marine species traditionally have high genetic diversity, which can be attributed to historically large population sizes and high reproductive potential (Carvalho and Hauser, 1995). Findings on *A. leucosternon* do not appear to deviate greatly from this generalization, both historically (h = 0.87-0.97) and contemporary (Ar = 9.03-10.86, H_E = 0.842-0.886). The high levels of microsatellite genetic diversity (H_O and H_E) are similar in range to those reported for *A. leucosternon* populations in the Eastern Indian Ocean and its congeners *Acanthurus nigricans* Linnaeus, 1758, *Acanthurus achilles* Shaw, 1803, and *Acanthurus japonicus* Schmidt, 1931 in the Pacific and Indian Oceans (DiBattista *et al.*, 2016). The similarity in contemporary genetic diversity estimates could suggest analogous population dynamics in these relatively young species that have akin morphology, ecology, and biology (DiBattista *et al*., 2016). However, unlike the Eastern Indian Ocean populations where only two loci (Ahy54 and Ahy203) deviated from the HWE (DiBattista *et al*., 2016), in the Eastern African populations four loci (Ahy54, Ahy75, Ahy182, and Ahy203) deviated from the HWE.

The range of haplotype and nucleotide diversity estimates observed in *A. leucosternon* are comparable to the estimates found in other coral reef fish in the Eastern African region, such as *L. kasmira* (Muths *et al.*, 2012) and *Epinephelus merra* Bloch, 1793 (Muths *et al.*, 2015), which

also used the cytochrome *b* marker. On a global scale, these estimates are similar to those obtained for *A. leucosternon* in the Eastern Indian Ocean and other *Acanthurus* species in the Atlantic, Indian, and Pacific Oceans (Rocha *et al.*, 2002; DiBattista *et al.*, 2016). The high haplotype and low nucleotide diversity may suggest an expansion of the Eastern African populations after a bottleneck (Grant and Bowen, 1998), which is consistent with the results of the mismatch distribution of HRI and SSD tests as well as the star-like topology of the haplotype network. Based on the mismatch distribution analysis this population expansion is estimated to have begun between 143,000 and 287,000 years ago, which corresponds to the mid-Pleistocene. However, *A. leucosternon* does not have a well-calibrated molecular clock, suggesting that these estimates may not accurately reflect the absolute demographic expansion time of this species. Nevertheless, the range of these estimates is can indicate the epoch and the period in which the expansion most likely occurred. During the Pleistocene glacial sea level low stands, a large proportion of the continental shelf became emergent, leading to loss of habitats and increased fragmentation within coral reef ecosystems of the Western Indian Ocean and Indo-Pacific (Grant and Bowen, 1998; Voris, 2000; Pellissier *et al.*, 2014). The loss of habitats and increased fragmentation may have led to the extirpation and drastic reduction of the *A. leucosternon* population in Eastern Africa. Decline in fish population due to a shortage of habitats can occur on very short time scales and has been shown in contemporary reef monitoring studies, which indicate that 62% of fish disappeared within 3 years of reduction of at least 10% coral cover (Wilson *et al.*, 2006). When the sea-level subsequently rose, increased suitable coral reef habitats could have enabled the population growth of *A. leucosternon*. Evidence of demographic expansion after a bottleneck has been reported in other surgeonfish species e.g. *Acanthurus nigrofuscus* Forsskål, 1775 (Eble *et al.*, 2011a), *Zebrasoma flavescens* Bennett, 1828 (Eble *et al.*, 2011b), and *Ctenochaetus strigosus* Bennett, 1828 (Eble *et al.*, 2009). For Eastern Africa, the hypothesis of population expansion after a bottleneck has also been supported in other reef fish

such as the blue-barred parrotfish *S. ghobban* (Visram *et al.*, 2010) and the skunk anemonefish *A. akallopisos* (Huyghe and Kochzius, 2017).

2.4.2. Genetic population structure

Microsatellite data showed a low but significant F_{ST} value (F_{ST} = 0.00252 p < 0.001, D_{EST} = 0.025 p = 0.0018) across our sampling area, which span potential biogeographic and oceanographic barriers in the Eastern African region. On the other hand, the mtDNA cytochrome *b* did not show significant structuring (Supplementary Table 2.2). The weak genetic differentiation revealed by microsatellites among *A. leucosternon* Eastern African population is in agreement with the finding reported for the Eastern Indian Ocean populations, albeit with a lower magnitude of genetic differentiation (Eastern Africa F_{ST} = 0.00252: distance of ~1500 KM and Eastern Indian Ocean F_{ST} = 0.0063: distance of ~ 6000 KM) (DiBattista *et al*., 2016). However, despite the significant F_{ST} and D_{EST} value, results for pairwise F_{ST}, PCOA, and hierarchical AMOVA failed to identify any genetic break among the Eastern African population, supporting the hypothesis of genetic homogeneity. It is likely that a homogeneous genetic pattern in *A. leucosternon* is facilitated by its lengthy post-larval stage (Randall, 2002), which can provide a mechanism for long-distance dispersal. Furthermore, estimates of genetic dispersals in marine fish with a PLD greater than 2 days, have shown that over 50% of the variance in spatial genetic patterns can be attributed to the duration of planktonic phase (Kinlan and Gaines, 2003). The finding of weak genetic differentiation broadly matches previous studies on other reef fish such as *L. kasmira* (Muths *et al.*, 2012), *A. akallopisos* (Huyghe and Kochzius, 2017), and *S. ghobban* (Visram *et al.*, 2010). However, contrary to these findings *Myripristis berndti* Jordan & Evermann, 1903 (Muths *et al.*, 2011) and *Epinephelus merra* Bloch, 1793 (Muths *et al.*, 2015) show pronounced genetic structures despite having relatively long PLDs (30-80 days). The discordant population structures among these coral reef species may be due to the sporadic permeability of Eastern African marine

barriers, which only restrict larval exchange but do not provide absolute vicariance in marine populations (Muths *et al*., 2011; Muths *et al*., 2015). On the other hand, the lack of a significant relationship between the genetic and geographic distance (F_{ST} R^2 = 0.081, p = 0.32 and D_{EST} R^2 = 0.0006, p = 0.42) confirms that the observed weak genetic differentiation is not attributed to distance restricted dispersal. The general congruence between mitochondrial DNA and nuclear markers in inferences of genetic homogeneity may suggest that connectivity among Eastern African population of *A. leucosternon* occurred deep in the past and has persisted to contemporary times.

Besides, having a lengthy post-larval stage, *A. leucosternon* populations are often found on the outer reef (seaward) habitats of Eastern African lagoons (Pers. obs.), an ecological characteristic that can enhance genetic homogeneity because the spawned larvae have a high chance of occurring in the path of the permanent flowing EACC, which can facilitate long-distance dispersal along the Eastern African coastline. Long distance dispersal among offshore Eastern African marine population has been supported by a recent model-based survey, which indicates that these populations exhibit a higher connectivity compared to their counterparts found in sheltered lagoons (Mayorga-Adame *et al*., 2017). However, recruitment and settlement of these dispersed larvae depend on the availability of suitable habitats as Alberto *et al*. (2010) showed that genetic distance increased with increasing habitat discontinuity and/or fragmentation. This suggests that the continuous Eastern African fringing reef that runs parallel to the Eastern African coastline from northern Mozambique (KL) to northern Kenya (KU), may also play a critical role in promoting larval exchange among different marine populations, preventing the deleterious outcomes of inbreeding and genetic isolation (Bowler and Benton, 2005; Keyghobadi, 2007). It is thus reasonable to argue that other factors (not only dispersal capacity) might also be responsible for the large-scale connectivity of *A. leucosternon* in Eastern Africa.

Interestingly, the STRUCTURE results of K = 2 showed that a few individuals from KR, MO, and TA were genetically distinct from the other populations (Figure 2.3). This pattern of differentiation is not consistent with the effect of barriers to dispersal in the Eastern African marine realm and could suggest occurrence of chaotic genetic patchiness in *A. leucosternon* as a result of pre-settlement selection, post-settlement selection (Johnson and Black, 1984), sweepstake reproduction success (Hogan *et al.*, 2010), variable source of larvae (Selkoe *et al.*, 2006) or kinship aggregations (Selwyn *et al.*, 2016). These phenomena can create an admixture of genetically differentiated individuals within a single local population by facilitating the accumulation of genetically distinct cohorts (Pusack *et al.*, 2014). Alternatively, the few differentiated individuals in these sample sites could be potential hybrids between *A. leucosternon* and *A. nigricans* as these two species are known to hybridize beyond their suture zone (DiBattista *et al*., 2016). However, no morphological differences were detected among the 336 individuals analysed in this study, suggesting that the potential hybrids will likely be backcrossed offsprings of F1 (without excluding F2 or later generations) hybrids and pure *A. leucosternon* parents. Most morphologically different hybrids were identified as an F1 generation, while no morphological difference was observed between backcrossed offsprings and pure parental species (DiBattista *et al*., 2016).

In conclusion, the genetic homogeneity established among *A. leucosternon* populations separated by more than 1000 km suggests that substantial larval exchange occurs among distant populations. Therefore, it is possible to manage these populations as a single unit, following a trans-boundary approach among the coral reef ecosystem of the four countries (Kenya, Tanzania, Mozambique, and Seychelles). This indicates that networks of marine protected areas (MPAs) are likely to be successful if they are implemented following a regional approach rather than a national approach because highly dispersive species such as *A. leucosternon* might have source and sink populations located in jurisdictions of two different Eastern African countries. The

consistency between the findings of our study and a recent larval based modeling study (Mayorga-Adame *et al*., 2017) underpins the need to consider species life history characteristics (e.g PLD) in marine species conservation.

The high level of genetic diversity displayed by *A. leucosternon* parallel to other regional studies on coral reef fishes indicates the ability of this species to withstand environmental changes among the sample sites. Nevertheless, the recovery of acanthurids after fisheries closure was remarkably slower compared to other fish families (McClanahan *et al*., 2007), which underscores the importance of monitoring and assessing artisanal and aquarium fisheries in Eastern Africa, especially with catch composition records from Kenyan reefs showing that *A. leucosternon* contribute up to 16% of all Acanthuridae caught for ornamental export (Okemwa *et al*., 2016).

Acknowledgement

Funding for this project was obtained from the Leibniz Centre for Tropical Marine Research, International Foundation for Science (IFS) (IFS grant no. A/5677-1), and Kenya Marine and Fisheries Research Institute (KMFRI). We thank Patroba Matiku (Tanzanian Fisheries Research Institute) for logistical support, Daniel Ocharo, Joshua Omweri, and Abubakar Athman (KMFRI), as well as January Ndagala, and Shabaan Chikambo for fieldwork assistance. We also thank Sonja Peters (ZMT) for laboratory assistance and Patrick Curry (ZMT) for proofreading the text. The Deutscher Akademischer Austausch Dienst (DAAD) together with National Council of Science and Technology, Kenya (NACOSTI) provided a scholarship to Levy Otwoma. We thank four anonymous Reviewers and the journal Editors for their comments that greatly improved the manuscript

3. CHAPTER - Connectivity of *Acanthurus triostegus*

Genetic population structure of the convict surgeonfish, *Acanthurus triostegus*: a phylogeographic reassessment across its range.

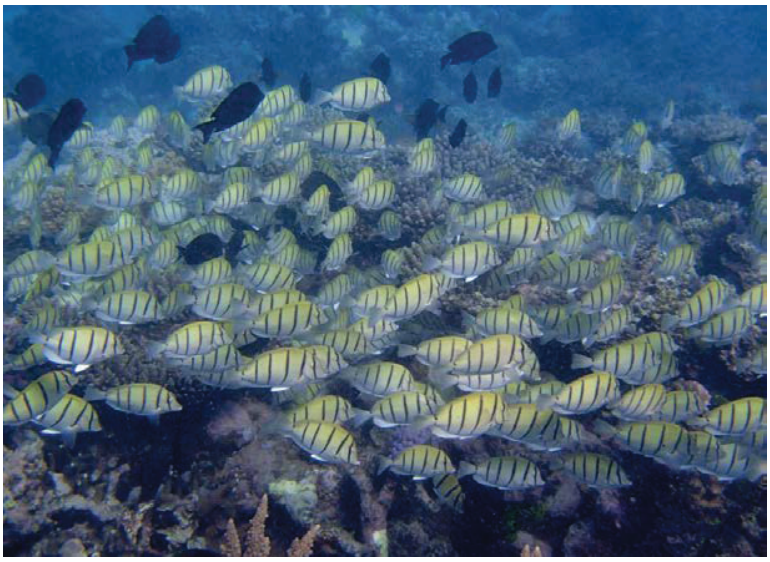
L. M. Otwoma[1,2,3], V. Diemel[1], H. Reuter[1,3], M. Kochzius[4], AND A. Meyer[1]

[1]Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany

[2]Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, Kenya

[3]Faculty Biology and Chemistry, University of Bremen, Germany

[4]Vrije Universiteit Brussel (VUB), Brussels, Belgium



A school of *Acanthurus triostegus* at Vamizi Island. © Tim McClanahan.

This is the authors' version of the work. Please cite the final version as follows:

Levy M. Otwoma, Valeska Diemel, Hauke Reuter, Marc Kochzius, Achim Meyer. (2018). Genetic population structure of the convict surgeonfish *Acanthurus triostegus*: A phologeographic reassessment across its range. **Journal of Fish Biology**. Doi: 1111/jfb.13686.

Abstract

This study investigates the genetic population structure and connectivity of *Acanthurus triostegus* in five Indo-Pacific biogeographic regions (Western Indian Ocean, Eastern Indian Ocean, Western Pacific, Central Pacific, and Eastern Pacific), using a mitochondrial DNA marker spanning the ATPase8 and ATPase6 gene regions. In order to assess the phylogeography and genetic population structure of *A. triostegus* across its range, 35 individuals were sampled from five localities in the Western Indian Ocean and complemented with 227 sequences from two previous studies (Lessios and Robertson, 2006; Liggins *et al*., 2016). Results from the overall analysis of molecular variance (AMOVA) without a priori grouping showed evidence of significant differentiation in the Indo-Pacific, with 25 (8.3 %) out of 300 pairwise Φ_{ST} comparisons being significant. However, the hierarchical AMOVA grouping of Indian and Pacific Ocean populations failed to support the vicariance hypothesis, showing a lack of a genetic break between the two ocean basins. Instead, the correlation between pairwise Φ_{ST} values and geographic distance showed that dispersal of *A. triostegus* in Indo-Pacific follows an isolation-by-distance model. Three haplogroups could be deduced from the haplotype network and phylogenetic tree, with haplogroup 1 and 2 dominating the Indian and the Pacific Ocean, respectively, while haplogroup 3 exclusively occurring in the Hawaiian Archipelago of the Central Pacific.

Key words: Genetic diversity, Kenya, Tanzania, Madagascar, mtDNA, Indo-Pacific Barrier

CHAPTER 3

3.1. Introduction

The Indo-Pacific Barrier (IPB) hinders the movement of tropical marine organisms between the Indian and the Pacific Ocean. Its exact location in the Indo-Australian Archipelago (IAA) is still being debated, but it is widely recognized that the efficacy of this barrier increased during the Pleistocene sea-level low stands (Gaither *et al*., 2010). During the Pleistocene (around 2.6 million to 11 700 years ago) glacial cycles, sea level repeatedly dropped up to 120 m below present, exposing the shallow Sunda and Sahul shelves. At the same time, the Torres Strait between New Guinea and Australia was closed and acted as a land bridge for 90000-100000 years until its inundation ~7000 years ago (Voris, 2000). The strong upwelling of cold water at the base of the Indonesian arc limited dispersal of tropical marine organisms through the few open narrow channels in the eastern Indonesian islands (Voris, 2000). This barrier divided populations that once freely exchanged migrants for tens of thousand years (Benzie, 1999). Although phylogeographic surveys across the Indo-Pacific are still at a nascent stage (Carpenter *et al*., 2011), studies on several taxa have shown a concordant genetic partition between the Indian and the Pacific Ocean. These include teleosts (McMillan and Palumbi, 1995; Planes and Fauvelot, 2002; Kochzius *et al.*, 2003; Bay *et al.*, 2004; Timm *et al.*, 2008; Timm and Kochzius, 2008; Gaither *et al.*, 2010; Mirams *et al.*, 2011), echinoderms (Benzie, 1999; Crandall *et al.*, 2008b; Kochzius *et al*., 2009; Otwoma and Kochzius, 2016), molluscs (Kochzius and Nuryanto, 2008; Kochzius *et al.*, 2009; Nuryanto and Kochzius, 2009; Hui *et al.*, 2016), crustacean (Lavery *et al.*, 1996), and seagrass (Hernawan *et al.*, 2017).

Although this concordant phylogeographic structure in numerous marine taxa may indicate that genetic divergence between the two ocean basins was caused by extrinsic factors such as the sea-level fluctuations during the Pleistocene (Hernawan *et al.*, 2017), a number of species lack this phylogeographic break. These include echinoderms *Eucidaris metularia* (Lamarck 1816) (Lessios *et al.*, 1999) and *Diadema savignyi* (Audouin 1809) (Lessios *et al.*, 2001); marine

gastropods *Echinolittorina reticulata* (Anton 1838) (Reid *et al.*, 2006) and *Thyca crystallina* (Gould 1846) (Kochzius *et al.*, 2009); and the teleost fishes *Naso vlamingii* (Valenciennes 1835) (Klanten *et al.*, 2007), *N. brevirostris* (Cuvier 1829) and *N. unicornis* (Forsskål 1775) (Horne *et al.*, 2008). The lack of genetic divergence in some of the species that span the Indo-Pacific has been interpreted as the loss of one of the two divergent lineages due to local extinction or selective sweeps (Grant and Bowen, 1998), or reestablishment of gene flow after the barriers were dissipated by sea-level rise (DeBoer *et al.*, 2008; Gaither *et al.*, 2011a; Liu *et al.*, 2012). On the other hand, it is also possible that the ranges of these species did not span the IAA during Pleistocene multiple glaciations (Crandall *et al.*, 2008a).

Several studies on marine shallow water species show a higher degree of genetic differentiation among the Indian Ocean populations as compared to their counterparts in the Pacific (Williams and Benzie, 1998; Benzie, 1999; Hui *et al.*, 2016; Otwoma and Kochzius, 2016; Huyghe and Kochzius, 2017). This suggests that not only are populations from the Indian and Pacific separated, but that species in each basin exhibit different patterns of population connectivity. The higher genetic differentiation in the Indian Ocean is attributed to the fewer reefs and island archipelagos available in this basin to facilitate long-distance dispersal through the stepping stone model (Williams and Benzie, 1998; Benzie, 1999). This is particularly true for species that disperse across the Indian Ocean while possessing a limited pelagic larval duration (PLD). Such species show limited larval exchange within the Indian Ocean (Williams and Benzie, 1998; Benzie, 1999; Hui *et al.*, 2016; Huyghe and Kochzius, 2017), possibly due to the vast distance between their suitable habitats (Spalding *et al.*, 2007). On the other hand, species with a PLD reaching 40 – 90 days show genetic uniformity across the Indian Ocean, suggesting that great dispersal ability may play a role in connecting Eastern and Western Indian Ocean population (Craig *et al.*, 2007; Horne *et al.*, 2008; Gaither *et al.*, 2010; Gaither *et al.*, 2011b; DiBattista *et al.*, 2016). However, the relationship between PLD and genetic population structure is not always

straightforward (Selkoe *et al*., 2014), as a positive correlation between the two is reported in some studies (Gaither *et al*., 2010; Gaither *et al*., 2011b), but not in others (Barber *et al.*, 2002; Weersing and Toonen, 2009). This ambiguity suggests that larval dispersal in marine species is not only influenced by PLD but also local oceanographic current conditions (Otwoma and Kochzius, 2016; DiBattista *et al.*, 2017), larval behaviour (Fisher *et al.*, 2005), and historical processes (Otwoma and Kochzius, 2016).

The convict surgeonfish *Acanthurus triostegus* L. 1758 is widely distributed in the lagoon and seaward reefs of the Indo-Pacific. It feeds predominantly on filamentous algae growing on coral reefs, thus helps to keep them in the coral-dominated state. Reproduction in this species occurs through large spawning aggregations that result in clouds of pelagic fertilized eggs (Hartup *et al.*, 2013). Studies on its post-recruitment stages report an average larval swimming speed of 0.56 m/s, which can be sustained for up to 194 hours (Leis and Carson-Ewart, 1997; Stobutzki and Bellwood, 1997; Fisher and Hogan, 2007). This suggests that *A. triostegus* larvae are capable of actively influencing their dispersal and settlement (Fisher *et al*., 2005). Otherwise, its pelagic larval phase of 40 to 60 days (McCormick, 1999) would facilitate long-distance dispersal, when the mean speed of ocean currents exceeds the average swimming speed of the larvae. The great dispersal potential and wide distribution of *A. triostegus* make it a suitable model to investigate the forces that shape the genetic structure and evolution of marine organisms in the Indo-Pacific. Previous genetic analyses of this species were based on allozymes (Planes, 1993; Planes *et al.*, 1998; Planes and Fauvelot, 2002) or mtDNA (Lessios and Robertson, 2006; Mirams *et al.*, 2011; Liggins *et al.*, 2016) and mainly focused on assessing the genetic structure of *A. triostegus* in the Eastern Indian Ocean (EIO), Western Pacific (WP), Central Pacific (CP), and Eastern Pacific (EP) (Planes, 1993; Planes *et al.*, 1998; Lessios and Robertson, 2006; Mirams *et al.*, 2011; Liggins *et al.*, 2016). Although the study by Planes and Fauvelot, (2002) covers the whole Indo-Pacific, only one population was sampled from the Indian Ocean (Mozambique).

In this study, newly sampled sequences from the Western Indian Ocean (WIO) were added to published sequences from two previous studies (Lessios and Robertson, 2006; Liggins *et al.*, 2016), in order to determine the genetic population structure of *A. triostegus* across its entire range. The aim was to assess the connectivity of *A. triostegus* among WIO reefs and the role contemporary or physical barriers (long-distance between suitable habitats) play in shaping its genetic structure. In addition, the influence of historical barriers on the phylogeography of *A. triostegus* across the IAA was examined. Based on the great dispersal potential of *A. triostegus* connectivity within Indian and Pacific basins was expected. However, the dispersal ability could have played a negligible role in connecting the Indian and Pacific *A. triostegus* populations during the Pleistocene sea-level low stands. Therefore, the intraspecific divergence between the two ocean basins was anticipated.

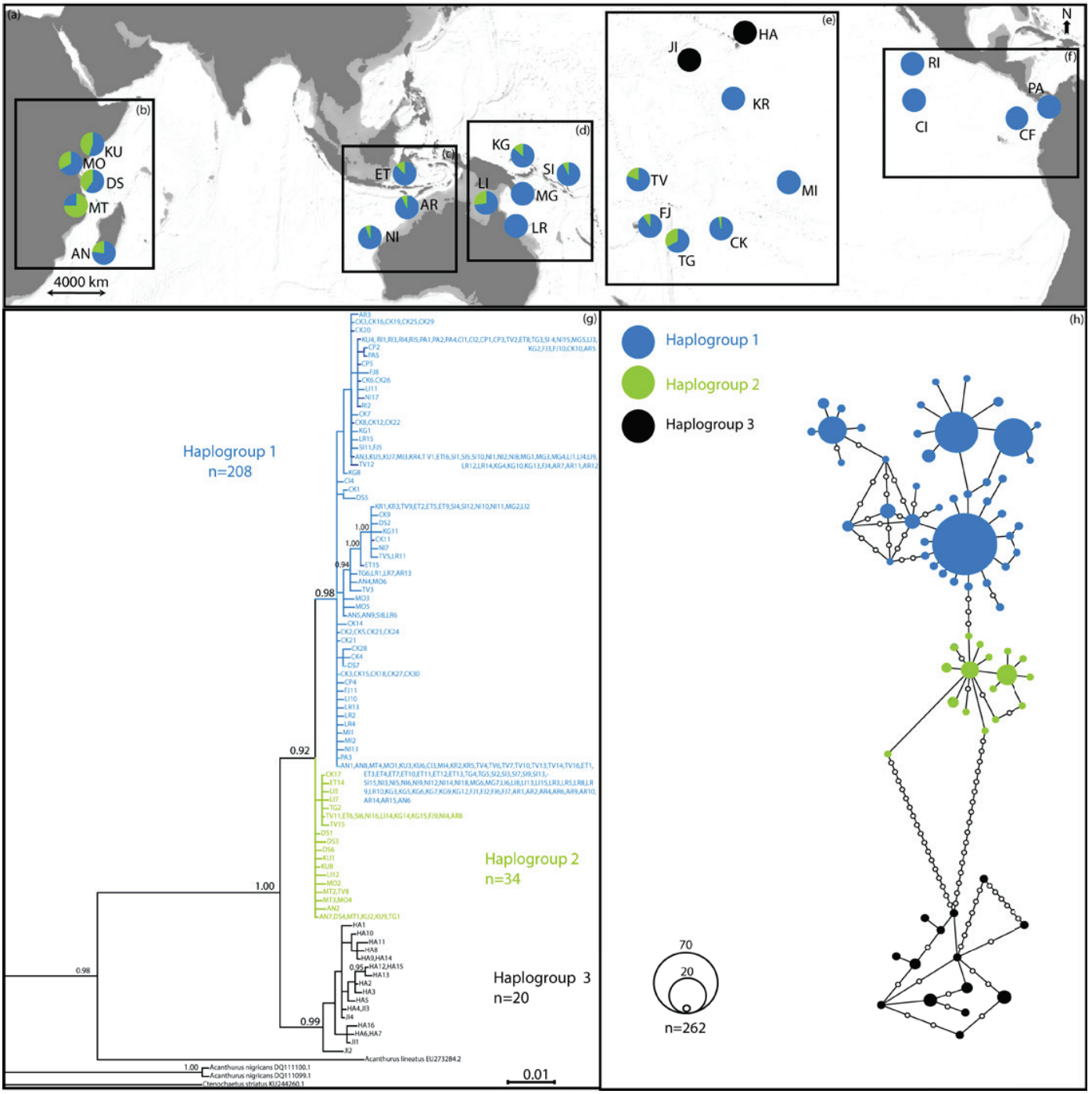


Figure 3.1 Map of Indo-Pacific (a) with *A. triostegus* sample sites, (b) Western Indian Ocean (WIO), (c) Eastern Indian Ocean (EIO), (d) Western Pacific (WP), (e) Central Pacific (CP), and (f) Eastern Pacific (EP). Light grey areas on the map indicate the Pleistocene sea-level low stands 120 m (Voris, 2000). (g) Majority rule consensus tree from the Bayesian phylogenetic analysis using the HKY +I + G model showing the three defined haplogroups. Posterior probabilities above 0.9 are shown at the respective nodes. (h) Minimum spanning network based on ATPase sequences. The filled circles represent haplotypes and their size proportional to their absolute frequencies. Lines represent single mutational steps, while small white circles are the missing intermediate haplotypes. Pie charts on the map (Figure 3.1(a)) illustrate the proportion of each haplogroup at different sampling sites. Abbreviation of sample sites is given in Table 3.1.

3.2. Materials and methods

3.2.1. Sampling and DNA extraction

Adult *A. triostegus* were caught between June and December 2015 by local fishermen using spear gun, gill net, basket trap, and beach seine from 5 localities in the WIO region (Figure 3.1(b) and Table 3.I). Fin clips were cut from each individual and stored in 96% ethanol prior to DNA extraction. The genomic DNA was extracted by the standard salting precipitation method (Sunnucks and Hales, 1996).

Table 3.1 Summary of *A. triostegus* genetic diversity indices for the georeferenced sequence samples. Number of sequences (n), number of haplotypes (NhP), haplotype diversity (h), and nucleotide diversity (π). WIO: Western Indian Ocean, EIO: Eastern Indian Ocean, WP: Western Pacific, CP: Central Pacific, and EP: Eastern Pacific.

Sample site	Biogeographical region	Sample code	n	NhP	h	π	Source of sequences
Dar Es Salaam, Tanzania	WIO	DS	7	7	1	0.009	Present study
Kiunga, Kenya	WIO	KU	9	6	0.92	0.006	Present study
Mombasa, Kenya	WIO	MO	6	6	1	0.008	Present study
Mtwara, Tanzania	WIO	MT	4	4	1	0.005	Present study
Anakao, Madagascar	WIO	AN	9	6	0.89	0.005	Present study
Ashmore Reef, Indian Ocean	EIO	AR	15	6	0.71	0.002	Liggins *et al*., 2016
East Timor, Indonesia	EIO	ET	16	7	0.74	0.006	Liggins *et al*., 2016
Ningaloo, Australia	EIO	NI	18	9	0.84	0.005	Liggins *et al*., 2016
Kavieng, Papua New Guinea	WP	KG	15	7	0.82	0.005	Liggins *et al*., 2016
Lihou Reefs, Australia	WP	LR	15	9	0.89	0.004	Liggins *et al*., 2016
Lizard Islands, Australia	WP	LI	15	10	0.91	0.007	Liggins *et al*., 2016
Motupore Island, Papua New Guinea	WP	MG	7	4	0.81	0.004	Liggins *et al*., 2016
Solomon Islands	WP	SI	15	7	0.82	0.005	Liggins *et al*., 2016
Cook Island	CP	CK	30	16	0.93	0.005	Liggins *et al*., 2016
Hawaii, USA	CP	HA	16	13	0.98	0.006	Liggins *et al*., 2016; Lessios & Robertson, 2006
Fiji	CP	FJ	11	7	0.87	0.004	Liggins *et al*., 2016
Johnston Island, USA	CP	JI	4	4	1	0.006	Lessios & Robertson, 2006
Kiritimati, Kiribati	CP	KR	5	3	0.8	0.006	Lessios & Robertson, 2006
Marquesas Islands, France	CP	MI	4	4	1	0.002	Lessios *et al*., 2006
Tonga	CP	TG	6	5	0.93	0.007	Liggins *et al*., 2016
Tuvalu	CP	TV	16	10	0.83	0.007	Liggins *et al*., 2016
Clipperton Island, France	EP	CF	5	4	0.9	0.003	Lessios & Robertson, 2006
Cocos Island, Costa Rica	EP	CI	4	3	0.83	0.003	Lessios & Robertson, 2006
Panama	EP	PA	5	3	0.7	0.002	Lessios & Robertson, 2006
Revillagigedos Islands, Mexico	EP	RI	5	2	0.4	0	Lessios & Robertson, 2006

3.2.2. Amplification and sequencing

A fragment of 842 bp of the ATPase8 and ATPase6 gene regions was amplified through polymerase chain reaction (PCR) using the primers described by Lessios and Robertson, (2006): ATP8.2 (5' AAAGCRTYRGCCTTTTAAGC 3') and CO3.2 (5' GTTAGTGGTCAKGGGCTTGGRTC 3'). All PCRs were conducted in a total volume of 25µl that included 2.5µl buffer C (Roboklon), 1µl dNTPs (10mM), 1µl $MgCl_2$ (25mM), 0.5µl BSA (10mg/ml), 0.5µl of each primer (10µM), 0.125µl Taq DNA polymerase (5U/µl) and 1µl of DNA template (100-300ng). The temperature profile consisted of 94 °C for 5 minutes, 39 cycles of 94 °C for 30 seconds, 54 °C for 40 seconds, 72 °C for 1 min and a final extension at 72 °C for 5 minutes, as described by Lessios and Robertson, (2006). Purification of the PCR products was done using the ExoSAP clean-up kit (ThermoFisher scientific) following the manufacturer's protocol. Sequencing was done using a DyeDeoxy terminator (Applied Biosystems) and an automatic sequencer (ABI PRISM 310 and 3100, Applied Biosystems).

These 35 sequences from five WIO localities were combined with 227 sequences (GenBank accession numbers: KJ779682.1-KJ779871.1 and DQ111127.1-DQ111163.1) from two previous studies (Lessios and Robertson, 2006; Liggins *et al.*, 2016) (Table 3.1).

3.2.3. Data analysis

Genetic diversity

Sequences were edited, trimmed and aligned using Muscle (Edgar, 2004) as implemented in Geneious version 8.1.6 (Kearse *et al.*, 2012). To ensure that only functional mitochondrial DNA sequences were used, all sequences were translated into amino acids in Squint Alignment Editor version 1.0.2 (Goode and Rodrigo, 2007). Thereafter, haplotypes were identified using the online web services of FaBox version 1.41 (Villesen, 2007). The haplotype (h) and nucleotide (π) diversity were calculated in Arlequin version 3.5 (Excoffier and Lischer, 2010).

Phylogenetic analysis

The phylogenetic inference was based on *A. triostegus* haplotypes, with sequences from the three sister species *Acanthurus lineatus* L. 1758 (EU273284.2), *Acanthurus nigricans* L. 1758 (DQ111100 and DQ111099), and *Ctenochaetus striatus* (Quoy & Gaimard, 1825) (KU244260) being used as outgroups. *Ctenochaetus striatus* was used to root the tree. The Akaike and all other criteria implemented in jModelTest version 2.1.10 (Posada, 2008) suggested the HKY+I+G as the best-fit model of evolution for the ATPase sequences. Bayesian phylogenetic analyses were conducted using MrBayes version 3.2.6 x64 (Huelsenbeck and Ronquist, 2001). Priors were set according to the HKY model with a gamma distribution and allowing for invariable sites (lset nst = 2 rates = invgamma). Two times four Markov chains run in parallel, three heated and one cold, using a random starting tree. All eight chains were run simultaneously for 10 million generations, with trees being sampled every 1000 generations for a total of 80,002 trees. The first 25 % of the trees were discarded as burn-in after confirming convergence of likelihood values of each chain using the commands *sump* and *sumt*. The majority-rule consensus tree containing the posterior probabilities of the phylogeny was determined from 60,002 trees. A spreadsheet program (Microsoft Excel 2010) was used to generate pie charts of the contribution of the different biogeographical regions for identified haplogroups (Figure 3.1(g)).

A minimum spanning network was created using the software PopART version 1.7 (Bandelt *et al.*, 1999) with default settings (Figure 3.1(h)).

Genetic population structure

The level of genetic differentiation among and between sampling locations was estimated by analysis of molecular variance (AMOVA), hierarchical AMOVA, and pairwise Φ_{ST} values in Arlequin, with a significance level of 0.05 and 10,000 permutations. Sequences sampled in

different geographical locations were hierarchically grouped in the AMOVA, according to specific biogeographic hypotheses. The division between the Indian and the Pacific Ocean was tested by contrasting Indian (all samples sites west of Torres Strait) and Pacific Ocean populations (all sample sites east of the Torres Strait). Division within each Indo-Pacific basin was tested by contrasting WIO and EIO populations in the Indian Ocean and WP, CP, and EP in the Pacific Ocean. The linear correlation between pairwise Φ_{ST} values and geographic distances was tested with the software car package in R version 3.2.2, with the shortest marine distance between sampling locations measured to the nearest 5 km in Google Earth. A multidimensional scaling (MDS) plot was drawn in XLstat version 7.5.2 to visualize the genetic differences between Indo-Pacific sample sites.

3.3. Results

3.3.1. Genetic diversity

In total, 262 individuals were used in the analyses, including 35 new sequences from the WIO region (GenBank accession numbers: MF139577-MF139611). The sequence alignment was trimmed to 796 bp, yielding 89 unique haplotypes, 91 substitutions, and 88 polymorphic sites. On the one hand, haplotype diversity was mostly ≥ 0.7 at all the sample sites, with the exception of one sample site in the EP (Revillagigedos Islands). The nucleotide diversity values, on the other hand, ranged from 0 to 0.009 within sample sites. In particular, the EP region had lower nucleotide diversity values than the WIO region; while the CP and WP were characterised by nucleotide diversity values ≥ 0.002 (Table 3.1).

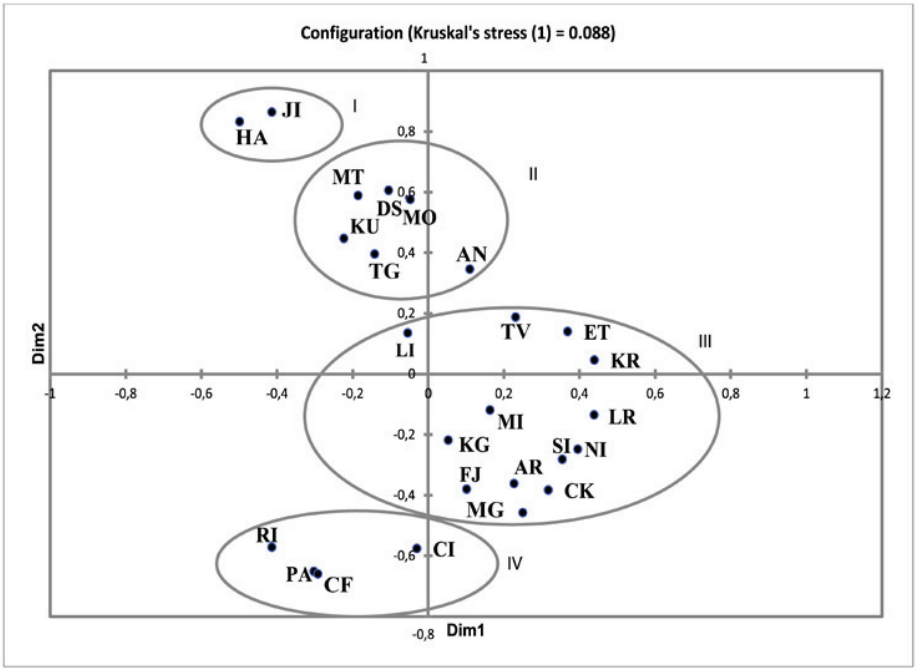


Figure 3.2 A multidimensional scale (MDS) plot of *A. triostegus* pairwise Φ_{ST} estimates among 25 georeferenced sample sites. Groups: I (Hawaii and Johnston Island), II (Western Indian Ocean), III (Central and Western Pacific), and IV (Eastern Pacific). Abbreviation of sample sites is given in Table 3.1.

Table 3.2 Pairwise Φ_{ST} values of georeferenced sequence samples of *A. triostegus* in the Indo-Pacific. Abbreviation of sample sites and biogeographical regions see Table 3.1.

		CP HA	CP MI	CP KR	CP TV	CP TG	CP JI	CP CK	CP FJ	WP KG	WP LR	WP LI	WP MG	WP SI	EP CF	EP PA	EP CI	EP RI	EIO NI	EIO AR	EIO ET	WIO DS	WIO KU	WIO MO	WIO MT
CP	HA	0.00																							
CP	MI	0.84*	0.00																						
CP	KR	0.83*	0.09	0.00																					
CP	TV	0.81*	-0.07	0.00	0.00																				
CP	TG	0.80*	-0.03	0.10	-0.07	0.00																			
CP	JI	0.12	0.86	0.82	0.79	0.77	0.00																		
CP	CK	0.85*	-0.07	0.02	0.01	0.09	0.85	0.00																	
CP	FJ	0.83*	-0.07	0.16	0.02	0.03	0.84	0.00	0.00																
WP	KG	0.83*	-0.10	0.07	-0.03	-0.02	0.82	-0.02	-0.04	0.00															
WP	LR	0.85*	-0.05	0.03	0.01	0.09	0.87*	0.00	0.06	0.02	0.00														
WP	LI	0.79*	-0.05	0.08	-0.03	-0.09	0.77*	0.05	-0.01	-0.03	0.08	0.00													
WP	MG	0.84*	-0.03	0.02	0.02	0.10	0.85	-0.06	-0.04	-0.04	0.02	0.03	0.00												
WP	SI	0.84*	-0.08	-0.01	-0.03	0.03	0.84	-0.04	-0.02	-0.04	-0.02	0.00	-0.06	0.00											
EP	CF	0.85*	0.33	0.35	0.20	0.24	0.87	0.16	0.07	0.16	0.32	0.15	0.09	0.19	0.00										
EP	PA	0.85*	0.36	0.37	0.20	0.25	0.87	0.16	0.07	0.16	0.32	0.14	0.10	0.19	-0.22	0.00									
EP	CI	0.84*	0.00	0.15	0.02	0.05	0.85	-0.03	-0.12	-0.04	0.07	-0.01	-0.09	-0.03	-0.04	-0.04	0.00								
EP	RI	0.86*	0.71	0.56	0.33	0.41	0.91	0.30	0.25	0.31	0.49	0.26	0.32	0.35	0.00	0.00	0.35	0.00							
EIO	NI	0.84*	-0.07	-0.02	-0.02	0.05	0.84	-0.04	0.00	-0.03	-0.02	0.02	-0.05	-0.06	0.19	0.18	-0.02	0.34	0.00						
EIO	AR	0.87*	-0.06	0.21	0.05	0.16	0.90	-0.01	-0.02	-0.01	0.02	0.07	-0.03	-0.01	0.28	0.28	-0.01	0.50	0.00	0.00					
EIO	ET	0.82*	-0.03	-0.07	-0.04	0.00	0.81	0.02	0.07	0.00	0.01	0.02	0.03	-0.02	0.26	0.26	0.07	0.39	-0.02	0.09	0.00				
WIO	DS	0.77*	0.16	0.20	0.09	-0.04	0.72	0.29	0.24	0.18	0.30	0.07	0.28	0.22	0.39	0.40	0.25	0.51	0.24	0.38*	0.14	0.00			
WIO	KU	0.80*	0.09	0.23	0.03	-0.09	0.79	0.17	0.09	0.05	0.22	-0.04	0.17	0.12	0.28	0.29	0.13	0.42	0.14	0.24	0.11	0.00	0.00		
WIO	MO	0.79*	0.08	0.13	0.02	-0.08	0.75	0.21	0.17	0.11	0.18	0.03	0.21	0.14	0.36	0.37	0.18	0.50	0.15	0.29	0.07	-0.01	0.02	0.00	
WIO	MT	0.80	0.47	0.46	0.22	0.06	0.79	0.46	0.42	0.34	0.51	0.16	0.51	0.42	0.63	0.65	0.52	0.78	0.42	0.61	0.32	-0.08	0.04	0.06	0.00
WIO	AN	0.82*	-0.04	0.07	-0.06	-0.09	0.82	0.05	0.06	-0.01	0.03	-0.03	0.10	0.00	0.32	0.32	0.10	0.48	0.02	0.12	-0.02	0.06	0.01	-0.05	0.23

*$p < 0.01$ (after Bonferroni correction)

3.3.2. Genetic population structure

AMOVA and pairwise Φ_{ST} values were non-significant among the WIO sample sites (Φ_{ST} = 0.024, P > 0.05 and Table 3.2), supporting the hypothesis of genetic homogeneity. The genetic similarity between the WIO sample sites is also shown in the MDS plot, with all the five sample sites clustering together (Figure 3.2).

Table 3.3 Hierarchical analysis (AMOVA) based on nucleotide diversity *of A. triostegus* with an alternative grouping of samples sites in the Indo-Pacific. For sample sites and biogeographical abbreviation see Table 3.1.

Grouping	Φ Statistics	P value
Indian Ocean		
WIO (DS,KU,MO,MT,AN)	Φ_{ST} = 0.024	> 0.05
Indian Ocean (DS,KU,MO,MT,AN,AR,ET,NI)	**Φ_{ST} = 0.124**	**<0.05**
WIO (DS,KU,MO,MT,AN) EIO (AR,ET,NI)	**Φ_{CT} = 0.152**	**<0.05**
Pacific Ocean		
(KG,LR,LI,MG,SI,CK,HA,FJ,JI,KR,MI,TG,TV,CF,CI,PA,RI)	**Φ_{ST} = 0.55**	**<0.05**
WP (KG,LR,LI,MG,SI) CP (CK,HA,FJ,JI,KR,MI,TG,TV) EP (CF,CI,PA,RI)	Φ_{CT} = -0.00738	>0.05
WP (KG,LR,LI,MG,SI) CP (CK,HA,FJ,JI,KR,MI,TG,TV)	Φ_{CT} = -0.04961	>0.05
CP (CK,HA,FJ,JI,KR,MI,TG,TV) EP (CF,CI,PA,RI)	Φ_{CT} = 0.03	>0.05
WP (KG,LR,LI,MG,SI) EP (CF,CI,PA,RI)	**Φ_{CT} = 0.23**	**<0.05**
Indo-Pacific		
Indian (DS,KU,MO,MT,AN,AR,ET,NI) Pacific (KG,LR,LI,MG,SI,CK,HA,FJ,JI,KR,MI,TG,TV,CF,CI,PA,RI)	Φ_{CT} = -0.02	>0.05
(DS,KU,MO,MT,AN,AR,ET,NI,KG,LR,LI,MG,SI,CK,HA,FJ,JI,KR,MI,TG,TV,CF,CI,PA,RI)	**Φ_{ST} = 0.53**	**<0.05**

In contrast, strong genetic differentiation was displayed when all sample sites from the Indian Ocean were considered (KU, MO, DS, MT, AN, AR, NI, and ET, Φ_{ST} = 0.124, P < 0.05) (Table 3.3). Further analysis of the hierarchical AMOVA indicated genetic differentiation between the WIO and EIO populations (Φ_{CT} = 0.152, P < 0.05) (Table 3.3).

Across the Pacific Ocean, AMOVA revealed a significant Φ_{ST} value of 0.55 (P < 0.05), with 55 % of variation being among populations and 45 % within populations. However, the hierarchical analysis involving the three biogeographical regions of the Pacific Ocean, i.e WP, CP, and EP did not reject the hypothesis of genetic homogeneity across the Pacific (Φ_{CT} = -0.00738, P > 0.05) (Table 3.3). In particular, the Central Pacific showed genetic similarity to both the WP and EP

(EP-CP Φ_{CT} = 0.03, P > 0.05, WP-CP Φ_{CT} = -0.04961, P > 0.05). However, the exclusion of the CP populations from the hierarchical grouping displayed a pronounced genetic structure (WP-EP Φ_{CT} = 0.23, P < 0.05) (Table 3.3).

On the scale of the entire Indo-Pacific, the overall AMOVA without a priori grouping showed evidence of significant differentiation (Φ_{ST} = 0.53, P < 0.05). The pairwise Φst estimates revealed 25 (8.3%) significant pairwise comparisons after sequential Bonferroni correction, with differences being mostly represented by Hawaii and Johnston Island (Table 3.2). Nevertheless, the hierarchical grouping of Indian (all samples west of the Torres Strait) and Pacific (all samples east of the Torres Strait) populations failed to support the vicariance hypothesis, showing a lack of genetic differentiation between the two ocean basins (Φ_{CT} -0.02 P > 0.05) (Table 3.3). However, the correlation between pairwise Φ_{ST} values and geographic distance was significant (r^2 = 0.19 P < 0.05; Figure 3.3), indicating that dispersal of *A. triostegus* in Indo-Pacific follows an isolation-by-distance model. The isolation-by-distance was also supported by the MDS plot, which showed samples sites from respective biogeographic regions clustering together (Figure 3.2).

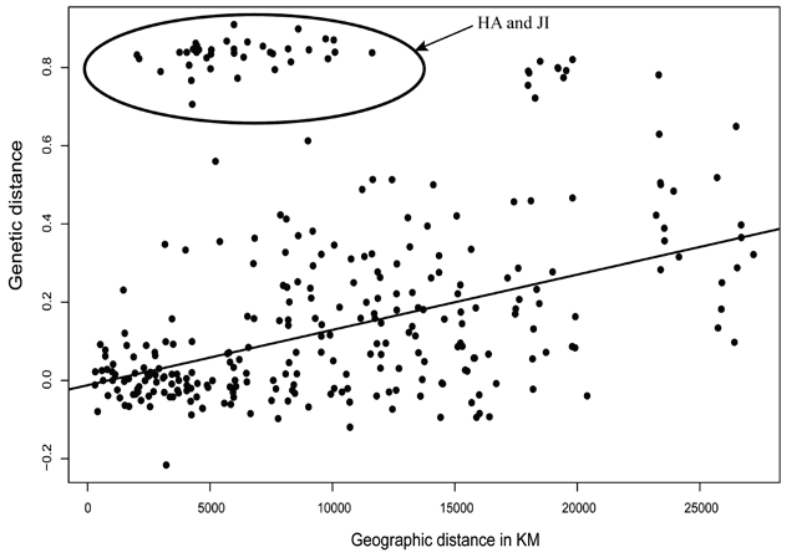


Figure 3.3 A scatter plot of the correlation between the geographic distance (km) and *A. triostegus* pairwise Φ_{ST} estimates for the 25 sampling locations in the Indo-Pacific (r^2 = 0.19 P < 0.05).

In total, three haplogroups can be deduced from the majority consensus tree of the Bayesian analysis (average standard deviation of split frequencies in the sampled trees = 0.007339) (Figure 3.1(g)). Haplogroup 1 (posterior probability = 0.98) and 3 (posterior probability = 0.99) are well supported, while haplogroup 2 appears like a conglomeration of haplotypes branching off haplogroup 1. With the exception of haplogroup 3, which is restricted to Hawaii and Johnston Island, the other two haplogroups are not arranged according to geographical locations. Haplogroup 1 has several shared haplotypes among the five biogeographical regions, with the most extreme sharing being between Panama (EP) and Kiunga (WIO) (Figure 3.1(g) and Figure 3.1 (h)). While haplogroup 2 is also shared between the two ocean basins, its frequency is higher in WIO sample sites (Figure 3.1(b) and Figure 3.1(g)). The haplotype network is characterised by a star-like structure, with dominant haplotypes connected to several singletons (Figure 3.1(h)).

3.4. Discussion

3.4.1 Genetic population structure

WIO and Indian Ocean connectivity

The AMOVA analysis reveals genetic connectivity (Φ_{ST} = 0.024, P > 0.05) across three WIO ecoregions: North Monsoon Current Coast (represented by Kiunga), East African Coral Coast (represented by Mombasa, Dar es Salaam, and Mtwara), and Western and Northern Madagascar (represented by Anakao) (Spalding *et al.*, 2007). Gene flow among *A. triostegus* WIO population is likely to be mediated by its pelagic larval phase and prevailing ocean currents in the WIO (supplementary Figure 3.1). Although the larvae of *A. triostegus* can swim at an average speed of 0.56 m/s (Leis and Carson-Ewart, 1997; Stobutzki and Bellwood, 1997), this is considerably less than the mean speed of the East African Coast Current (1 m/s) and Mozambique channel eddies (> 0.5 m/s) (Swallow *et al.*, 1991; Lumpkin and Johnson, 2013). The interaction of *A. triostegus*

larvae with the strong WIO currents can limit their ability to influence dispersal and settlement (self-recruitment), favouring long-distance dispersal. However, it is unlikely that dispersal in *A. triostegus* is entirely a function of ocean currents (passive), as a large number of larvae would be lost through this mechanism, thinning out its population over ecological time scales (Cowen *et al*., 2000). It is, thus, possible that this species employ both active (short) and passive (long) dispersal mechanisms. Active dispersal between coral reef habitats in the WIO might be mediated primarily by the late stages of *A. triostegus* larvae (Leis and Carson-Ewart, 1997; Stobutzki and Bellwood, 1997), which can sustain their swimming ability for up to 194 hours, covering a distance of 60 nautical miles in a single bout (Stobutzki and Bellwood, 1997). Overall, the results of genetic homogeneity in *A. triostegus* are consistent with the findings of biophysical modeling of connectivity, which indicates that population connectivity in the WIO increases with increase in dispersal ability (Crochelet *et al.*, 2016; Mayorga-Adame *et al.*, 2017). Genetic homogeneity in the WIO has also been observed in other reef fish such as *Lutjanus kasmira* (Forsskål 1775) (Muths *et al.*, 2012), *Scarus ghobban* (Forsskål 1775) (Visram *et al.*, 2010), *Amphiprion akallopisos* (Bleeker 1853) (Huyghe and Kochzius, 2017), *Dascyllus trimacullatus* (Rüppell 1829) (O'Donnell *et al.*, 2017), and *Acanthurus leucosternon* (Bennet 1833) (Otwoma *et al.*, 2018). Nevertheless, the findings of lack of structure in the WIO for *A. triostegus* have to be interpreted with caution as the number of individuals analysed for this region is low.

The overall AMOVA involving all Indian Ocean sample sites show a strong genetic differentiation (Φ_{ST} = 0.124, P < 0.05), rejecting the hypothesis of genetic homogeneity within the Indian Ocean. Further analysis in the hierarchical AMOVA suggests a differentiation between EIO and WIO *A. triostegus* populations (Φ_{CT} = 0.152, P < 0.05). This genetic differentiation between EIO and WIO has previously been shown in species with PLDs not longer than 22 days such as *Linckia laevigat*a L. 1758 (22 days; Williams and Benzie, 1998; Otwoma and Kochzius, 2016), *Tridacna* spp. (Bruguière 1797) (9-12 days; Hui *et al.*, 2016), *Amphiprion akallopisos* (7-

22 days; Huyghe and Kochzius, 2017), *Acanthaster planci* L. 1758 (14-21 days; Benzie, 1999; Vogler *et al.*, 2012), and *Penaeus monodon* (Fabricius 1798) (~14 days; Duda Jr and Palumbi, 1999; Benzie *et al.*, 2002). However, species with PLDs reaching up to 40 to 90 days display genetic homogeneity across the Indian Ocean. These include *Myripristis berndti* (Jordan & Evermann 1903) (55 days; Craig *et al.*, 2007), *Naso* spp. (60-90 days; Horne *et al.*, 2008), *Acanthurus leucosternon* (~55 days; DiBattista *et al.*, 2016), *Coris cuvieri* (Bennett 1831) (53 days; Ahti *et al.*, 2016), and *Lutjanus kasmira* (20-44 days; Gaither *et al.*, 2010). The findings of this study present the first report of an EIO-WIO differentiation in a species with great dispersal potential (*A. triostegus*; PLD 44-60 days), which is inconsistent with previous studies (Craig *et al*., 2007; Horne *et al*., 2008; Gaither *et al*., 2010; Ahti *et al*., 2016; DiBattista *et al*., 2016). This discordance of genetic patterns in different species spanning the Indian Ocean underpins the suggestion that marine species respond uniquely to the dynamic marine environment (Crandall *et al.*, 2008a). Besides, marine barriers solely based on distance (e.g the barrier between WIO and EIO) are semipermeable in nature and may allow sporadic dispersal across them when conditions are favourable (DiBattista *et al*., 2012), leading to discordant population structures, even in species possessing similar life history characteristics (Lessios and Robertson, 2006; DiBattista *et al*., 2012). This also indicates that PLD alone cannot adequately predict the genetic population structure of marine populations.

Indo-Pacific

Despite the addition of sequences from two peripheral biogeographic regions (WIO and EP) to the Liggins *et al*., (2016) dataset (a largely EIO, WP, and CP dataset), the results of this study do not support the vicariance hypothesis (Φ_{CT} -0.02, $P > 0.05$). This genetic pattern largely matches the findings of an earlier study on *A. triostegus* using COI as a marker (Mirams *et al.,* 2011). The general concordance between ATPase (present study) and COI (Mirams *et al*., 2011) in

inferences of the phylogeographic pattern is due to the same mode of inheritance, as both markers are found on the mitochondrial locus. In contrast, an allozyme study on *A. triostegus* across the Indo-Pacific shows a significant genetic differentiation between the Indian and Pacific populations (Planes and Fauvelot, 2002). Similar discordances between mitochondrial DNA and allozymes have been shown in other marine organisms (Elliott, 1996; Williams *et al.*, 2002), with allozymes displaying a higher level of genetic differentiation than mitochondrial DNA. A possible explanation for this difference is that allozymes (nuclear) take a longer time to reach equilibrium between genetic drift and migration than mitochondrial DNA (Williams *et al.*, 2002; Larmuseau *et al.*, 2010), thus, are more reflective of the effect of past historical barriers to dispersal than present-day gene flow. Overall, this finding adds to the growing number of studies that report a lack of genetic divergence at the Indo-Pacific Barrier (IPB) in other shallow water marine taxa (Lessios *et al.*, 1999; Lessios *et al.*, 2001; Reid *et al.*, 2006; Klanten *et al.*, 2007; Horne *et al.*, 2008; Kochzius *et al.*, 2009; Gaither *et al.*, 2010; Gaither *et al.*, 2011*b*) and is in contrast to the effect of lowered sea level during Pleistocene glacial cycles. Sea level repeatedly dropped to up to 120 m below present levels, limiting genetic exchange between the Indian and Pacific populations of various taxa (reviewed extensively by Carpenter *et al*., 2011). The absence of a genetic break in *A. triostegus* is not a confirmation that the Pleistocene sea-level low stands had no effect on this species, but most likely an indication of the quick re-establishment of substantial gene flow between the Indian and Pacific populations of *A. triostegus* since the last isolation by sea level low stands (Horne *et al.*, 2008). This hypothesis is supported by *A. triostegus* great dispersal potential and generalist nature. Unlike other habitat-specific species, *A. triostegus* can occur in highly unstable environments such as tide pools, and bays that could have enabled it to quickly colonize the new habitats along the IPB during sea-level transgression (Mirams *et al.*, 2011).

A lack of a genetic break between the Indian and the Pacific Ocean is also corroborated by the geographical distribution of haplogroups in the Indo-Pacific. On the one hand, haplogroup 1 is found at all samples sites albeit at a lower frequency in the WIO. On the other hand, haplogroup 2 dominates in the WIO but is found at a lower frequency in the EIO, WP, and CP and is absent in EP. Haplogroup 3 is the most divergent group and occurs exclusively in Hawaii and Johnston Island (Figure 3.1). These two sample sites are the documented range for the subspecies *Acanthurus triostegus sandvicensis* (Streets 1877). Streets (1877) noted the differences in the fin ray number, and colouration pattern of *A. triostegus* from Hawaii and Johnston Island, without intergradations to *A. triostegus* from other sites (Schultz and Woods, 1948). This observation led Streets to suggest a separate species *Acanthurus sandvicensis*. However, this is disputed by Randall (1956), who attributes the differentiation to differences in water temperature and geographical isolation of Hawaii and Johnston Island and suggests the rank of a subspecies (*Acanthurus triostegus sandvicensis*). Both Lessios and Roberts (2006) and Liggins *et al*. (2016) report a genetic divergence between *Acanthurus triostegus sandvicensis* and the remaining CP, WP, and EP populations (*Acanthurus triostegus triostegus*). The evolution of this subspecies in Hawaii and Johnston Island is consistent with recent evidence, indicating that peripheral habitats such as Hawaii and Johnston Island are not just evolutionary graveyards, but also produce and export new species to central biodiversity hotspot areas (Eble *et al.*, 2011b; Fitzpatrick *et al.*, 2011; Bowen *et al.*, 2013). The wide distribution of dominant haplotypes in the Indo-Pacific indicates genetic exchange at small and large scale (Figure 3.1 and Figure 3.4). This suggests that the genetic population structure of *A. triostegus* can be explained by a metapopulation migrant pool model, where each population has an equal chance of providing colonizers. Such a dispersal mechanism could have enabled frequent larval exchange between the Indian and Pacific populations, gradually eroding the genetic break between these two basins (Horne, 2014). The great dispersal ability and cosmopolitan nature of *A. triostegus* support this view (Stobutzki and Bellwood, 1997; McCormick, 1999; Leis and Carson-Ewart, 1997; Fisher and Hogan, 2007).

Although the hierarchical AMOVA does not support the existence of a genetic break in the Indo-Pacific, the overall AMOVA without a priori grouping display a strong genetic differentiation in the Indo-Pacific (Φ_{ST} = 0.53, P < 0.05). This can be attributed to a dispersal model that follows isolation-by-distance in *A. triostegus* (r^2 = 0.19 P < 0.05), which has also been demonstrated in a previous allozymes study (Planes and Fauvelot, 2002). The finding of isolation-by-distance is not surprising given the sample sites of this study spread across a geographic distance of more than 28,000 km that is characterised by discontinuous reef habitats. Notably, pairwise comparisons with Hawaii and Johnston Islands populations exhibit higher Φ_{ST} values even at a relatively short distance (Figure 3.3 and Table 2.2), possibly due to self-recruitment presumed to occur at these sites (Wren *et al.*, 2016). The spatial arrangement of samples sites in the MDS plot corresponds to the genetic similarity of sample sites, providing further evidence of isolation-by-distance (Figure 3.2).

3.4.2. Genetic diversity

The genetic diversity estimates revealed mostly high haplotype and low nucleotide diversity values, a pattern common to other marine fishes. High haplotype diversity in this species could be a result of mixing between the Indian and Pacific populations, which is made possible by the great dispersal ability of *A. triostegus*. These molecular diversity indices and star-shaped network signal a population expansion after a period of small effective population size, which is consistent with the effect of Pleistocene multiple glaciations (Grant and Bowen, 1998).

Acknowledgements

Funding for this project was obtained from the Leibniz Centre for Tropical Marine Research (ZMT) and International Foundation for Science (IFS) (IFS grant no. A/5677-1). We thank P. Matiku (Tanzanian Fisheries Research Institute) for logistical support, D. Ocharo, J. Omweri, and A. Athman (KMFRI), as well as J. Ndagala, H. Ratsimbazafy (VUB), and S. Chikambo for sampling and fieldwork assistance. We also thank S. Peters (ZMT) for laboratory assistance. The Deutscher Akademischer Austausch Dienst (DAAD) together with the National Council of Science and Technology, Kenya (NACOSTI) provided a scholarship to L. Otwoma

4. CHAPTER - Genetic population structure and mating behaviour

Comparative phylogeography of surgeonfishes in the Indian Ocean: Genetic population structure of two sympatric sister species with divergent mating behaviour.

Levy Michael Otwoma[1,2,3], Hauke Reuter[1,2], Achim Meyer[1]

[1]Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany

[2]Faculty Biology and Chemistry, University of Bremen, Germany

[3]Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, Kenya

Email: levyot@yahoo.com; levy.otwoma@leibniz-zmt.de

This work is **in preparation** for submission to a journal

Abstract

Disentangling the contribution of biotic/intrinsic and abiotic/extrinsic factors in the structuring of the genetic diversity of reef species is critical to illuminating the diversification of evolutionary lineages in marine environments. However, previous studies have mainly focused on determining the influence of pelagic larval duration (PLD) on the connectivity of reef fishes, whereas few studies have examined the effects of other biotic factors such as mating behaviour, egg mass deposition, and mode of larval development. Here we use mitochondrial DNA (ATPase 6/8) and microsatellite loci to compare the population genetic structure of the spawning aggregating *Acanthurus triostegus* and monogamous spawning *Acanthurus leucosternon*, to determine whether mating behaviour has an influence on the connectivity of *Acanthurus* species. Given that the site fidelity associated with spawning aggregations can enhance larval retention, lower levels of genetic diversity and connectivity were expected in *A. triostegus* compared to *A. leucosternon*. However, contrary to expectation both species displayed genetic homogeneity in the Western Indian Ocean, indicating that mating behaviour has no influence larval dispersal of these *Acanthurus* species. At the scale of the Indian Ocean, the survey of the two *Acanthurus* species revealed divergent population structures, with populations of *A. triostegus* displaying significant genetic differentiation in the Indian Ocean, while *A. leucosternon* exhibits no genetic structure. However, the connectivity patterns displayed by *A. triostegus* was inconsistent with the influence of mating behaviour, suggesting the divergent population structures might be as a result of other factors such as differences in larval swimming ability.

CHAPTER 4

4.1. Introduction

Understanding dispersal in the marine environment is essential because it has a profound influence on species evolution and persistence (Mora and Sale, 2002). For most shallow marine species with a bipartite life cycle, dispersal through the pelagic larvae represents the only mechanism of linking populations between distant sites. However, tracking dispersal in the marine environment remains a major challenge, because marine larvae are minute and suffer high rates of mortality (Sale *et al.*, 2005). Consequently, the application of genetic markers to infer dispersal in marine organisms is increasingly a common practice (Hellberg *et al.*, 2002; Jones *et al.*, 2009). Because larvae of most marine species spend times ranging from days to months in the pelagic marine environment (Sale *et al*., 2005; Almany *et al*., 2007), the population genetic approach predicts that species with a long pelagic larval duration (PLD) will have a high dispersal and weak genetic structure. Indeed, previous studies have shown a correlation between PLD and gene flow (Dawson *et al.*, 2002; Teske *et al.*, 2007; Faurby and Barber, 2012; Barbosa *et al.*, 2013; Riginos *et al.*, 2014; DiBattista *et al.*, 2016). But there is growing number of studies, which demonstrate that the influence of PLD on dispersal distance is often overestimated (Barber *et al.*, 2002; Weersing and Toonen, 2009; Selkoe and Toonen, 2011; Riginos *et al.*, 2013). Furthermore, other features such as past biogeographic events (Barber *et al.*, 2002; Otwoma and Kochzius, 2016), ocean currents (DiBattista *et al.*, 2017), larva swimming ability (Leis and Carson-Ewart, 1997; Fisher *et al.*, 2005; DiBattista *et al.*, 2017), differences in habitat (Rocha *et al.*, 2002), and local adaptation (Imron *et al.*, 2007) have been found to profoundly affect the genetic population structure of marine species.

Comparative phylogeography offers invaluable insights into the factors that drive spatial genetic structuring in codistributed taxa (Papadopoulou and Knowles, 2016). This approach uses the concordance-discordance criterion to determine whether the genetic structure of sympatric species is impacted by abiotic or biotic factors (Papadopoulou and Knowles, 2016). The

assumption of most comparative phylogeographic studies is that taxa evolving in a certain environment respond the same way to extrinsic factors that cause genetic divergence. Nevertheless, co-occurring taxa often show discordant phylogeographic structures, suggesting that species respond uniquely to environmental changes or historical processes (Crandall *et al.*, 2008a; DiBattista *et al.*, 2012; Weber *et al.*, 2015; Puritz *et al.*, 2017). According to Papadopoulou and Knowles, (2016), taxon-specific traits need to be incorporated into comparative phylogeography studies, so as to provide a better understanding of the mode and rate of phylogeographic diversification. For example, Puritz *et al*., (2017) compared the population genetics of the planktonic-developing *Meridiastra calcar* and benthic-developing *Parvulastra exigua* in the temperate waters of Australia and linked their divergent responses to Pleistocene glacial cycles to species-specific traits. Similarly, Weber *et al*., (2015) found that the brooding lineages of the brittle star, *Ophioderma longicauda* displays a higher genetic structure than the broadcast spawner lineage, suggesting that integrating species-specific traits into comparative phylogeographic tests can help to disentangle the existing discrepancy between dispersal ability and genetic structuring of marine species.

Many reef fishes have evolved a reproductive strategy that involves the temporal gathering of sexually mature males and females (100 to 10,000s) at a specific location to spawn (Claydon *et al.*, 2014). Most of the reef fish species that form these temporal spawning gatherings (spawning aggregations) are found in the families Acanthuridae, Scaridae, Serranidae, Pomacentridae, Lethrinidae, Lutjanidae, and Siganidae (Sala *et al.*, 2003; De Mitcheson Yvonne *et al.*, 2008; Gerhardinger *et al.*, 2009; Hartup *et al.*, 2013; Claydon *et al.*, 2014). These spawning aggregations occur at a specific location over a long period of time, indicating a strong degree of site-fidelity by aggregate spawners. Previous studies suggest that connectivity between multiple spawning aggregation sites may be restricted by ocean currents, philopatry (spawning site fidelity), and larval behaviour (Lobel and Robinson, 1988; Cherubin *et al.*, 2011; Jackson *et al.*,

2014). This suggests that substantial genetic differentiation between and among various spawning aggregations may exist among reef fishes (Beldade *et al.*, 2014; Jackson *et al.*, 2014) but see (Zatcoff *et al.*, 2004; Portnoy *et al.*, 2012; Bernard *et al.*, 2016). Therefore, assuming that the site fidelity associated with spawning aggregation does enhance larval retention; species forming spawning aggregations would be expected to have lower genetic diversity and connectivity patterns than non-aggregate spawners (Beldade *et al.*, 2014; Jackson *et al.*, 2014).

To test the influence of reproductive behaviour on the genetic structuring of reef species, we focus on two phylogenetically related surgeonfishes, the powder blue-tang, *Acanthurus leucosternon* and convict surgeonfish, *Acanthurus triostegus* (Sorenson *et al.*, 2013). These two species have clear differences in their range-sizes but are sympatric in large parts of the Indian Ocean (Randall, 1956). Despite being phylogenetically related (Sorenson *et al.*, 2013), these two species differ in aspects of spawning behaviour. *Acanthurus leucosternon* forms monogamous pairings (1 male and 1 female) dispersed throughout the reef (Robertson *et al.*, 1979; Kuiter and Debelius, 2001). In this mating system, the species do not leave their permanent territories to spawn; thus, avoids the risk of losing home territories to non-territory holders (Robertson *et al.*, 1979). *Acanthurus triostegus*, on the other hand, forms resident spawning aggregations, with dense streams of individuals (1000-10,000) migrating to specific sites to reproduce. Generally, the aggregation sites are located approximately 2 km away from the adult home range (Claydon *et al.*, 2014), suggesting that spawning aggregations in *A. triostegus* should occur in every reef where it is present. Previous studies suggest that resident spawning aggregation can persist at a specific site for 12 to ~20 of years (Colin, 1996; Claydon *et al.*, 2014).

PLD estimates among *Acanthurus* species are not remarkably different and range between 40 and 70 days (Thresher, 1984; McCormick, 1999; Rocha *et al.*, 2002). However, Leis and Carson-Ewart, (1997) observed that larvae of *A. triostegus* swam two times (55.7 cm/s) faster than other *Acanthurus* species (24.7 cm/s), suggesting that this species may be more capable of self-

recruitment than other *Acanthurus* species. This prediction of high self-recruitment in *A. triostegus* is consistent with the genetic divergence reported between two geographically close sites (Moorea and Bora-Bora separated by approximately 259 KM) in the Pacific Ocean (Planes and Fauvelot, 2002). Given that the larvae of *A. triostegus* are concentrated at a particular aggregation site, connectivity between multiple spawning aggregations can be restricted by its strong swimming larvae. Therefore, using mitochondrial and nuclear DNA markers we compare the population genetic structure of *A. leucosternon* and *A. triostegus*, to determine whether the reproductive mating behaviour has an effect on the genetic structuring of these coral reef fishes. We expected a higher genetic structuring among populations of *A. triostegus* than *A. leucosternon* in the Indian Ocean because the larvae of the former are concentrated at specific locations during spawning and connectivity between spawning sites may be restricted by its efficient swimming larvae. In addition, we reconstructed the demographic history of these two species to determine whether differences in species-specific traits or habitats played a role in shaping their present phylogeographic structure.

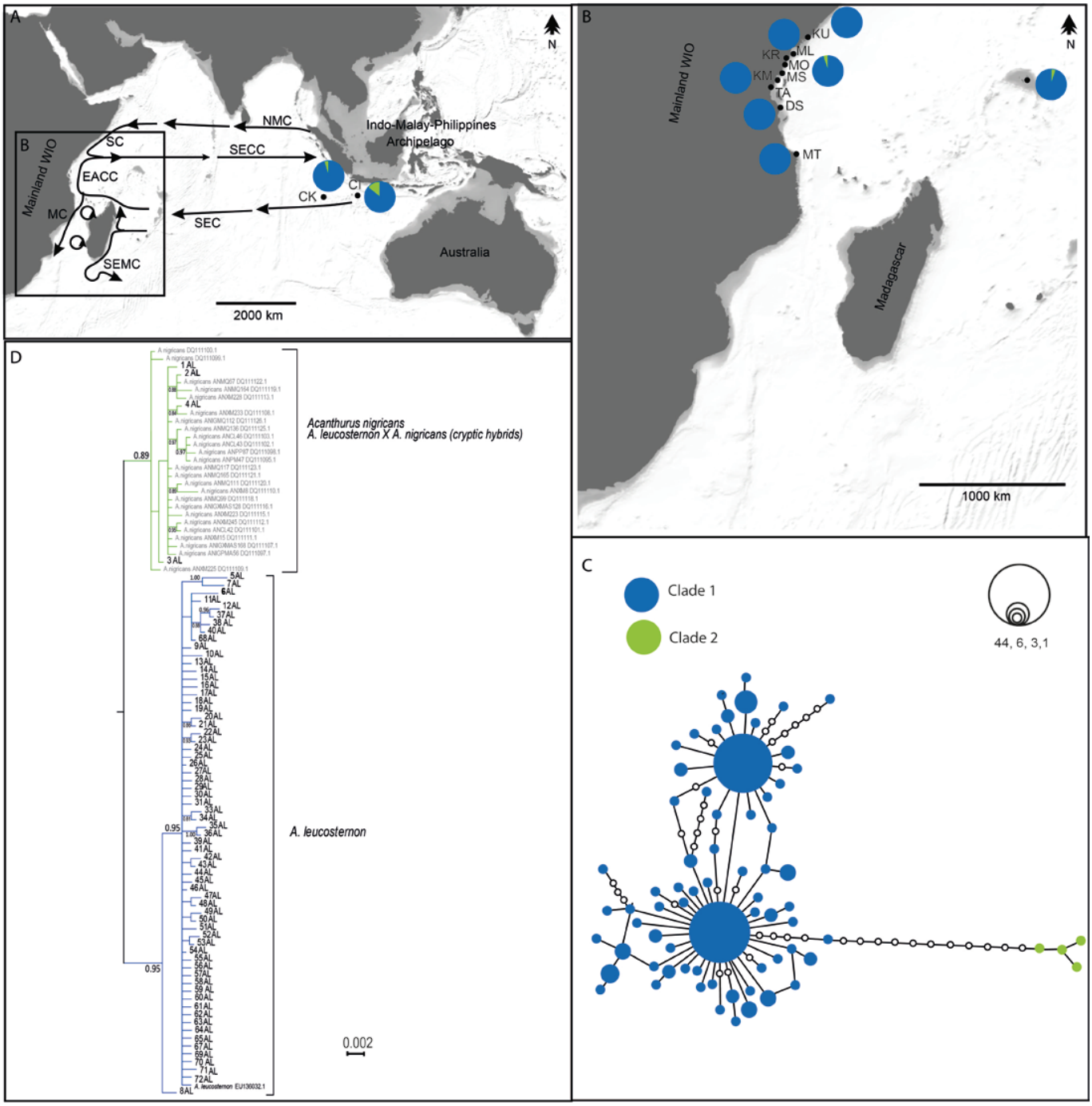


Figure 4.1 Maps showing (A) *A. leucosternon* sample sites in the Indian Ocean, (B) Western Indian Ocean, dominant surface ocean currents (For sample sites abbreviations see Table 4.1 and 4.2). NMC; Northeast Monsoon Current, SECC; South Equatorial Counter Current, SEC; South Equatorial Current, SEMC; Southeast Madagascar Current, MC; Mozambique Current, EACC; East African Coastal Current, and SC; Somali Current. (C) Haplotype network constructed from 785bp fragment spanning the ATPase6 and ATPase8 gene regions of *A. leucosternon*. Large circles and lines represent haplotypes and one mutational step, respectively, while small circles represent intermediate missing haplotypes. (D) A section of a majority consensus Bayesian phylogenetic tree showing only the clade separation in *A. leucosternon* (for the full Bayesian tree see Figure 4.5).

4.2. Materials and methods

4.2.1. Sampling and DNA extraction

Samples of adult *A. triostegus* and *A. leucosternon* were collected at 15 locations in the Indian Ocean, between 2011 and 2015 (Figure 4.1 and Figure 4.2). Fin clips from individual fishes were obtained and stored in 96% ethanol or saturated salt-DMSO solution. DNA extraction was done following the standard salting-out protocol (Sunnucks and Hales, 1996).

4.2.2. Amplification and sequencing of ATPase fragment

A partial fragment spanning the mitochondrial ATPase gene region was amplified for both species through the polymerase chain reaction (PCR) using ATP8.2 (5AAAGCRTYRGCCTTTTAAGC 3') and CO3.2 (5' GTTAGTGGTCAKGGGCTTGGRTC 3') primers (Lessios and Robertson, 2006). The PCR reactions were conducted according to the original protocol (Lessios and Robertson, 2006). Purification of the PCR products was done by incubating with 5U exonuclease I and 1U alkaline phosphatase (both Thermoscientific) following the manufacturer's protocol. Thereafter, sequencing was performed on a DyeDeoxy terminator (Applied Biosystems) and an automatic sequencer (ABI PRISM 310 and 3100, Applied Biosystems). The ATPase dataset for *A. triostegus* was supplemented with sequences from Otwoma *et al*., (2018) and Liggins *et al*., (2016) (Table 4.1).

4.2.3. Amplification and genotyping

Individuals of each species were amplified through PCR using 10 published microsatellites loci: Ahy49, Ahy54, Ahy65, Ahy75, Ahy, Ahy112, Ahy119, Ahy170, Ahy178, Ahy182, and Ahy203 (Dibattista *et al*., 2011). PCR reactions and conditions followed protocol described in Otwoma *et al*. (2018). Labelled PCR products were pooled for genotyping and resolved on ABI 3730 genetic analyser alongside a labelled internal size standard (AlexaFluor 660 (IBA GmbH)-labelled). Microsatellite allele sizes were manually scored using Geneious version 8.1.6 (Kearse *et al.*,

2012). Genotyping was repeated for 80 randomly chosen individuals for each species to check for possible misamplification and scoring errors. It should be noted that only samples from nine WIO samples sites were genotyped (Table 4.2), because the remainder of the samples either became available at a later stage of the study (Seychelles, Christmas Island, and Cocos-Keeling) or were completely not available to us (Ningaloo, Ashmore Reef, and East Timor).

4.2.4. Data analysis

ATPase

ATPase sequences were aligned and trimmed in Geneious version 8.1.6 (Kearse *et al.*, 2012). Thereafter, sequences were deposited in GenBank under accession numbers (xxx-xxx). Arlequin version 3.5 was used to calculate haplotype and nucleotide diversities at each sampling location and in each species. Genetic differentiation among and between sample sites was tested using single-level analysis of molecular variance (AMOVA), hierarchical AMOVA, and pairwise comparison in Arlequin. All analyses were permuted 10,000 times at a significance level of 0.05. We used the online IBDWS services to test the relationship between geographic distance and all Indian Ocean Φ_{ST} estimates in both species. Corrected Akaike Information Criterion (AICc) implemented in jModelTest version 2.1.9 (Darriba *et al.*, 2012) was used to select the best substitution model for our datasets: HKY+G for *A. leucosternon* and HKY+G for *A. triostegus*.

The neutral evolution of the ATPase marker was tested by FU' FS tests for each species (Fu, 1997). Significant negative FU' FS values indicate either selective sweeps, purifying selection, or population expansion after a genetic bottleneck (Fu, 1997). The signature of population expansion after a bottleneck was confirmed by comparing simulated and observed mismatch distribution in Arlequin (Fu, 1997; Schneider and Excoffier, 1999). A unimodal mismatch distribution indicates a population that has undergone a recent and fast demographic expansion, while a multimodal mismatch distribution suggests a population under demographic equilibrium. The Bayesian Skyline Plot (BSP) in BEAST version 1.8.4 (Drummond and Rambaut, 2007) was

used to examine changes in effective population size (Ne) through time. The BSP analyses were run under HKY+G (*A. leucosternon*) and HKY+G (*A. triostegus*) substitution models, employing a strict clock. We used the ATPase 8 and 6 average within species substitution rate of 1.3 x 10^{-8} per site per year (Lessios & Robertson, 2006) under a fixed prior distribution. The program Tracer version 1.5 was employed to visualize the BSP (Drummond *et al.*, 2005).

Phylogenetic and haplotype network analysis

Newly generated and all public available ATPase6/8 sequences from Acanthuridae plus *Paracanthurus hepatus* KT826539.1 (outgroup) were aligned using Mafft (Katoh *et al*., 2002) with the default options (*-linsi)*. The resulting alignment of 624 Sequences was trimmed to the same length of 785bp in BioEdit (Hall, 1999). The software Alter (Glez-Pena *et al*., 2010) was used to collapse identical haplotypes resulting in the final alignment of 217 sequences. Subsequently the best suited substitution model was selected using the corrected Akaike Information Criterion (AICc) as implemented in jmodeltest (Posada, 2008). A phylogenetic tree was constructed from MrBayes version 3.2.6 x 64 (Huelsenbeck and Ronquist, 2001). Priors were set according to the suggested HKY model with gamma distribution. Two times four Markov chains run in parallel, three heated and one cold, searching from a random starting tree. All eight chains were run simultaneously for 10 million generations with sampling every 1000 generations. The first 25% of the trees were discarded as burn-in after confirming convergence of likelihood values of each chain using the command *sump*. The majority-rule consensus tree with posterior probabilities was determined from the remaining 60,002 trees using the command *sumt conformat=simple* and visualized in Mega 6.0 (Tamura *et al*., 2013). A minimum spanning network was created using the software PopART version 1.7 (Bandelt *et al*., 1997) using the default settings.

Microsatellites

The deviation from the expectations of Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) was examined for each locus and sample site using GENEPOP version 4.2 (Raymond and Rousset, 1995; Rousset, 2008). Micro-checker version 2.2.3 was used to screen for the presence of null alleles and large allele dropout (van Oosterhout *et al.*, 2004). For each sample site, the mean number of alleles (Na), expected heterozygosity (He), observed heterozygosity (Ho), and private alleles were estimated in GenAlex version 6.5 (Peakall and Smouse, 2012). The average allelic richness (Ar) and inbreeding coefficient (F_{IS}) were calculated for each sample site using FSTAT version 2.9.3.2 (Goudet, 1995).

The hypothesis of homogeneous allele frequency and genotype distributions among sample sites was tested using FreeNA (Chapuis and Estoup, 2007). FreeNA was chosen because it uses the ENA (Excluding Null Alleles) method to provide for an accurate estimation of F_{ST} in the presence of null alleles (Chapuis and Estoup, 2007). Additionally, the relationship between genotypes and geographical locations was evaluated using the discriminant analysis of principal components (DAPC) in Adegenet version 2.0.2 (Jombart *et al.*, 2010). Unlike Bayesian clustering methods, DAPC can be performed in situations where the assumptions of Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) have not been met.

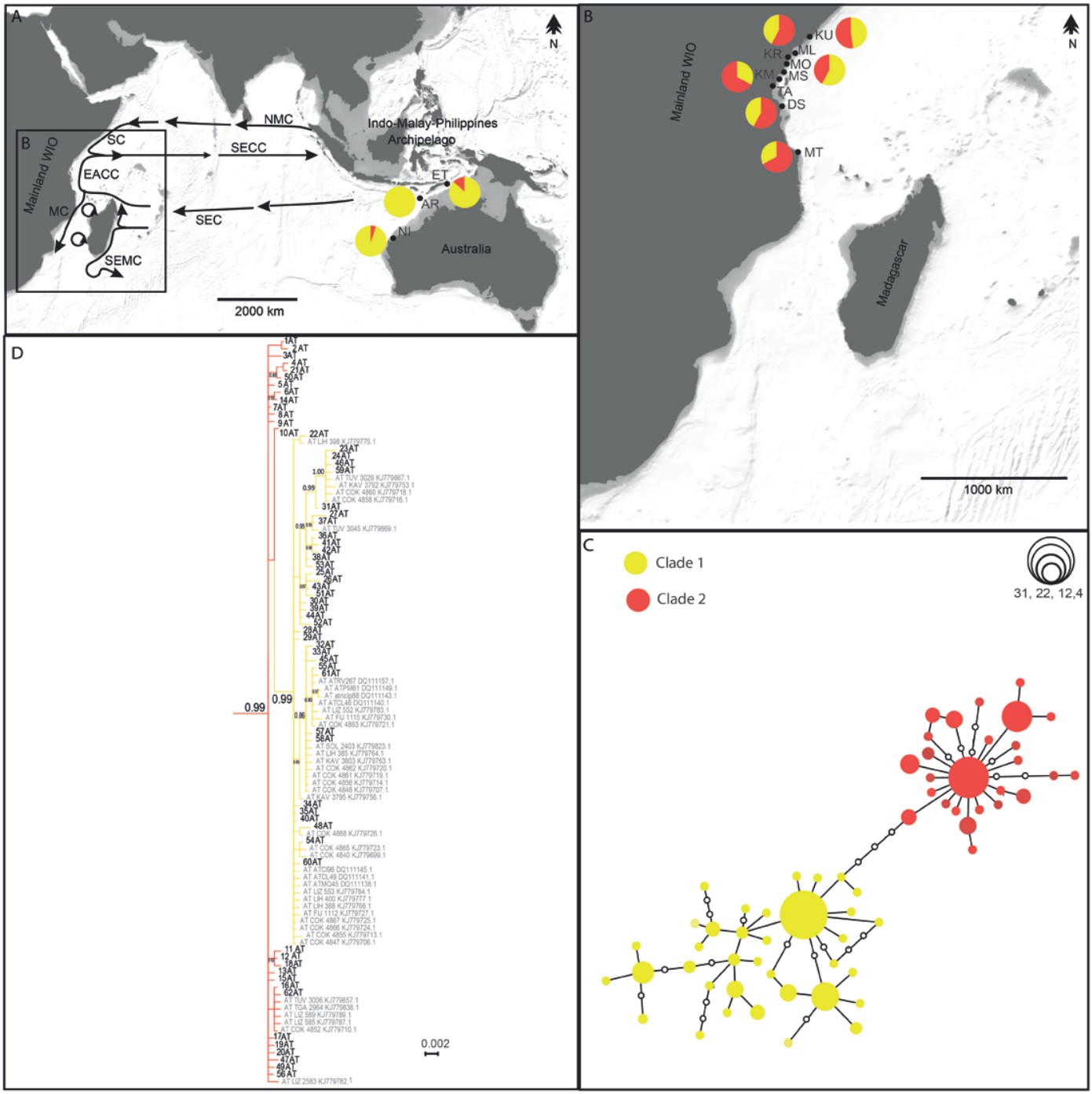


Figure 4.2 Maps showing (A) *A. triostegus* sample sites in the Indian Ocean (B) Western Indian Ocean, dominant surface ocean currents (For sample sites abbreviations see Table 4.1 and 4.2). NMC; Northeast Monsoon Current, SECC; South Equatorial Counter Current, SEC; South Equatorial Current, SEMC; Southeast Madagascar Current, MC; Mozambique Current, EACC; East African Coastal Current, and SC; Somali Current. (C) Haplotype network constructed from 785bp fragment spanning the ATPase6 and ATPase8 gene regions of *A. triostegus*. Large circles and lines represent haplotypes and one mutational step, respectively, while small circles represent intermediate missing haplotypes. (D) A section of a majority consensus Bayesian phylogenetic tree showing only the clade separation in *A. triostegus* (For the full Bayesian tree see Figure 4.5).

The sequential Bonferroni correction was used to adjust the confidence interval of all analysis involving multiple tests (Rice, 1989). The relationship between geographic and genetic distance

was evaluated using a Mantel test in GenAlex for both species. The distance between sampling locations was measured to the nearest 5 km in Google Earth.

4.3. Results

4.3.1. Genetic diversity

A total of 179 *A. leucosternon* and 169 *A. triostegus* were analysed. The sequence alignments were trimmed to 785 bp for *A. leucosternon* and 785 bp for *A. triostegus*, revealing 72 and 62 unique haplotypes, respectively. Haplotype diversity was almost similar between the two species, ranging from 1 to 0.71 (mean = 0.94) in *A. triostegus* and 0.98 to 0.8 (mean = 0.89) in *A. leucosternon* sampling sites. Nevertheless, the mean nucleotide diversity was twofold higher in *A. triostegus* (0.0074 vs 0.0034) (Table 4.1). A two-sample t-test confirmed the significant difference between the nucleotide diversities of the two species (t = 2.11, df = 16, P = 0.0006).

Table 4.1 Genetic diversity of *A. leucosternon* and *A. triostegus* deduced from a fragment spanning 785 bp gene regions of ATPase8 and ATPase6. (n) the number of sequences, (Nhp) number of haplotypes, (h) haplotype diversity, (π) nucleotide diversity, FU'FS, (SSD) sum of square deviations, and (HRI) Harpendig's raggedness index.

Location	code	Biogeographical region	n	Nhp	h	π	FU' FS	SSD	HRI
Acanthurus leucosternon									
Kiunga	KU	WIO	25	14	0.86	0.0035	-7.27**	0.133*	0.025^{ns}
Malindi	ML	WIO	21	15	0.94	0.0035	-10.54***	0.002^{ns}	0.033^{ns}
Mombasa	MO	WIO	20	9	0.8	0.0034	-2.16^{ns}	0.006^{ns}	0.047^{ns}
Kisite-Mpunguti	KM	WIO	19	10	0.84	0.0022	-5.71***	0.0039^{ns}	0.071^{ns}
Dar es Salaam	DS	WIO	15	13	0.98	0.0037	-9.88***	0.023^{ns}	0.101^{ns}
Mtwara	MT	WIO	25	15	0.89	0.0026	-11.31***	0.004^{ns}	0.064^{ns}
Mahe	MH	WIO	25	13	0.88	0.0039	-4.99**	0.0078^{ns}	0.049^{ns}
Cocos-Keeling Island	CK	EIO	22	15	0.92	0.0049	-7.16**	0.039*	0.152^{ns}
Christmas Island	CI	EIO	7	6	0.95	0.0032	-2.71*	0.044^{ns}	0.224^{ns}
All sample sites			**179**	**72**	**0.89**	**0.0034**	**-26.49*** **	**0.0015^{ns}**	**0.041^{ns}**
Acanthurus triostegus									
Kiunga	KU	WIO	21	11	0.91	0.0065	-1.45^{ns}	0.039^{ns}	0.051^{ns}
Malindi	ML	WIO	19	14	0.94	0.0062	-5.72**	0.039^{ns}	0.051^{ns}
Mombasa	MO	WIO	12	12	1	0.0084	-6.74**	0.015^{ns}	0.026^{ns}
Kisite-Mpunguti	KM	WIO	21	15	0.94	0.0076	-4.94*	0.0078^{ns}	0.011^{ns}
Dar es Salaam	DS	WIO	24	20	0.99	0.0085	-10.61**	0.0103^{ns}	0.013^{ns}

Mtwara	MT	WIO	24	14	0.93	0.0066	-3.49^{ns}	0.018^{ns}	0.02^{ns}
East Timor	ET	EIO	16	7	0.74	0.0059	0.69^{ns}	0.052^{ns}	0.13^{ns}
Ashmore Reef	AR	EIO	15	6	0.71	0.0023	-1.06^{ns}	0.059^{ns}	0.17^{ns}
Ningaloo	NI	EIO	18	9	0.84	0.0048	-1.31^{ns}	0.019^{ns}	0.041^{ns}
All sample sites			**169**	**62**	**0.94**	**0.0074**	**-25.01*****	**0.0045^{ns}**	**0.0073^{ns}**

Ns: not significant; *0.05 ≥ P ≥ 0.01; ** 0.01 > P ≥ 0.001; *** P < 0.001

All the ten loci amplified successfully in 305 *A. leucosternon*, while only four (Ahy 49, Ahy 119, Ahy 170, and Ahy 178) amplified consistently in 320 *A. triostegus*. After Bonferroni correction, 1 out of 36 loci in *A. triostegus* and 19 out of 90 loci in *A. leucosternon* deviated from the expectations of HWE. Analysis in Micro-checker suggested that deviations at 5 markers (one in *A. triostegus* (Ahy170 (Tanga)) and four in *A. leucosternon* (Ahy 54 (all populations), Ahy 75 (Malindi, Kuruwitu, Kisite-Mpunguti, and Kiunga) Ahy 182 (Mombasa, Tanga, and Kiunga), and Ahy 203 (Kisite-Mpunguti, Tanga, and Kiunga)) could be due to the presence of null alleles. Nevertheless, there was no evidence of linkage disequilibrium between the loci in both *A.triostegus* and *A. leucosternon* datasets. The mean allelic richness varied between 9.03 (Kuruwitu) and 10.9 (Dar es Salaam) in *A. leucosternon*, and between 5.75 (Tanga) and 6.53 (Mtwara) in A. *triostegus*. Observed and expected heterozygosity in *A. leucosternon* (Ho = 0.81-0.88 and He = 0.84-0.89) were slightly higher than those of *A. triostegus* (Ho = 0.63-0.85 and He = 0.66-0.73) (Table 4.2).

Table 4.2 Microsatellite genetic diversity characteristics of *A. leucosternon* and *A. triostegus*. (n) number of individuals, (Na) number of alleles, (Ne) number of effective alleles, (Ar) allelic richness, (Ho) observed heterozygosity, (He) expected heterozygosity, (PVA) private alleles, and (F_{IS}) inbreeding index.

		Acanthurus leucosternon								***Acanthurus triostegus***							
Location	Code	n	Na	Ne	Ar	Ho	He	PVA	F_{IS}	n	Na	Ne	Ar	Ho	He	PVA	F_{IS}
Kiunga	KU	25	10.3	6.62	9.08	0.82	0.86	1	0.05^{ns}	32	10.75	5.56	6.33	0.82	0.72	1	-0.15^{ns}
Malindi	ML	40	13.5	7.17	10.1	0.86	0.87	1	0.01^{ns}	47	12.5	5.76	6.05	0.68	0.66	1	-0.02^{ns}
Kuruwitu	KR	35	11.7	6.18	9.03	0.86	0.84	3	-0.02^{ns}	46	11.25	6.16	6.19	0.79	0.68	2	-0.11^{ns}
Mombasa	MO	33	13.7	1.73	10.8	0.87	0.89	0	0.02^{ns}	23	8.75	5.55	6.48	0.72	0.73	1	0.02^{ns}
Msambweni	MS	35	13.3	7.04	10.2	0.84	0.86	3	0.03^{ns}	43	11	6.22	6.21	0.79	0.71	4	-0.11^{ns}
Kisite-Mpunguti	KI	51	15	7.21	10.3	0.82	0.86	7	0.05*	34	12	5.92	6.24	0.77	0.69	3	-0.17^{ns}
Tanga	TA	29	11.5	6.65	9.8	0.81	0.85	3	0.05*	26	6.75	3.95	5.75	0.63	0.67	0	0.063*

Dar es Salaam	DS	16	11.2	6.32	10.9	0.86	0.86	2	-0.01^{ns}	33	10	5.66	5.96	0.85	0.71	1	-0.19^{ns}
Mtwara	MT	41	14.7	7.06	10.3	0.88	0.86	5	0.01^{ns}	36	12.5	6.22	6.53	0.69	0.69	7	0.002^{ns}
All sample sites		**305**	**12.7**	**6.93**	**10.1**	**0.84**	**0.86**	**25**	**0.01^{ns}**	**320**	**10.61**	**5.67**	**6.31**	**0.75**	**0.69**	**20**	**-0.074^{ns}**

Ns: not significant; *0.05 ≥ P ≥ 0.01; ** 0.01 > P ≥ 0.001; *** P < 0.001

4.3.2. Genetic population structure

Analysis of molecular variance (AMOVA), based on the ATPase marker indicated genetic homogeneity among the samples of *A. leucosternon* (Φ_{ST} = -0.0047, P = 0.72) and *A. triostegus* (Φ_{ST} = 0.0035, P = 0.35) in the WIO. Correspondingly, pairwise comparisons between and among WIO locations were all nonsignificant for both species (Table 4.3 and Table 4.4). However, AMOVA involving all Indian Ocean locations (WIO and EIO), revealed significant genetic differentiation Φ_{ST} value (Φ_{ST} = 0.15, P < 0.0001) among samples of A. *triostegus*, but remained nonsignificant in *A. leucosternon* (Φ_{ST} = -0.00067, P = 0.49). Further analysis in the hierarchical AMOVA and pairwise comparison suggested that the heterogeneity in *A. triostegus* Indian Ocean samples was due to the differentiation between EIO and WIO (Φ_{CT} = 0.27, P = 0.01) (Table 4.3). The relationship between all Indian Ocean pairwise Φ_{ST} estimates and geographic distance indicated a significant isolation-by-distance in *A. triostegus* (r^2 = 0.75 P < 0.0001), but not in *A. leucosternon* (r^2 = 0.0082, P = 0.59) (Table 4.3, Table 4.4, Supplementary Figure 4.1, and Supplementary Figure 4.2) .

Table 4.3 Pairwise comparison between Indian Ocean populations of *A. triostegus* based on ATPase derived Φ_{ST} estimates. For sample sites, abbreviations see Table 1 and 2.

	KU	**ML**	**MO**	**KM**	**DS**	**MT**	**ET**	**AR**
ML	0.007^{ns}							
MO	0.048^{ns}	0.049^{ns}						
KM	0.009^{ns}	-0.028^{ns}	0.046^{ns}					
DS	-0.001^{ns}	-0.015^{ns}	-0.026^{ns}	-0.009^{ns}				
MT	0.007^{ns}	-0.022^{ns}	0.076^{ns}	-0.025^{ns}	0.006^{ns}			
ET	**0.191****	**0.286*****	**0.095***	**0.237****	**0.146****	**0.275*****		
AR	**0.315*****	**0.454*****	**0.272*****	**0.396*****	**0.288*****	**0.428*****	0.092^{ns}	
NI	**0.241*****	**0.359*****	**0.173****	**0.312*****	**0.214*****	**0.346*****	-0.023^{ns}	-0.002^{ns}

Ns: not significant; *0.05 ≥ P ≥ 0.01; ** 0.01 > P ≥ 0.001; *** P < 0.001

For microsatellites, ENA corrected estimates of AMOVA, revealed low but significant F_{ST} values among WIO samples of *A. leucosternon* (F_{ST} = 0.0025 P < 0.001) and *A. triostegus* (F_{ST} = 0.011 P < 0. 001). Nevertheless, the majority of the variation was explained by differences within locations (*A. leucosternon* 99% and *A. triostegus* 95%). For *A. leucosternon*, the ENA corrected pairwise F_{ST} estimates ranged from 0 to 0.0081 and were all not significant from zero after Bonferroni adjustment (significance level = 0.001) (Supplementary Table 4.1).

Table 4.4 Pairwise comparison between Indian Ocean populations of *A. leucosternon* based ATPase derived Φ_{ST} estimates. For sample sites, abbreviations see Table 1 and 2.

	KU	ML	MO	KM	DS	MT	MH	CI
ML	-0.012^{ns}							
MO	0.009^{ns}	-0.005^{ns}						
KM	0.015^{ns}	-0.009^{ns}	-0.016^{ns}					
DS	0.028^{ns}	0.006^{ns}	-0.003^{ns}	-0.014^{ns}				
MT	0.002^{ns}	-0.008^{ns}	-0.006^{ns}	-0.018^{ns}	0.001^{ns}			
MH	0.002^{ns}	-0.017^{ns}	-0.026^{ns}	-0.014^{ns}	-0.005^{ns}	-0.009^{ns}		
CI	-0.006^{ns}	-0.019^{ns}	-0.026^{ns}	0.002^{ns}	-0.013^{ns}	-0.011^{ns}	-0.019^{ns}	
CK	$\mathbf{0.046}^{**}$	0.023^{ns}	-0.024^{ns}	0.011^{ns}	0.014^{ns}	0.026^{ns}	-0.014^{ns}	-0.023^{ns}

Ns: not significant; *0.05 ≥ P ≥ 0.01; ** 0.01 > P ≥ 0.001; *** P < 0.001

For *A. triostegus*, the ENA corrected pairwise F_{ST} estimates ranged between 0 and 0.0127, with only one pairwise comparison (between Malindi and Kuruwitu) remaining significant after Bonferroni adjustment (Supplementary Table 4.2). The DAPC assignment also supported the lack of significant spatial structure among WIO sample sites in both species (K = 1, Figure 4.3). The isolation-by-distance test using all the nine WIO samples sites analysed with microsatellites was similarly not significant in both species (*A. triostegus* r^2 = 0.03 P = 0.28 and *A. leucosternon* r^2 = 0.07 P = 0.15) (data not shown).

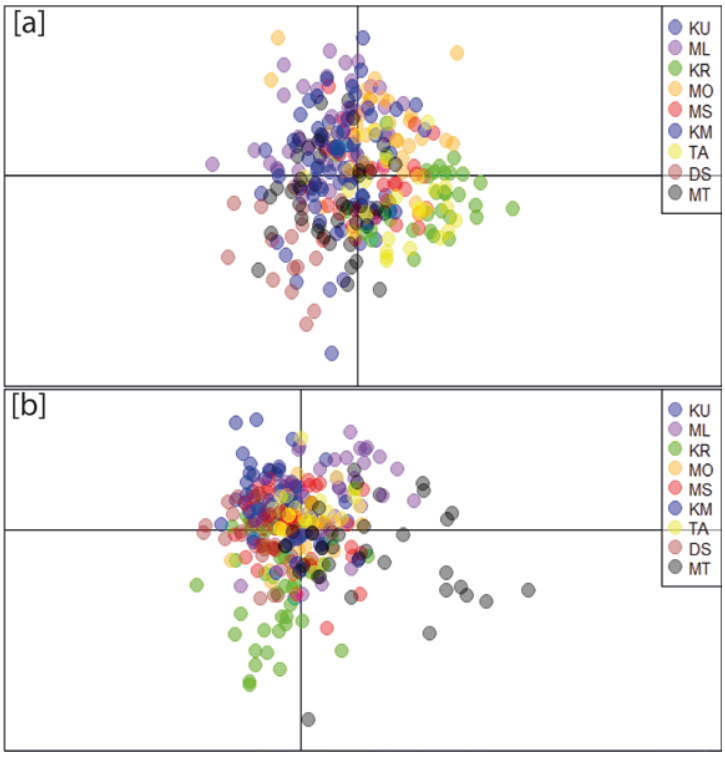


Figure 4.3 Scatter plots of the Discriminant analysis of principal components (DAPC) for (a) *A. leucosternon* and (b) *A. triostegus*, with the legend showing the corresponding locations.

4.3.3. Demographic and phylogeographic analysis

For *A. leucosternon*, the neutral evolution of the ATPase marker was rejected in all the sample sites with the exception of Mombasa in the WIO. On the contrary, negative and significant FU' FS values were only revealed in 5 out of 9 *A. triostegus* sampling sites (Table 4.1). Nevertheless, the mismatch distribution analysis using both the SSD and HRI goodness-of-fit indicated that the model of sudden population expansion could not be rejected in all the Indian Ocean populations for both species (Table 4.1). Similarly, BSP rejected the hypothesis of constant Ne, indicating a population expansion that began ~ 60,000 years ago in *A. leucosternon* (Late Pleistocene) and ~ 125,000 years ago in *A. triostegus* (Mid-Pleistocene) (Figure 4.4).

The phylogenetic analysis and haplotype network revealed two clades for each species (Figure 4.1, Figure 4.2, and Figure 4.5). While clade 1 in *A. triostegus* is found in both eastern and western Indian Ocean, clade 2 is mainly dominant in the western Indian Ocean (Figure 4.2). On the other hand, in *A. leucosternon* almost all individuals are components of clade 1 which is

found throughout the Indian Ocean, while clade 2 appears to be *A. leucosternon* individuals with introgressed *A. nigricans* genes (Figure 4.1).

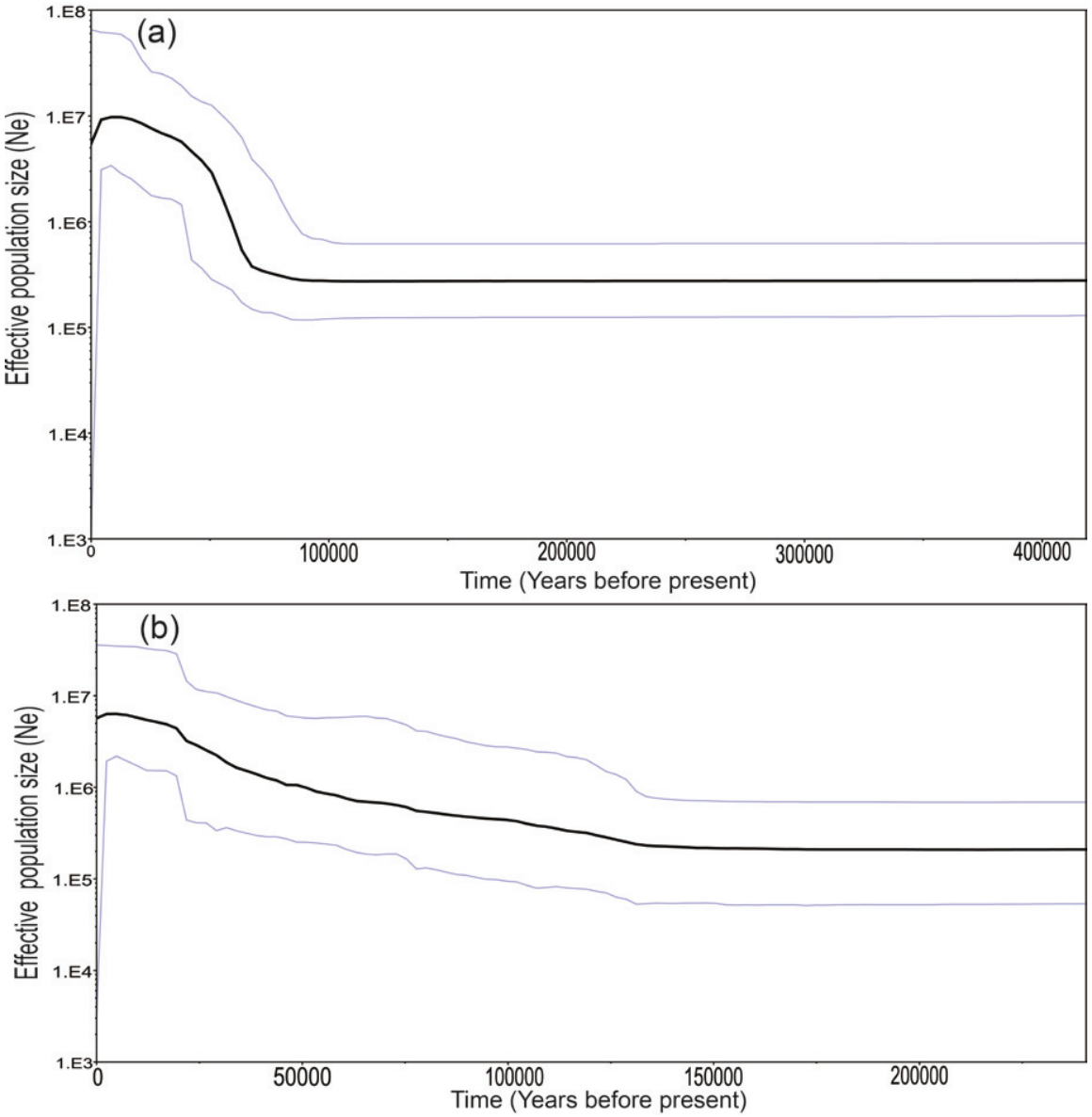


Figure 4.4 Bayesian skyline plots showing the transition of effective population size in a) *A. leuocsternon* and b) *A. triostegus* Indian Ocean populations over time. The thick solid lines are the estimated medians, while the light grey lines represent the 95% posterior density interval.

4.4. Discussion

The present study investigated the genetic population structure of *A. triostegus* and *A. leucosternon*, to determine whether differences in their mating behaviour could lead to differing

connectivity patterns in the Indian Ocean. *A triostegus* aggregates during spawning (Hartup *et al.*, 2013; Claydon *et al.*, 2014), while *A. leucosternon* spawns through the monogamous pairing of 1 male and 1 female (Robertson *et al.*, 1979). Given that the site fidelity associated with spawning aggregations can enhance larval retention, lower levels of genetic diversity and connectivity were expected in *A. triostegus* compared to *A. leucosternon*.

4.4.1. WIO connectivity

Contrary to expectation pairwise comparisons and DAPC showed that both species exist as single panmictic populations in the WIO, rejecting the hypothesis that populations of *A. triostegus* are more structured than *A. leucosternon*. Similar patterns of connectivity in these two *Acanthurus* species can be explained by two common factors. First, the long PLD and year-round spawning of acanthurids (Randall, 1956; Thresher, 1984; Craig, 1998; McCormick, 1999; Rocha *et al*., 2002), could expose the larvae of these two species to the full spectrum of the prevailing ocean currents in the WIO, promoting long-distance dispersal. Interestingly, almost all the WIO sample sites are located in the vicinity of the permanent north-flowing East African Coastal Current (EACC), which flows faster (mean velocity of EACC = 100 cm/s) than *A. triostegus* (55.7 cm/s) or other *Acanthurus* species larvae (24.7 cm/s) (Swallow *et al.*, 1991; Leis and Carson-Ewart, 1997). This suggests that the effect of ocean currents (EACC) could override the influence of other factors in determining the dispersal distances for larvae of both species.

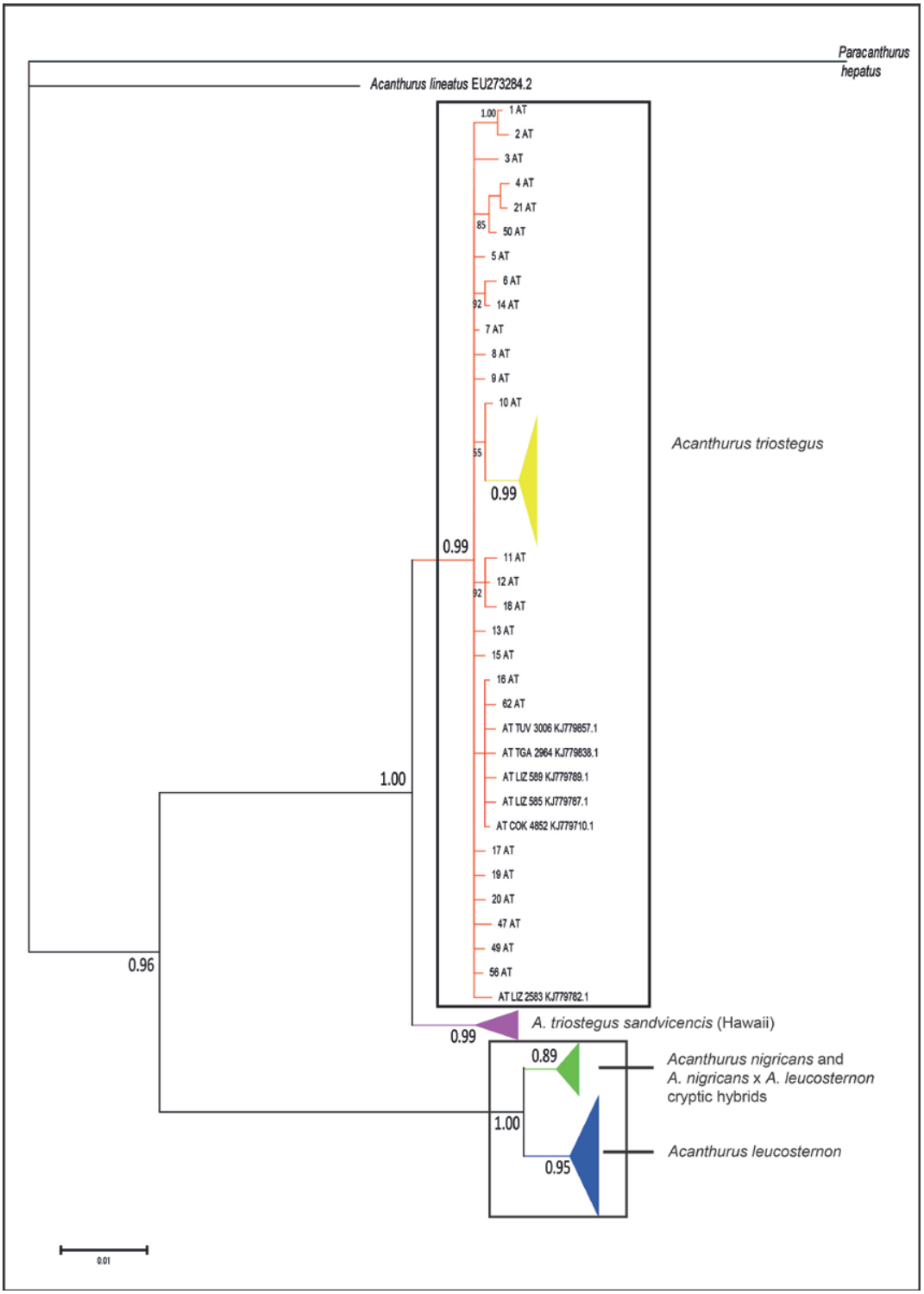


Figure 4.5 A majority consensus Bayesian phylogenetic tree based on combined dataset (*A. leucosternon* and *A. triostegus*) and reference sequences from GenBank. The tree is rooted using *Paracanthurus hepatus* downloaded from GenBank. Only posterior probabilities above 0.5 (50%) are shown

Second, the linear arrangements of coral reef habitats along the Eastern African coastline may be acting as stepping stones for active larval dispersal (through larval swimming) between the

different sampling locations or multiple spawning aggregations, leading to genetic connectivity among *A. triostegus* and *A. leucosternon* populations. However, such a dispersal mechanism often results in isolation-by-distance (Puebla *et al.*, 2009), which was not detected in our microsatellite datasets of the two species. Nonetheless, the magnitude of F_{ST} value revealed by the overall AMOVA in the WIO was far higher in *A. triostegus* (microsatellite: F_{ST} = 0.01 and mitochondrial DNA: Φ_{ST} = 0.0035, P = 0.35) than in *A. leucosternon* (microsatellite: F_{ST} = 0.0025 and mitochondrial DNA: Φ_{ST} = -0.0047, P = 0.72), indicating there are additional factors, which might affect dispersal that differs between these two species. Previous studies on other shallow water marine species have also shown a lack of genetic differentiation between multiple spawning aggregations (Zatcoff *et al.*, 2004; Shaw *et al.*, 2010; Carson *et al.*, 2011; Portnoy *et al.*, 2012; Bernard *et al.*, 2016) but see (Beldade *et al.*, 2014; Jackson *et al.*, 2014).

4.4.2. Indian Ocean divergence

The survey of the two surgeonfishes across the Indian Ocean (EIO and WIO) revealed divergent population structures. Populations of *A. triostegus* display significant genetic differentiation in the Indian Ocean, while *A. leucosternon* exhibits no genetic structure. Although these results are generally consistent with our predictions that *A. triostegus* will have a higher genetic differentiation than *A. leucosternon*, it seems unlikely that these differences stem from behaviour related to their mating strategies. Spawning aggregation events in *A. triostegus* draws individuals to a spawning site located approximately 2 km away from the adult home range (Robertson *et al.*, 1979; Claydon *et al.*, 2014), suggesting that each sampling locations analysed for this species (in the present study) represent a spawning aggregation site. Therefore, if the signature of genetic differentiation in *A. triostegus* is driven by fidelity to spawning aggregation sites, we would expect spatial genetic differences between nearby and distant sampling locations. These expectations are contradicted by *A. triostegus* pairwise comparison (Table 4.3) estimates, which

show that most of the significant pairwise Φ_{ST} values were between distant sites (EIO and WIO sampling localities), rather than within biogeographical regions.

A more feasible explanation for the disparity in the phylogeographic structures could be that the 2 species differ in their larval swimming capabilities. Leis and Carson-Ewart, (1997) determined the average swimming speed of *A. triostegus* larvae (55.6 cm/s) to be twofold higher than that of other *Acanthurus* species (24.7 cm/s). Given that East Timor, Ashmore Reef, Christmas Island, and Cocos-Keeling are located in the slow flowing South Equatorial Current (6.5^{o} S - 12^{o} S, mean velocity = 20 - 24cm/s) (Schott and McCreary Jr, 2001; Lumpkin and Johnson, 2013), it is possible that the larvae of *A. triostegus* interacting with this current have the potential to limit their dispersal distances, while *A. leucosternon* larvae are transported to the WIO. The finding of an isolation-by-distance signature in *A. triostegus* seems to support this prediction, indicating that its strong swimming larvae may favour dispersal between geographically near populations (Puebla *et al.*, 2009), while long distance dispersal may be more sporadic (Planes and Fauvelot, 2002). *Acanthurus leucosternon*, on the other hand, does not exhibit a significant isolation-by-distance, possibly due to substantial long-distance dispersal. In fact, declining populations of *A. leucosternon* at Cocos Keeling and Christmas Island (Marie *et al.*, 2007) may indicate that long-distance dispersal (passive dispersal) exceeds self-recruitment (active dispersal) at these sites, because the latter is required to sustain stable populations at a given location (Cowen *et al.*, 2006). In general, our prediction on the effect of larval swimming capability is consistent with emerging empirical and biophysical models, which suggest that active larval dispersal favour philopatry, larval retention, and self-recruitment (Jones *et al.*, 1999; Cowen *et al.*, 2000; Gerlach *et al.*, 2007; Burgess *et al.*, 2016). Nevertheless, without direct estimates of larval dispersal in *A. triostegus* and *A. leucosternon*, this hypothesis remains largely speculative.

The phylogenetic analysis revealed two clades for each species. In *A. triostegus*, clade 1 is distributed throughout the Indian Ocean, while clade 2 is mainly found in the WIO and occurs at a lower frequency in the EIO. The dominance of clade 2 in the WIO could suggest that it

developed there, after a long-term absence of gene flow between EIO and WIO. But its appearance in the EIO (at lower frequencies) and the wide-distribution of clade 1 in the Indian Ocean, suggest that separation between EIO and WIO populations of *A. triostegus* was not absolute (Figure 4.5). In *A. leucosternon*, the two clades are non-geographic but clade 1 is dominant in all sampling locations, while Clade 2 is rare and appears to be individuals with introgressed *A. nigricans* genes. The occurrence of clade 2 at Mombasa and Mahe in the WIO is consistent with available evidence, suggesting that introgression of *A. leucosternon* with *A. nigricans* genes is more widespread (DiBattista *et al.*, 2016; Otwoma *et al.*, 2018) than previously thought and may result in the merging of the two species into one (Marie *et al.*, 2007).

4.4.3. Demographic history

Both species experienced demographic expansion that dates back to the Pleistocene period when sea-level fluctuations profoundly affected habitat availability (Lambeck and Chappell, 2001; Lambeck *et al.*, 2002). In the Indian Ocean, reef habitats may have been reduced by approximately 90%, when the sea level dropped up to 130 m below present levels (Ludt and Rocha, 2014). This loss of habitats could have restricted the population growth of *A. triostegus* and *A. leucosternon*, which started to expand after the habitats were restored as the sea-level rose. However, the demographic expansion was more dramatic and recent in *A. leucosternon* (expansion time ~ 60,000 years ago: Late Pleistocene) than in *A. triostegus* (expansion time ~125,000 years ago: mid-Pleistocene), possibly due to the differences in species-specific habitat requirements. Unlike *A. leucosternon*, which is often restricted to coral reef habitats, *A. triostegus* can be found inhabiting turbid waters in bays, harbors, and tide pools (Randall, 1956; Robertson *et al*., 1979). According to Kotiaho et al. (2005), species with narrow niche breadth are usually sensitive to habitat disturbance and face a higher risk of extinction. It is, thus, possible that the strict dependence of *A. leucosternon* on coral reefs may have lagged its population expansion

until suitable habitats were available. In contrast, the older expansion time in *A. triostegus* suggests that it may have been able to colonize the unstable and low-quality habitats that became available immediately when sea-level started to rise. This inference is supported by the findings of higher nucleotide diversity in *A. triostegus* than in *A. leucosternon* (Table 4.1), which suggest that the former might have had multiple isolated populations in different refugia that came into contact as sea-level rose to inflate its genetic diversity (Ludt *et al.*, 2012).

In principle, the differences in the levels of nucleotide diversity values may also indicate divergent evolutionary histories in the two *Acanthurus* species (Delrieu-Trottin *et al.*, 2017). *Acanthurus leucosternon* is a young species that diverged from its ancestral clade in the mid-Pleistocene (~600,000 years ago) (Sorenson *et al.*, 2013; DiBattista *et al.*, 2016) and low nucleotide diversity could suggest recent extinction or recolonization events in the Indian Ocean (Pellissier *et al.*, 2014). In contrast, *A. triostegus* diverged from the *Acanthurus* and *Ctenochatus* clade in the Miocene (>20Mya) (Sorenson *et al.*, 2013) and the high nucleotide diversity may suggest that it has had a stable and long demographic history in the Indian Ocean (Pellissier *et al.*, 2014).

Acknowledgements

Funding for this project was obtained from the Leibniz Centre for Tropical Marine Research (ZMT) and International Foundation for Science (IFS) (IFS grant no. A/5677-1). We thank P. Matiku (Tanzanian Fisheries Research Institute) for logistical support, D. Ocharo, J. Omweri, and A. Athman (KMFRI), as well as J. Ndagala, H. Ratsimbazafy (VUB), and S. Chikambo for sampling and fieldwork assistance. We also thank S. Peters (ZMT) for laboratory assistance. The Deutscher Akademischer Austausch Dienst (DAAD) together with the National Council of Science and Technology, Kenya (NACOSTI) provided a scholarship to L. Otwoma.

5. CHAPTER - Exploitation and life history characteristics

Response to exploitation and life history characteristics of two *Acanthurus* species with divergenet mating behaviour along the Kenyan coastline

L. M. Otwoma[1,2,3], H. Reuter[1,3]

[1]Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany

[2]Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, Kenya

[3]Faculty Biology and Chemistry, University of Bremen, Germany

Email: levyot@yahoo.com; levy.otwoma@leibniz-zmt.de

Telephone: +49(0)421 238 00-158

FAX: +49 (0) 421 238 00-30

This work is **in preparation** for submission to a journal

Abstract

Growth parameters (length at first maturity, length at optimum yield, asymptotic length, growth constant, and growth performance index) and mortality from two *Acanthurus* species were compared, to deduce whether differences in species-specific traits (spawning mating behaviour) could lead to differences in exploitation rate. Despite comparable estimates of the von Bertalanffy asymptotic length ($L\infty$) in the two species (*A. triostegus* (27.9-29.6) and *A. leucosternon* (26.1-27.4)), the growth coefficient constant (K) estimates for *A. triostegus* (0.85-1.23) were almost three to fivefold higher than those of *A. leucosternon* (0.29-0.38), suggesting that *A. triostegus* attains its maximum length faster than *A. leucosternon*. Length at first maturity (Lm), length at optimum yield (Lopt), and exploitation rate (E) were also different between the two species, specifically indicating exploitation beyond the sustainable yield in *A. triostegus* but not in *A. leucosternon*. These results are consistent with other findings in the Western Indian Ocean, which report an exceptionally higher exploitation rate in spawning aggregating species compared to the counterparts forming monogamous pairs and may suggest that regardless of body size spawning aggregation increases the susceptibility of species to exploitation.

CHAPTER 5

5.1. Introduction

As fish landings continue to decline in lagoonal reef fisheries (Samoilys *et al.*, 2017), the focus is shifting towards smaller and less preferred species to sustain the increasing effort (Pauly *et al.*, 1998). Among the small-sized individuals under pressure from the increasing fishing effort are surgeonfishes from the family Acanthuridae (Hicks and McClanahan, 2012; Rehren *et al.*, 2018). Although the majority of the individuals in this family are considered low valued, they play important functional roles in maintaining coral reef resilience. In particular, they exhibit a wide range of feeding behavior that includes the consumption of algal and plant communities, which not only facilitate coral recruitment but also provide a linkage for energy flow to higher trophic levels (Crossman *et al.*, 2005). Overharvesting of herbivorous Acanthuridae can impair the ability of the reef ecosystem to maintain its resilience against change to algal-dominated state (Marshell and Mumby, 2015), yet considerably less is known about the demographic information and the exploitation status of most the species in this family.

The genus *Acanthurus* is the most conspicuous and dominant in the family Acanthuridae, representing 40 nominal species. Most of these species occur in the Indian and Pacific Ocean, where they are abundant and occupy more reef habitats than any other fish genus (Randall, 1956; Bellwood *et al.*, 2014; Marshell and Mumby, 2015). *Acanthurus* species are distinguished by the presence of multi-denticulate teeth specialized for cropping the fast-growing reef epilithic algal community (Wismer *et al.*, 2009; Bellwood *et al.*, 2014). However, they display a wide range of length sizes (11 cm in *Acanthurus polyzona* to 70 cm in *Acanthurus xanthopterus*) and demographic or growth parameters (asymptotic length and growth constant) (Choat and Axe, 1996; Choat and Robertson, 2002), suggesting that some species in this genus might be more vulnerable to exploitation than others. More specifically, larger species (in terms of length and weight) may be more vulnerable to exploitation than smaller ones (Taylor *et al.*, 2014). But vulnerability to exploitation among these species may also vary with natural abundance, life

history characteristics, or ease of capture (Sadovy de Mitcheson *et al.*, 2013). Regardless of the causes of vulnerability, it is important to understand the growth parameters and exploitation rate of individual *Acanthurus* species, because application of a uniform management strategy on species with different responses or vulnerability to exploitation would be inappropriate.

To test the species-specific response to exploitation among *Acanthurus* species, we focus on the powder blue-tang (*Acanthurus leucosternon*) and the convict surgeonfish (*Acanthurus triostegus*). These two species are amongst the most conspicuous and dominant groups of coral reef fishes. *Acanthurus triostegus* occurs throughout the Indo-Pacific, whereas *A. leucosternon* is restricted to the Indian Ocean (Randall, 1956). Like other *Acanthurus* species, these two species are primarily herbivores, feeding on benthic algae that inhibits coral recruitment (Crossman *et al.*, 2005). However, they differ in important aspects of their reproductive behaviour, which might affect their susceptibility to fishing. *Acanthurus triostegus* forms massive spawning aggregations of 1000 to 10,000s individuals throughout the year (Hartup *et al.*, 2013), while *A. leucosternon* spawns through monogamous pairing that usually involves one female and one male (Robertson *et al.*, 1979). Because fishing is efficient at removing a large proportion of conspecific individuals when they are gathered at a specific site (Grüss *et al.*, 2014), *A. triostegus* is likely to be more vulnerable to capture and exploitation than the pair occurring *A. leucosternon*. In addition, these two species also appear to differ in their habitat-specificity, while *A. leuocsternon* is often restricted to coral reef habitats; *A. triostegus* can also be found inhabiting turbid waters in bays, lagoons, and harbours (Randall, 1956), which could suggest that *A. triostegus* is able to maintain it is optimum growth performance over a wide range of habitats in marine environments than *A. leucosternon*.

In Kenya, *A.triostegus* and *A. leucosternon* are important components of subsistence, commercial, and aquarium fishery (McClanahan and Hicks, 2011; Hicks and McClanahan, 2012; Okemwa *et al.*, 2016), providing essential protein and income for livelihoods of coastal

communities. The harvesting of these species in Kenya presents an opportunity to assess species-specific response to exploitation in the genus *Acanthurus*. Therefore, in this study, we use fisheries dependent data collected along the Kenyan coastline to evaluate the life history status of *A. leucosternon* and *A. triostegus*. The main objective of the study was to compare the growth parameters (Length at first maturity, length at optimum yield, asymptotic length, growth constant, and growth performance index) and mortality of the two species, to deduce whether differences in species-specific traits (spawning behaviour) could lead to differences in exploitation rate. We expected the exploitation rate of *A. triostegus* to be higher than that of *A. leucosternon* because the former's spawning aggregating behaviour can make it more susceptible to fishing.

5.2. Materials and methods

5.2.1 Study area, state of fishery, and fishing gears

The study was conducted at four sampling sites located along a stretch of approximately 120 km of the southern Kenyan coastline. Two of the landing sites Bamburi and Vipingo are located north of Mombasa city, while Msambweni and Shimoni are found south of Mombasa (Figure 5.1). The four sites were chosen because they represent typical artisanal reef fisheries in Kenya. Marine artisanal fishing in Kenya is mainly carried out in the coral reef ecosystem, with fishermen using human power and simple gear technology to generate large catches (McClanahan and Mangi, 2004; Hicks and McClanahan, 2012). Current estimates on overall artisanal landings show a declining trend despite the increase in effort (McClanahan and Mangi, 2001; Samoilys *et al.*, 2017). To compensate for the dwindling stock, Kenyan fishermen are increasingly adopting fishing gears that are less selective in terms of fish species and size (McClanahan and Mangi, 2004; Samoilys *et al.*, 2017). Gears that are currently used by artisanal fishermen include basket trap, spear gun, beach seine, gillnet, and hand line. Although beach

seine and spear gun are currently banned by fisheries laws (Kenya Gazette Notice No. 7565), they are still in use in large parts of the coastline (Hicks and McClanahan, 2012).

In general, fishermen have a preference for certain species, but small and low valued species are rarely discarded (Obura, 2001). Consequently, small-sized species have become an important component of fishermen harvest, putting them at risk of decline. Nevertheless, previous life history characteristics and exploitation studies in Kenya have focused on species that dominates fishermen catch such as *Siganus sutor*, *Lethrinus lentjan, Lutjanus fulviflamma*, and *Leptoscarus vaigensis* (McClanahan and Hicks, 2011; Hicks and McClanahan, 2012; Tuda *et al.*, 2016), while there is no evaluation of the status of *Acanthurus* species despite long-term Under Visual Census (UVC) surveys, indicating that the abundance of Acanthuridae is significantly lower than estimates made during the last three decades (Samoilys *et al.*, 2017).

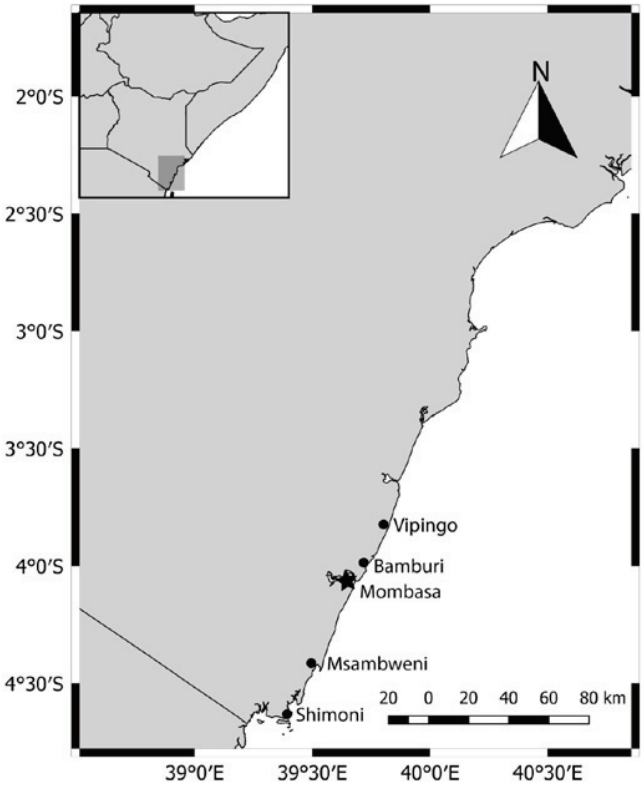


Figure 5.1 Map of the Kenyan coastline showing the four fish landing sites sampled in the present study.

5.2.2. Catch assessments

Landings of *A. leucosternon* and *A. triostegus* were recorded over a period of one year (between September 2015 and April 2017) at the four sites (Figure 5.1). At each sampling site, a data collector measured the total length (distance between the snout and the tip of the longest lobe of the caudal fin) of *A. leucosternon* and *A. triostegus* to the nearest 0.1cm and recorded the type of gear used. Because only two species were being monitored, specimens were identified or confirmed using photographic images of the two species that were made available to every data collector. In total 2432 fish specimens were measured, representing 489 *A. leucosternon* and 1943 *A. triostegus*.

5.2.3. Data analysis

Length-frequency distributions and fishing gears

Length-frequency data (LFQ) of the two species was binned into thirty 1 cm size classes, ranging from 5 cm to 31 cm. The size class of 1 cm was determined based on the maximum length of the fish species (Neumann *et al*., 2012), which maximized the spread of the data for the length-frequency distributions. The quantity of fish landed for each species was graphically examined between gears, with relative abundance (%) being used to represent the numerical dominance of each gear and length groups.

Life history calculations

The length at first maturity (Lm) and length at maximum possible yield (Lopt) were calculated to determine whether growth or recruitment overfishing was occurring in *A. leucosternon* and *A. triostegus*. While growth overfishing occurs when there are not enough juveniles left in the stock to mature and spawn, recruitment overfishing occurs when too many reproductively immature individuals are taken from the stock causing the recruitment potential to be impaired (Hilborn and

Walters, 2001). Both Lm and Lopt were estimated as a function of the asymptotic length (L∞) as shown in equation 1 and 2 (Froese and Binohlan, 2000):

$$Log_{10}\ Lm = 0.8979*Log_{10}L\infty - 0.0782 \quad (1)$$

$$Log10\ Lopt = 1.042*\ Log10L\infty - 0.2742 \quad (2)$$

The estimated Lm and Lopt were compared to the length frequency distributions so as to determine the effect of fishing on the size structure of each species (growth and recruitment overfishing).

The main von Bertalanffy Growth Function (VBGF) parameters L∞, *ø'* (growth performance index), and K (growth constant) of the two species were estimated from the LFQ data, using the R package TropFishR (Mildenberger *et al.*, 2017). This package not only implements the Powel-Wetherall and ELEFAN (Electronic Length Frequency Analysis) methods in R program but also introduces two new algorithms in ELEFAN i.e ELEFAN_SA (simulated annealing algorithm) (Xiang *et al.*, 2013) and ELEFAN_GA (genetic algorithm) (Scrucca, 2013). These new algorithms optimise the VBGF growth curve fitting through the specifications of C (constant indicating the amplitude of oscillation) and t_s (the fraction of the year where the sine wave oscillation turns positive) values, which reduce the stochasticity of the search process to find the best scores for L∞ and K (Mildenberger *et al.*, 2017; Taylor and Mildenberger, 2017).

The Powel-Wetherall method (Wetherall, 1986) was used to obtain the initial estimate of the asymptotic (L∞) in TropfishR. Thereafter, the range of the L∞ estimates from the Powel-Wetherall method was used as seed values to determine the optimum growth coefficient constant (K) and asymptotic length (L∞) in K-scan, response surface analysis (RSA), simulated annealing (SA), and genetic algorithm (GA). All the ELEFAN functions modelled growth parameters following the VBGF equation (3):

$$Lt = L\infty* [1 - e\ (-K\ (t - t_o)] \quad (3)$$

Where Lt is the total length of the fish at a particular age (t), $L\infty$ is the theoretical asymptotic length, K is the VBGF growth constant, and t_0 is the theoretical age when the fish has a length = 0.

Mortality and exploitation rates

The total instantaneous mortality (Z) was estimated by the linearized length converted catch curve (equation 4) described by Pauly, (1984) and as implemented in TropfishR.

$$Ln (C/dti) = a + b*ti \quad (4)$$

Where C is the fish catch grouped in length class, dt is the time change needed for fish to grow through the length class, ti is the relative age of fish of a given length class, and b is the instantaneous total mortality (Z).

Because the empirical estimation of imstantaneous natural mortality (M) varies widely between methods, it is a common practice to employ multiple estimators to characterize the uncertainty (Then *et al.*, 2015). In this study, we used both the one parameter K (equation 5) and the updated Pauly, (1980) (equation 6) methods to determine the natural mortality of *A. leucosternon* and *A. triostegus* in the absence of t_{max} (maximum age) as follows:

$$M = 1.692K \quad (5)$$

$$M = 4.118K^{0.73} L\infty^{-0.33} \quad (6)$$

The instantaneous fishing mortality (F) was obtained by subtracting the instantaneous natural mortality (M) calculated in equation 5 and 6 above from the total instantaneous mortality (Z) estimated in equation 4 as follows:

$$F = Z-M \quad (7)$$

The exploitation rate (E) for each fish species was calculated based on the (Pauly, 1984) method as follows:

$$E = F / Z \quad (8)$$

Where E is the exploitation rate, M is the natural mortality, and F is the fishing mortality. The exploitation rate values were compared to the (Gulland, 1971) index, to characterize the stock as either underexploited, optimum, or overexploited.

5.3. Results

5.3.1. Length-frequency distributions and fishing gears

Landings of *A. leucosternon* and *A. triostegus* were recorded from the four common Kenyan gears i.e. beach seine, gillnet, spear gun, and traditional trap. Among the gears, beach seine caught the highest proportion of *A.leucosternon* individuals below Lm (64%), while gill net captured the most *A. triostegus* individuals under the Lm (46%) (Figure 5.2).

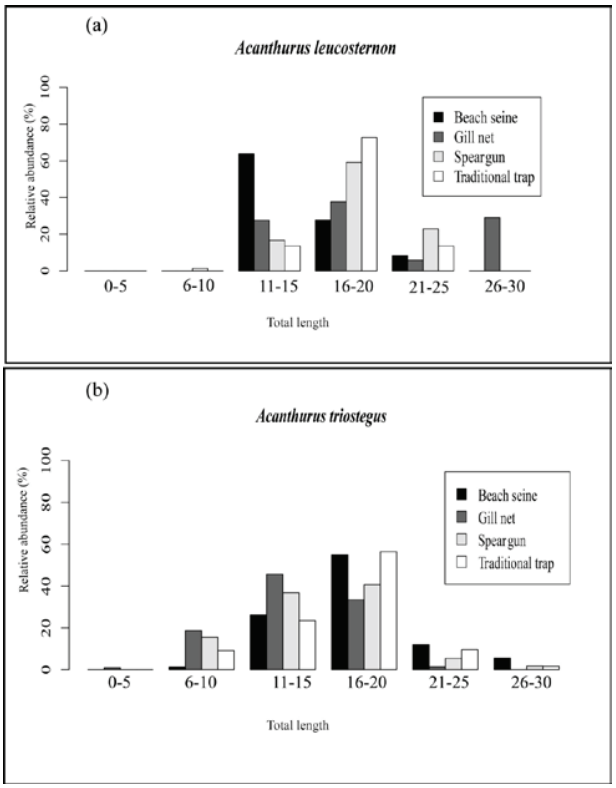


Figure 5.2 Catch composition and size distribution by gear for a) *A. leucosternon* and b) *A. triostegus* caught along the southern Kenyan coastline.

Thirty-one percent of all *A. leucosternon* caught were below the Lopt (15.9 cm), whereas twenty-seven percent were below the Lm (15.6cm) calculated from ELEFAN GA growth parameters

(Table 5.I), suggesting neither growth nor recruitment overfishing was occurring in this species (Figure 5.3). In contrast, seventy-one percent of all caught *A. triostegus* were below Lm (17.5 cm) and seventy-six percent were below the Lopt (18.2 cm) calculated from ELEFAN GA growth parameters (Table 5.1), indicating both growth and recruitment overfishing were occurring in this species (Figure 5.3).

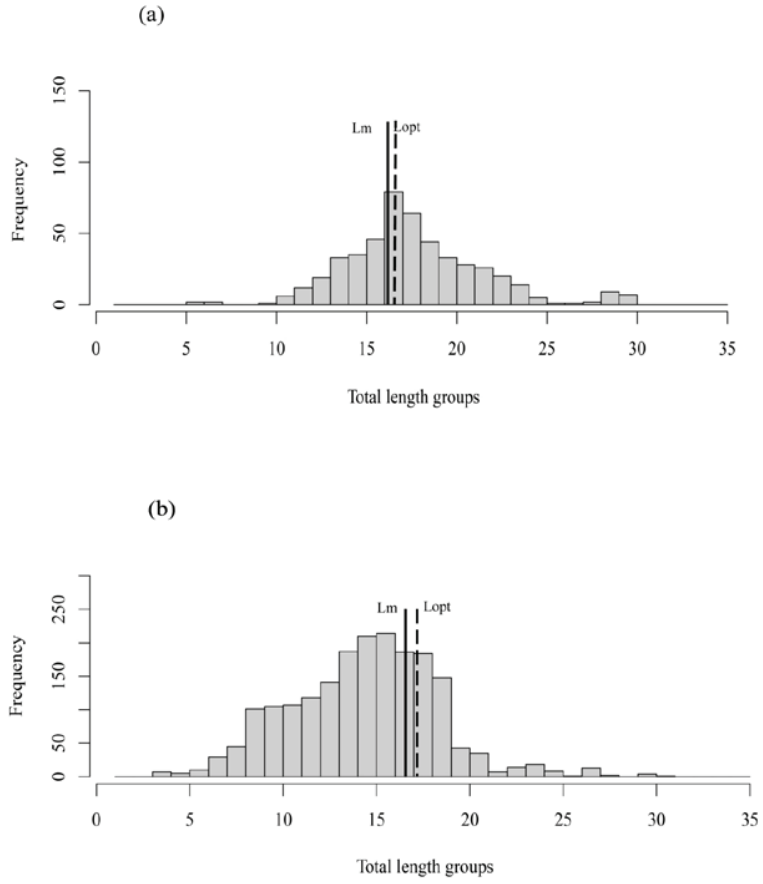


Figure 5.3 Length-frequency distribution histograms for (a) *A. leucosternon* and (b) *A. triostegus* caught off the southern Kenyan coastline.

5.3.2. Growth parameters, mortality, and exploitation

The estimates of VBGF parameters varied slightly between the four ELEFAN methods as shown in Table 5.1. While the L∞ estimates were comparable between *A. leuocosternon* (26.1 - 27.4) and *A. triostegus* (27.9-29.6), the estimated growth coefficient constants (K) were higher in *A. triostegus* (0.85-1.23) compared to *A. leucosternon* (0.29-0.35), suggesting that *A. triostegus*

attains its maximum length faster than *A. leucosternon*. The growth performance index values for *A. triostegus* (2.88-2.98) were slightly higher than those estimated for *A. leucosternon* (2.33-2.38) (Table 5.1).

The instantaneous total mortalities (Z) estimate from the length converted catch curves for *A. triostegus* (2.91-4.98) were almost three to fivefold higher than those of *A. leucosternon* (0.79-0.82). This translated to fishing mortalities (F) range of 1.47 to 3.41 for *A. triostegus* and 0.17 to 0.33 for *A. leucosternon*, indicating an exploitation rate ranging from 0.20 to 0.40 in *A. leucostern*on and 0.51 to 0.68 in *A. triostegus*. The two natural mortality (N) estimators yielded contrasting estimates in the two species. The updated Pauly (1980) formula estimator revealed lower estimates than the one parameter K estimator in *A. triostegus*, while *A. leucosternon* estimates from the updated Pauly (1980) formula were higher than the estimates of the one parameter K estimator (Table 5.1, Figure 5.4, and Figure 5.5).

Table 5.1 The von Bertalanffy growth parameters and mortality estimates of *A. leucosternon* and *A. triostegus*, using one parameter and two parameter natural mortality estimators' in TropFishR package (Then *et al*.. 2015; Mildenberg *et al*., 2017). L∞ (Asymptotic length), K (growth constant), M (natural mortality), Z (total mortality), F (fishing mortality), and E (exploitation rate).

				Updated Pauly $_{nls-T}$				One parameter K					
Species	**L∞**	**K**	**ø'**	**M**	**Z**	**F**	**E**	**M**	**F**	**E**	**Status**	**Lm**	**Lopt**
A. leucosternon													
ELEFAN with K scan	27.2	0.29	2.33	0.56	0.79	0.23	0.29	0.49	0.30	0.38	Underexploited	16.2	16.6
ELEFAN with RSA	27.4	0.31	2.37	0.59	0.82	0.24	0.29	0.53	0.29	0.35	Underexploited	16.3	16.8
ELEFAN with SA	27.2	0.29	2.35	0.57	0.82	0.24	0.30	0.49	0.33	0.41	Underexploited	16.2	16.6
ELEFAN with GA	26.1	0.35	2.38	0.66	0.82	0.17	0.20	0.59	0.23	0.28	Underexploited	15.6	15.9
Powell-Wetherall	27 ± 3												
A. triostegus													
ELEFAN with K scan	27.9	1.23	2.98	1.59	4.98	3.38	0.68	2.08	2.89	0.58	Overexploited	16.6	17.1
ELEFAN with RSA	28.0	1.21	2.98	1.58	4.98	3.41	0.68	2.05	2.93	0.59	Overexploited	16.6	17.1
ELEFAN with SA	29.6	0.85	2.88	1.21	2.91	1.71	0.59	1.44	1.47	0.51	Overexploited	17.5	18.2
ELEFAN with GA	29.6	0.98	2.96	1.33	3.34	2.02	0.60	1.65	1.69	0.51	Overexploited	17.5	18.2
Powell-Wetherall	28 ± 4												

5.4. Discussion

We compared the life history characteristics, mortality, and exploitation of *A. triostegus* and *A. leucosternon*, to deduce whether differences in their spawning behaviour (pairing or aggregation) could lead to differences in their exploitation rate. Consistent with expectations, our results demonstrate that the species that forms spawning aggregations (*A.triostegus*) year around had a higher exploitation rate than the pair spawning species (*A. leucosternon*). Furthermore, estimates of length at maturity and length at optimum yield also indicated that growth and recruitment overfishing were occurring in *A. triostegus*, but not in *A. leucosternon*.

Despite comparable estimates of the von Bertalanffy asymptotic length ($L\infty$) in the two species (*A. triostegus* (27.9-29.6) and *A. leucosternon* (26.1-27.4)), the growth constant (K) estimates for *A. triostegus* (0.85-1.23) are almost three to fivefold higher than those of *A. leucosternon* (0.29-0.38), suggesting that *A. triostegus* attains its maximum length faster than *A. leucosternon*. Intra-genera differences in growth coefficient constant (K) have previously been shown in other reef fishes genus such as *Lutjanus*, *Lethrinus*, and *Plectropomus* (Currey *et al.*, 2013; Prince *et al.*, 2015) and can be due to differences in habitat preferences, but exploitation can also lead to different growth rates (Gust *et al.*, 2002). Considering estimates from FishBase (Froese and Pauly, 2018), the growth performance index (*ø*') value 2.76 reported for *A. leucosternon* in Lakshawdeep India is slightly higher than the range (2.33-2.38) reported for the Kenyan population, which suggest superior growth conditions in the Indian reefs. However, *A. triostegus* Kenyan populations displayed slightly higher growth performance indices (2.88-2.96) than the Lakshawdeep Indian population (2.60), underlining the ability of this species to maintain its optimum growth in a wide range of habitats. Unlike *A. leucosternon* which is restricted to coral reef habitats, *A. triostegus* can be found living in turbid marine environments such as coastal bays, ship harbors, and creeks (Mirams *et al.*, 2011).

Our length at maturity estimates for *A. triostegus* (16.6-17.5) were clearly larger than the 12 cm reported by (Mangi and Roberts, 2006) and may reflect the increasing area closures and gear management at the Kenyan coastline (McClanahan and Hicks, 2011). Nevertheless, the finding of growth and recruitment overfishing in *A. triostegus* indicates that either this species has not fully recovered from previous overexploitation or that the study by Mangi and Roberts (2006) did not capture its entire size distribution at the southern Kenyan coastline. Unfortunately, there are no published Lm and Lopt estimates for *A. leucosternon* to compare with the estimates presented in our study. Overall, the length at maturity and length at optimum yield estimates differed marginally in both species, indicating that both species may be adapted to start egg production at maximum biomass (Beverton, 1992).

Mortality (F, M, and Z) and exploitation rates (E) were different between the two species, with *Acanthurus triostegus* experiencing a higher level of exploitation (E = 0.51 – 0.68) than *A. leucosternon* (E = 0.20 – 0.41). The finding of a high level of exploitation in *A. triostegus* is unlikely to be due to differences in consumer preference because both species are considered to be of low value by local fishermen and fetch only ~1USD per Kg (Pers. obs.). It is also unlikely that the observed differences in the exploitation rate (E) are as a result of differences in natural abundance of the two species. Because the most abundant species in Kenya's fishing grounds are rarely landed by fishermen (McClanahan *et al.*, 2010), suggesting that natural abundance has little influence on the species caught by local fishermen. A plausible explanation for the high-level exploitation in *A. triostegus* is its spawning and mating behaviour. Unlike *A. leucosternon*, *A. triostegus* is less territorial and forms large spawning aggregations throughout the year that might be susceptible to fishing (Robertson *et al.*, 1979; Hartup *et al.*, 2013; Claydon *et al.*, 2014). Indeed, individuals of *A. triostegus* were landed en mass by a particular fishing crew (Pers. obs.), indicating they were most likely fished in groups. Although this rationale might explain why *A. triostegus* is the most abundant *Acanthurus* spp. caught in Kenya, where it contributes approximately 3% of the total number of fish landed (Hicks and McClanahan, 2012), *Acanthurus*

nigrofuscus also often forms spawning aggregation but constitutes lower proportion fishermen catch in Kenya (Hicks and McClanahan, 2012). This suggests that other factors not explored by the present study may also influence the catch composition of Kenyan fishermen.

In general, the modified Pauly (1980) method yielded higher natural mortality estimates than the one parameter K method in the underexploited *A. leucosternon*, whereas in *A. triostegus* the one parameter K method displayed higher natural mortalities than the modified Pauly (1980) method. This pattern is consistent with the observation made by Rehren *et al*. (2018) on *Siganus sutor*, *Lethrinus borbonicus*, *Leptoscarus vaigiensis*, *Lethrinus lentjan*, *Lutjanus fulviflamma*, and *Scarus ghobban* and may suggest that the one parameter K method tends to overestimate natural mortality in overexploited species.

In Kenya, the use of spear gun and beach seine is forbidden by fisheries laws because of their destructive nature (McClanahan and Mangi, 2004). However, compliance by fishermen has been challenging as both gears involve low expenditure and high economic return (Mangi *et al.*, 2007; Tuda *et al.*, 2016). Sixty-four percent of *A. leucosternon* catch from beach seine comprised of individuals below the Lm, supporting previous reports that this gear captures a large proportion of immature individuals (Hicks and McClanahan, 2012; Tuda *et al.*, 2016).

On the contrary, a large proportion of *A. triostegus* individuals under the Lm were captured by gill net, indicating a possibility that fishermen were using gill nets with a mesh size smaller than the recommended 6cm size limit. This underlines the minor difference existing between beach seine and gill net, when the mesh size of the latter is too small (McClanahan and Mangi, 2004). Nevertheless, the application of mesh size restriction in a multispecies fishery is often challenging, considering that different species mature at different sizes (Tuda *et al*., 2016). In Kenya, the difficulty of enforcing the required mesh size is also exacerbated by the loose arrangement of landing sites along the coastline.

Even though unabated fishing activities are predicted to cause collapse of large, long-lived species that form large aggregations like Serranidae (Sadovy de Mitcheson *et al.*, 2013; Robinson

et al., 2015) and Trachichthyidae (Clark, 2001), our findings of high level exploitation in *A. triostegus* suggests that fast life histories species might also be at risk of declining to lower abundance just as larger, slower-growing species. This means that fast-growing species should not be overlooked by fisheries management plan that aims to protect fish species from collapse.

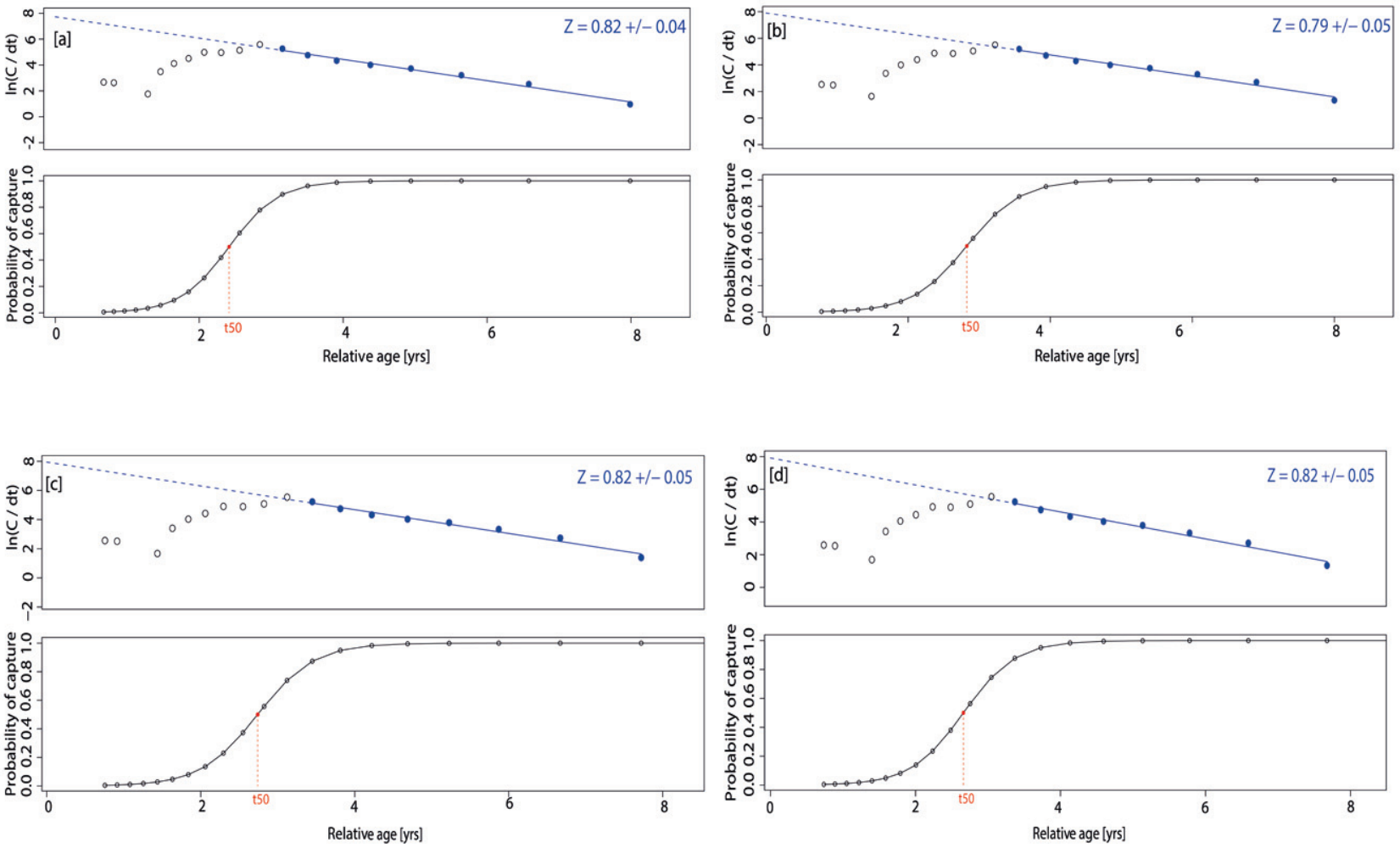


Figure 5.4 The length converted catch curves for *A. leucosternon* based on (a) ELEFAN GA, (b) ELEFAN with Kscan, (c) ELEFAN with SA, and (d) ELEFAN with RSA, showing the relationship between the length of the catch (ln (C/dt)) and relative age in years.

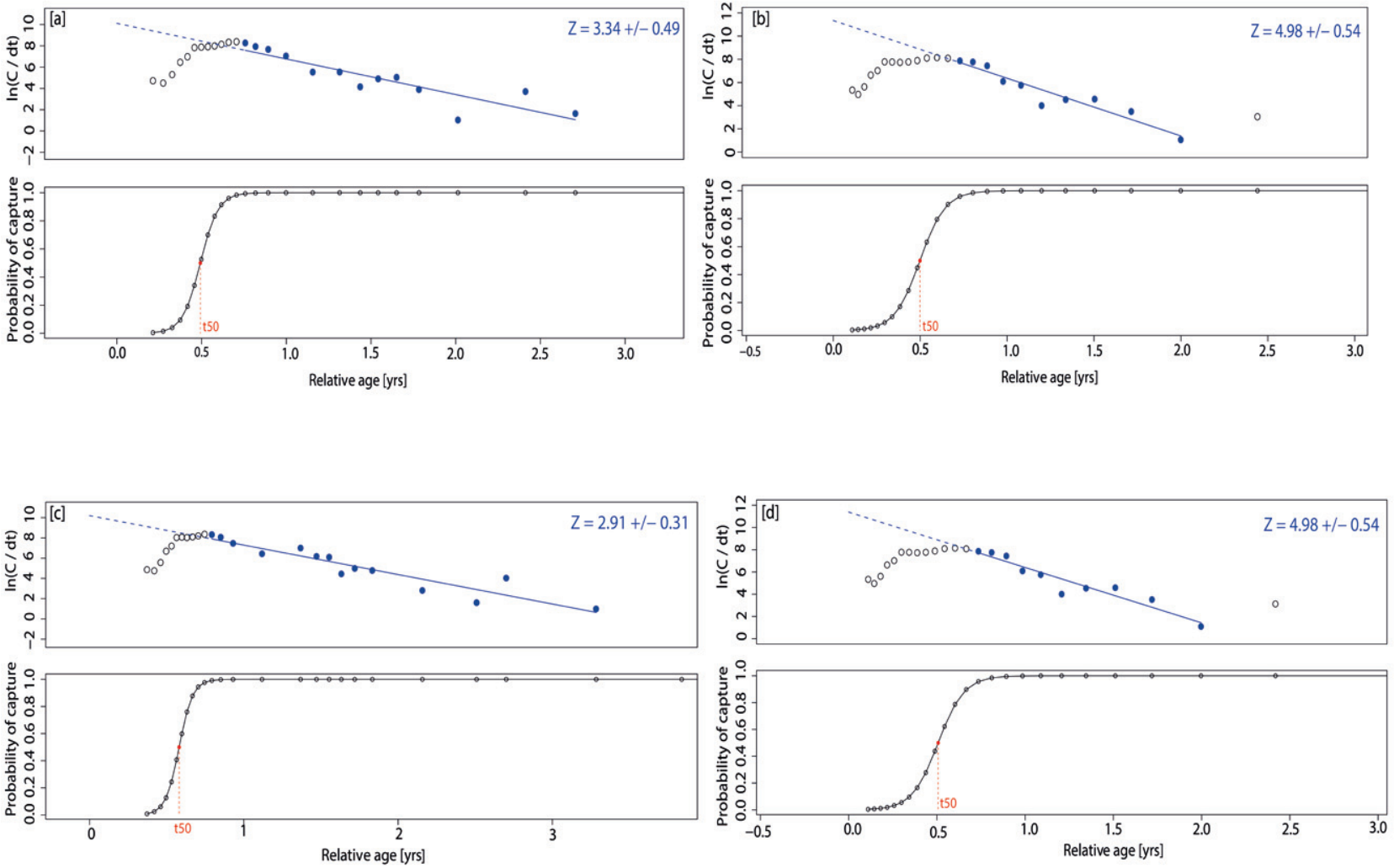


Figure 5.5 The length converted catch curves for *A. triostegus* based on (a) ELEFAN GA, (b) ELEFAN with Kscan, (c) ELEFAN with SA, and (d) ELEFAN with RSA, showing the relationship between the length of the catch (ln (C/dt)) and the relative age in years.

Acknowledgements

Funding for this project was obtained from the Leibniz Centre for Tropical Marine Research (ZMT) and International Foundation for Science (IFS) (IFS grant no. A/5677-1). We thank P. Matiku (Tanzanian Fisheries Research Institute) for logistical support, D. Ocharo, J. Omweri, and A. Athman (KMFRI), as well as J. Ndagala, H. Ratsimbazafy (VUB), and S. Chikambo for sampling and fieldwork assistance. We also thank S. Peters (ZMT) for laboratory assistance. The Deutscher Akademischer Austausch Dienst (DAAD) together with the National Council of Science and Technology, Kenya (NACOSTI) provided a scholarship to L. Otwoma.

6. CHAPTER - General discussion and synopsis

The general aim of this thesis was to assess the genetic population structure (connectivity, demographic history, and genetic diversity) and growth parameters of *A. leucosternon* and *A. triostegus*, to determine the influence of biotic and abiotic factors on the connectivity and exploitation patterns of these species in the Western Indian Ocean and the Indian Ocean at large. In particular, I evaluated the influence of mating behaviour on the connectivity and exploitation of these two species. In the following sections, I summarize and discuss the main findings of this thesis to provide insights into the factors that drive connectivity and the exploitation of *Acanthurus* species in the Western Indian Ocean and other parts of the Indian Ocean. Finally, I draw conclusions from these findings, their implications for marine resource management and recommendations for future studies.

6.1. Major findings and discussion

6.1.1 Connectivity of *A. leucosternon* and *A. triostegus* in the Western Indian Ocean and Indo-Pacific

The Western Indian Ocean (WIO) was recognized as one of the richest marine biodiversity areas in the Indo-Pacific region, hosting about 11,200 marine species, including 2,200 fish species, over 350 coral species, 11 species of mangroves, 12 species of seagrass, 3,000 species of mollusks, 450 species of crabs, and 300 species of echinoderms (WWF, 2004; WWF, 2006). Over the last two decades, genetic and taxonomic studies in the WIO have helped us to understand the origin of this diversity. However, these inferences have been based on only a few taxa, limiting our understanding of the historical and contemporary processes responsible for the distribution of this diversity (Borsa *et al.*, 2016). The limited number of genetic studies in the WIO has also precluded the use of this information in biodiversity conservation and management.

While aiming to increase the number of genetic surveys in the WIO, chapter 2 of this thesis investigated the genetic population structure of *A. leucosternon* in three WIO countries (Kenya, Tanzania, and Mozambique). The data from mitochondrial and nuclear DNA showed widespread genetic homogeneity in *A. leucosternon* WIO populations, substantiating the prediction that long PLD do confer high connectivity to marine species. The pelagic larval duration of acanthurids ranges from 40 to 60 days and could expose the larvae of *A. leucosternon* to the full spectrum of prevailing WIO currents (Schott and McCreary Jr, 2001), facilitating long-distance dispersal. These findings are consistent with other studies on high dispersal reef fishes in the WIO such as *Lutjanus kasmira* (Muths *et al.*, 2012), *Scarus ghobban* (Visram *et al.*, 2010), *Lutjanus fulviflamma* (Dorenbosch *et al.*, 2006), and *Dascyllus trimacullatus* (O'Donnell *et al.*, 2017). However, the relationship between PLD and genetic population structuring remains tenuous (Bernardi *et al.*, 2003; Kelly and Palumbi, 2010; Riginos *et al.*, 2011), as some life history traits that are hard to evaluate such as the larval swimming ability, egg type, and spawning strategies have been shown to influence the realized dispersal in marine species (Riginos *et al.*, 2011).

Genetic surveys comparing the WIO to other regions in the Indo-Pacific have uncovered complex phylogeographic structures. While some studies designate this region as a possible source of haplotypes (Muths *et al.*, 2015; Huyghe and Kochzius, 2017), others show it sharing dominant haplotypes with all Indo-Pacific biogeographies (Craig *et al.*, 2007; Klanten *et al.*, 2007; Horne *et al.*, 2008; Gaither *et al.*, 2010; Ahti *et al.*, 2016). Nevertheless, the sampling in WIO and central-Indian Ocean has been substantially low, indicating that more systematic work is required to fully understand the evolutionary and dispersal processes that shape the phylogeographic structure of this region (Borsa *et al*., 2016). Chapter 3 of this thesis used *Acanthurus triostegus* as a model species to explore the connectivity of WIO populations to other Indo-Pacific biogeographies (the eastern Indian Ocean, Western Pacific, Central Pacific, and Eastern Pacific). The results from the AMOVA, haplotype network, and the phylogenetic tree did not support the hypothesis of genetic

differentiation between Indian and Pacific *A. triostegus* populations, but WIO populations were significantly different from the Eastern Indian Ocean and appeared to harbor a unique haplogroup that was only present at lower frequencies in other biogeographies. The unique haplogroup that dominates the WIO is likely to have arisen there, after a long-term absence of gene flow between the WIO and the other Indo-Pacific populations. This finding seems to corroborate other studies that indicate that the WIO is a centre of origin (Borsa *et al.*, 2016; Huyghe and Kochzius, 2017), but it is unlikely the diversification and persistence of species in the WIO occurs through only one mechanism, especially with the finding that the four hypotheses used to explain speciation (centre of origin, centre of accumulation, centre of survival, and centre of overlap) are not mutually exclusive (Barber, 2009; Gaither and Rocha, 2013).

6.1.2. Exploring the links between mating behaviour and genetic population structure in *A. leucosternon* and *A. triostegus*

Chapter 4 of this thesis compared the genetic population structure of *A. triostegus* and *A. leucosternon*, to determine whether differences in their mating behaviour could lead to different connectivity patterns in the Indian Ocean. These two species share several behavioural and feeding ecologies (Randall, 1956), but have major differences in their mating and spawning strategies. While *A. leucosternon* spawns through monogamous pairing, *A. triostegus* forms massive spawning aggregations (Robertson *et al.*, 1979; Whiteman and Côté, 2004; Hartup *et al.*, 2013; Claydon *et al.*, 2014). Because the site fidelity associated with spawning aggregations can enhance larval retention, lower genetic diversity and connectivity patterns were expected in *A. triostegus* as compared to *A. leucosternon*. The results from mitochondrial and nuclear DNA revealed intriguing genetic patterns in the two *Acanthurus* species, but a common pattern consistent with the effect of divergent mating systems was not evident at both local (WIO) and broad (Indian Ocean: EIO and WIO) scales. At the scale of Western Indian Ocean, both species

are found to exhibit panmixia, rejecting the prediction that populations of *A. triostegus* will be more structured than *A. leucosternon*. Panmixia in both *Acanthurus* species is likely to be facilitated by the permanently north-flowing East African Coast Current, which flows faster than the average swimming speed of *Acanthurus* larvae (24 cm/s). This may indicate that EACC overrides the influence of other factors such as mating behaviour, larval swimming ability, or geographic distance in determining dispersal distances of sedentary marine species.

Across the Indian Ocean (EIO and WIO), analysis of mitochondrial DNA reveals divergent population structures in the two *Acanthurus* species, while *A. leucosternon* display panmixia across the Indian Ocean, *A. triostegus* exhibit significant genetic partition between the WIO and EIO. However, the spatial structuring observed in *A. triostegus* seems to be inconsistent with the effect of resident spawning aggregation of *A. triostegus* which are reef restricted (Hartup *et al.*, 2013; Claydon *et al.*, 2014). Thus, if the genetic partition between EIO and WIO in *A. triostegus* is driven by spawning aggregation, we would expect spatial genetic differences at finer scales, including between sample sites that were separated by 20 km e.g. between Kisite Mpunguti and Msambweni. Yet the pairwise comparisons in chapter 4 indicate that the genetic differences were mainly between EIO and WIO sample sites. These findings seem to confirm previous studies on groupers, snappers, and squids, which also found no relationship between mating behaviour and genetic population structure (Zatcoff *et al.*, 2004; Shaw *et al.*, 2010; Carson *et al.*, 2011; Portnoy *et al.*, 2012; Bernard *et al.*, 2016).

Contrary to expectation, *A. triostegus* was also found to exhibit higher nucleotide diversities than *A. leucosternon*. This difference in nucleotide diversities is likely to suggest that the two species had a contrasting evolutionary history. A reconstruction of their past demographic history using the BEAST software indicates that both species experienced demographic expansion during the Pleistocene (11,700- 2.5 Mya), but the demographic expansion in *A. leucosternon* (demographic expansion time 60,000 years ago) was more recent and sudden than *A. triostegus* (demographic

expansion time 125,000 years ago). This seems to reflect their differences in habitat requirements. *A. triostegus* is a generalist species, which can be found inhabiting turbid waters in bays, tide pools, and harbor, whereas *A. leucosternon* is often restricted to coral reef habitats (Randall, 1956). The strict dependence of *A. leucosternon* on coral reefs may have restricted or lagged its population growth during the sea level low stands, while *A. triostegus* may have been able to colonize low-quality habitats that became available immediately when the sea level started to rise. This might suggest that *A. triostegus* had multiple isolated populations in different refugia that came into contact when sea level rose increasing its current nucleotide diversity.

6.1.3. Mating behaviour and vulnerability of *A. leucosternon* and *A. triostegus* to exploitation

In chapter 5, fisheries dependent data were used to test the influence of mating behavior on the exploitation rate of *A. leucosternon* and *A. triostegus*. Because fishing is efficient at removing conspecific individuals when they are gathered at a given place, a higher exploitation rate was expected in the aggregate spawning *A. triostegus* as compared to the monogamous spawning *A. leucosternon*. Consistent with these expectations, the length converted catch curve in TropfishR (Mildenberger *et al*., 2017) shows that the exploitation rate is beyond the critical level of 0.5 (Gulland, 1971) in *A. triostegus* but not in *A. leucosternon*, suggesting that the former is being exploited unsustainably (over-exploited) at the southern Kenyan coastline. These findings are also confirmed by the empirical relationship between Length at maturity (Lm), Length at optimum yield (Lopt), and Length at instantaneous ($L\infty$), which indicate that growth and recruitment overfishing are occurring in *A. triostegus* but not in *A. leucosternon*. Growth overfishing occurs when fish are harvested before they grow to an optimal size, while recruitment overfishing occurs when fishing impairs the recruitment potential of an exploited stock.

The results of chapter 5 seem to be supportive of previous research findings in the western Indian Ocean, which report an exceptionally higher exploitation rate in the spawning aggregating

Siganus sutor compared to the monogamous spawning species such as *Scarus ghobban* (Rehren *et al*., 2018), confirming that spawning aggregation increases susceptibility of species to exploitation. However, other factors such as consumer preferences and natural abundance have been shown to influence the reef fish catchability (Sadovy de Mitcheson *et al.*, 2013). In the case of *A. triostegus* and *A. leucosternon*, both species fetch approximately 1 USD per kg at the southern Kenyan coastline, suggesting that consumer preference is not likely to explain the differences in exploitation rate reported. Potential differences in natural abundance seem also unlikely to account for the observed differences in the exploitation of the two *Acanthurus* species because underwater visual census studies indicate that the most abundant species in Kenya's fishing ground are not necessarily those commonly landed by fishermen, suggesting that natural abundance does not influence catch composition in Kenya (McClanahan *et al.*, 2010). *A. triostegus* was typically landed en masses using mainly gillnets and beach seine, indicating that the caught individuals occurred in aggregates (Per. Obs.). In contrast, *A. leucosternon* were mostly landed singly by spear gun fishermen. Besides local fishermen suggested that spear gun was inefficient at capturing *A. triostegus* because it notably dispersed individuals of this species when they were gathered. This anecdotal evidence reinforces the notion that *A. triostegus* were mainly targeted in aggregates or occurred mostly in aggregates. However, in some rare cases, both species can gather for purposes of foraging, shelter, or resting, which might be exploited by fishermen. But unlike spawning aggregations that are predictable in space and time, gathering for purposes of foraging, shelter, or resting occurs spontaneously or opportunistically, making it difficult for fishermen to target them (Robinson *et al.*, 2014).

6.2. Conclusions and implications for management

Coral reef species have undergone severe decline due to global warming, overfishing, and coastal pollution (Hughes *et al.*, 2017a; Hughes *et al.*, 2017b) such that their baselines today is dramatically different from 500 or 100 years ago (Gardner *et al.*, 2003). These shifts are difficult

to reverse and often affect local communities in numerous ways, including loss of fishing livelihoods, loss of tourism revenue, increased coastal erosion, and protein deficiencies (Bruno and Valdivia, 2016). Although sound management strategies can be successful in reversing/mitigating this trend, they have often relied on population dynamics models to determine stock-recruitment dynamics and to define sustainable harvesting limits (Bernatchez *et al.*, 2017). These strategies are usually confined to particular administrative units, which are often not aligned with the structure and dynamics of the ecological systems and population biology of several species (Osio *et al.*, 2015; Borja *et al.*, 2016). Consequently, vulnerable populations that are intermingled with abundant ones during parts of their life history may not be fully protected.

The western Indian Ocean comprising of 10 countries (Comoros, France, Kenya, Madagascar, Mauritius, Mozambique, Seychelles, Somalia, South Africa, and Tanzania) is an example of a region where conservation actions are often planned and undertaken by individual countries independently of their neighbouring countries (Mazor *et al.*, 2013; Levin *et al.*, 2018), with the exception of a few recent projects such as WIO-SAP (Western Indian Ocean Strategic Action Plan) and Smart Fish. Although country-based initiatives are important for sustainable management of local marine resources, they are often deficient in protecting highly dispersal species that cross international boundaries, because the source and sink populations of these species may be located in the jurisdictions of two different countries. For example, a recent study on whale sharks (*Rhincodon typus*) found that their population off the coastline of Mozambique acted as a source for the Seychelles population (Andrzejaczek *et al.*, 2016). Similarly, genetic studies in the WIO indicate that the population structure of sedentary species is mainly shaped by ocean currents, which flow across international boundaries and several ecoregions (Visram *et al.*, 2010; Muths *et al.*, 2012; Otwoma and Kochzius, 2016; Huyghe and Kochzius, 2017; Otwoma *et al.*, 2018; Ratsimbazafy and Kochzius, 2018). This suggests that collaboration between and

among mainland and island countries in the region is necessary for the effective conservation of species and ecosystems that are continuous across their borders.

The results presented in chapter 2, 3, and 4 of this study indicate that *A. triostegus* and *A. leucosternon* exist as single panmictic populations in the five WIO countries (Kenya, Tanzania, Mozambique, Madagascar, and Seychelles) sampled. This suggests that a network of transboundary MPAs and common fisheries regulations in the region would ensure the source and sink populations of *A. triostegus* and *A. leucosternon* in different countries are protected, maintaining a high genetic diversity in their populations. So far two transboundary MPA's have been proposed in Eastern Africa (WIO), the future Ruvuma-Palma National Reserve (between southern Tanzania and northern Mozambique borders) and Lubombo Ponta do Ouro-Kosi Bay and Coastal Transfrontier Conservation and Resource Area (between southern Mozambique and northern South Africa borders) (Guerreiro *et al.*, 2010; Grilo *et al.*, 2012; Levin *et al.*, 2018). The proposed Ruvuma-Palma National Reserve is particularly important because this location seems to be the entry point for propagules coming from the larger Indian Ocean (Obura, 2012). Chapter 2 and 3 showed *that A. triostegus* and *A. leucosternon* individuals collected at this location are not distinct from northern Tanzania and Kenyan population, which indicate that it might be the source populations for other East African reef fauna given that dispersal is mainly driven by EACC which permanently flows northwards up to the border of Kenya and Somali. On the other hand, propagules from the WIO mainland region are exported to other parts of the Indian Ocean through the South Equatorial Counter Current (SECC), which arises when EACC converges with Somali Current at the border between Kenya and Somali. Consequently, establishing a transboundary MPA at the border between Kenya and Somali would ensure the reef fauna that export propagules to the larger Indian Ocean are protected. However, insecurity and absence of central government in Somali make this currently impossible to achieve (Levin *et al*., 2018). A solution might involve the extension of the Kiunga Marine National Reserve (1°42.25'S

41°31.78'E to 2°2.58'S 41°14.80'E) further south to ensure that a large proportion of coral reef, mangrove, and seagrass habitats are protected.

While conservation actions should be taken at the regional level, attention should also be paid to the differences in life history traits among reef fauna, because different life history strategies can lead to different exploitation rate. For example, chapter 5 of this study demonstrates that exploitation rate is higher in the spawning aggregating *A. triostegus* compared to monogamous spawning *A. leucosternon*, despite both species having similar length sizes. This suggests that regardless of body size, spawning aggregation can increase vulnerability to exploitation. However, previous studies have emphasized protection of larger, long-lived species that form spawning aggregation from uncontrolled fishing (Clark, 2001; Sadovy de Mitcheson *et al.*, 2013), overlooking the fact that small-sized species forming spawning aggregation may also be at risk of declining to lower abundance just as the larger species. Therefore, formulating fisheries laws and regulations that consider species life history characteristics rather than body size could greatly improve fisheries management in the Western Indian Ocean and the Indian Ocean at large.

6.3. Recommendations and future directions

This thesis investigated the genetic population structure and growth parameters of *A. leucosternon* and *A. triostegus*, to determine whether species-specific traits can lead to different connectivity and exploitation patterns among *Acanthurus* species. The findings attempted to disentangle the contribution of biotic/intrinsic and abiotic/extrinsic factors in the genetic structuring and exploitation of *Acanthurus* species, but much work remains to be done. Chapter 2 and 3 demonstrate genetic homogeneity in *A. leucosternon* and *A. triostegus* in Kenya, Tanzania, northern Mozambique, and southwest Madagascar. However, recent findings on lethrinids (*Lethrinus nebulosus* and *L. mahsena*) have shown that their southern Mozambican, South African, and Seychelles populations were genetically different from Kenyan, Tanzanian, Malagasy and Mauritian populations (Healey *et al.*, 2018a; Healey *et al.*, 2018b). It is thus

possible that additionally sampling of *A. triostegus* in southern Mozambique, South Africa, Mauritius, and eastern Madagascar could reverse the conclusion of genetic homogeneity in the WIO, but this is unlikely to be the case in *A. leucosternon* that exhibits widespread genetic homogeneity througout the whole Indian Ocean.

In Chapter 4, mitochondrial DNA and microsatellite datasets showed that mating behaviour has no influence on genetic connectivity pattern of *A. leucosternon* and *A. triostegus*. However, with the increase of next-generation sequencing in population genetics, it will be interesting to see if the results using larger SNP datasets are comparable to the current findings using traditional markers (mtDNA and microsatellites). There is already evidence indicating that the higher resolution SNPs marker is capable of detecting structuring patterns that are not visible in mitochondrial DNA or microsatellites datasets (DiBattista *et al.*, 2017).

Finally, the finding of a higher exploitation rate in *A. triostegus* as compared to *A. leucosternon* is based on one-year monitoring data, but McClanahan and Hicks, (2011) showed that the length of most coral reef fishes off the Kenyan coast changes with time, suggesting that the results presented in chapter5 of this thesis may not be definitive. Therefore, future studies testing the influence of mating behaviour on the exploitation of *Acanthurus* species should consider increasing the temporal resolution of the study.

REFERENCES

Ahti, P. A., Coleman, R. R., DiBattista, J. D., Berumen, M. L., Rocha, L. A. & Bowen, B. W. (2016). Phylogeography of Indo-Pacific reef fishes: sister wrasses Coris gaimard and C. cuvieri in the Red Sea, Indian Ocean and Pacific Ocean. *Journal of Biogeography* **43**, 1103-1115.

Alberto, F., Raimondi, P. T., Reed, D. C., Coelho, N. C., Leblois, R., Whitmer, A. & Serrão, E. A. (2010). Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. *Ecology* **91**, 49-56.

Alcazar, D., Sr. & Kochzius, M. (2015). Genetic population structure of the blue sea star *Linckia laevigata* in the Visayas (Philippines). *Journal of the Marine Biological Association of the United Kingdom* **96**, 707-713.

Almany, G. R., Berumen, M. L., Thorrold, S. R., Planes, S. & Jones, G. P. (2007). Local replenishment of coral reef fish populations in a marine reserve. *Science* **316**, 742-744.

Almany, G. R., Hamilton, R. J., Bode, M., Matawai, M., Potuku, T., Saenz-Agudelo, P., Planes, S., Berumen, M. L., Rhodes, K. L., Thorrold, S. R., Russ, G. R. & Jones, G. P. (2013). Dispersal of grouper larvae drives local resource sharing in a coral reef fishery. *Current Biology* **23**, 626-630.

Andrzejaczek, S., Meeuwig, J., Rowat, D., Pierce, S., Davies, T., Fisher, R. & Meekan, M. (2016). The ecological connectivity of whale shark aggregations in the Indian Ocean: a photo-identification approach. *Royal Society Open Science* **3**.

Antoro, S., Na-Nakorn, U. & Koedprang, W. (2006). Study of genetic diversity of orange-spotted grouper, Epinephelus coioides, from Thailand and Indonesia using microsatellite markers. *Marine Biotechnology* **8**, 17-26.

Ayre, D. J. & Hughes, T. P. (2000). Genotypic diversity and gene flow in brooding and spawning coral corals along the Great Barrier Reef, Australia. *Evolution* **54**, 1590-1605.

Bandelt, H. J., Forster, P. & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* **16**, 37-48.

Barber, P. H. (2009). The challenge of understanding the Coral Triangle biodiversity hotspot. *Journal of Biogeography* **36**, 1845-1846.

Barber, P. H., Erdmann, M. V. & Palumbi, S. R. (2006). Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the Coral Triangle. *Evolution* **60,** 8125-1839.

REFERENCES

Barber, P. H., Palumbi, S. R., Erdmann, M. V. & Moosa, M. K. (2002). Sharp genetic breaks among populations of Haptosquilla pulchella (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. *Molecular Ecology* **11**, 659-674.

Barbosa, S. S., Klanten, S. O., Puritz, J. B., Toonen, R. J. & Byrne, M. (2013). Very fine-scale population genetic structure of sympatric asterinid sea stars with benthic and pelagic larvae: influence of mating system and dispersal potential. *Biological Journal of the Linnean Society* **108**, 821-833.

Bay, L. K., Choat, J. H., van Herwerden, L. & Robertson, D. R. (2004). High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? *Marine Biology* **144**, 757-767.

Beldade, R., Jackson, A. M., Cudney-Bueno, R., Raimondi, P. T. & Bernardi, G. (2014). Genetic structure among spawning aggregations of the gulf coney *Hyporthodus acanthistius*. *Marine Ecology Progress Series* **499**, 193-201.

Bellwood, D. R., Goatley, C. H. R., Brandl, S. J. & Bellwood, O. (2014). Fifty million years of herbivory on coral reefs: fossils, fish and functional innovations. *Proceedings of the Royal Society B: Biological Sciences* **281**.

Benny, P. N. (2002). Variability of Western Indian Ocean Currents. *Western Indian Ocean Journal of Marine Science* **1**, 81-90.

Benzie, J. A. H. (1999). Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations in the Indian and Pacific Oceans. *Evolution* **53**, 1782-1795.

Benzie, J. A. H., Ballment, E., Forbes, A. T., Demetriades, N. T., Sugama, K., Haryanti & Moria, S. (2002). Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon*. *Molecular Ecology* **11**, 2553-2569.

Bernard, A. M., Feldheim, K. A., Nemeth, R., Kadison, E., Blondeau, J., Semmens, B. X. & Shivji, M. S. (2016). The ups and downs of coral reef fishes: the genetic characteristics of a formerly severely overfished but currently recovering Nassau grouper fish spawning aggregation. *Coral Reefs* **35**, 273-284.

Bernardi, G., Findley, L. & Rocha-Olivares, A. (2003). Vicariance and dispersal across Baja Carlifornia in disjunct marine fish populations. *Evolution* **57**, 1599-1609.

Bernatchez, L., Wellenreuther, M., Araneda, C., Ashton, D. T., Barth, J. M. I., Beacham, T. D., Maes, G. E., Martinsohn, J. T., Miller, K. M., Naish, K. A., Ovenden, J. R., Primmer, C. R., Young Suk, H., Therkildsen, N. O. & Withler, R. E. (2017). Harnessing the Power of Genomics to Secure the Future of Seafood. *Trends in Ecology & Evolution* **32**, 665-680.

REFERENCES

Berumen, M. L., Walsh, H. J., Raventos, N., Planes, S., Jones, G. P., Starczak, V. & Thorrold, S. R. (2010). Otolith geochemistry does not reflect dispersal history of clownfish larvae. *Coral Reefs* **29**, 883-891.

Beverton, R. J. H. (1992). Patterns of reproductive strategy parameters in some marine teleost fishes. *Journal of Fish Biology* **41**, 137-160.

Bird, C. E., Holland, B. S., Bowen, B. W. & Toonen, R. J. (2007). Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. *Molecular Ecology* **16**, 3173-3186.

Borja, A., Elliott, M., Andersen, J. H., Berg, T., Carstensen, J., Halpern, B. S., Heiskanen, A.-S., Korpinen, S., Lowndes, J. S. S., Martin, G. & Rodriguez-Ezpeleta, N. (2016). Overview of Integrative Assessment of Marine Systems: The Ecosystem Approach in Practice. *Frontiers in Marine Science* **3**.

Borsa, P., Durand, J.-D., Chen, W.-J., Hubert, N., Muths, D., Mou-Tham, G. & Kulbicki, M. (2016). Comparative phylogeography of the western Indian Ocean reef fauna. *Acta Oecologica* **72**, 72-86.

Bowen, B. W., Rocha, L. A., Toonen, R. J. & Karl, S. A. (2013). The origins of tropical marine biodiversity. *Trends in Ecology & Evolution* **28**, 359-366.

Bowen, W., Bass, A. L., Rocha, L. A., Grant, W. S. & Robertson, D. R. (2001). Phylogeography of the trumpetfishes (*Aulostomus*): ring species complex on a global scale. *Evolution* **55**, 1029-1039.

Bowler, D. E. & Benton, T. G. (2005). Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews* **80**, 205-225.

Bruno, J. F. & Valdivia, A. (2016). Coral reef degradation is not correlated with local human population density. *Scientific Reports* **6**, 29778.

Burgess, S. C., Baskett, M. L., Grosberg, R. K., Morgan, S. G. & Strathmann, R. R. (2016). When is dispersal for dispersal? Unifying marine and terrestrial perspectives. *Biological Reviews* **91**, 867-882.

Carpenter, K. E., Barber, P. H., Crandall, E. D., Ablan-Lagman, M. C. A., Ambariyanto, Mahardika, G. N., Manjaji-Matsumoto, B. M., Juinio-Menez, M. A., Santos, M. D., Starger, C. J. & Toha, A. H. A. (2011). Comparative phylogeography of the Coral Triangle and implications for marine management. *Journal of Marine Biology* **2011**, 14.

Carson, E. W., Saillant, E., Renshaw, M. A., Cummings, N. J. & Gold, J. R. (2011). Population structure, long-term connectivity, and effective size of mutton snapper (*Lutjanus analis*) in the Caribbean Sea and Florida Keys. *Fishery Bulletin* **109**, 416-428.

REFERENCES

Carvalho, G. R. & Hauser, L. (1994). Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries* **4**, 326-350.

Chapuis, M.-P. & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* **24**, 621-631.

Cherubin, L. M., Nemeth, R. S. & Idrisi, N. (2011). Flow and transport characteristics at an *Epinephelus guttatus* (red hind grouper) spawning aggregation site in St. Thomas (US Virgin Islands). *Ecological Modelling* **222**, 3132-3148.

Choat, H. & Robertson, D. R. (2002). Age-based studies. *In: Sale PF (ed) Dynamics and diversity in a complex ecosystem. Academic Press, San Diego*, 57-80.

Choat, J. H. & Axe, L. M. (1996). Growth and longevity in acanthurid fishes; an analysis of otolith increments. *Marine Ecology Progress Series* **134**, 15-26.

Clark, M. (2001). Are deepwater fisheries sustainable? — the example of orange roughy (*Hoplostethus atlanticus*) in New Zealand. *Fisheries Research* **51**, 123-135.

Claydon, J. A. B., McCormick, M. I. & Jones, G. P. (2014). Multispecies spawning sites for fishes on a low latitude coral reef: spatial and temporal patterns. *Journal of Fish Biology* **84**, 1136-1163.

Clement, M., Posada, D. & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657-1659.

Colin, P. L. (1996). Longevity of some coral reef fish spawning aggregations. *Copeia* **1996**, 189-192.

Comeros-Raynal, M. T., Choat, J. H., Polidoro, B. A., Clements, K. D., Abesamis, R., Craig, M. T., Lazuardi, M. E., McIlwain, J., Muljadi, A., Myers, R. F., Nañola, C. L., Jr., Pardede, S., Rocha, L. A., Russell, B., Sanciangco, J. C., Stockwell, B., Harwell, H. & Carpenter, K. E. (2012). The likelihood of extinction of iconic and dominant herbivores and detritivores of coral reefs: The parrotfishes and surgeonfishes. *PLoS ONE* **7**, e39825.

Cowen, R. K., Lwiza, K. M. M., Sponaugle, S., Paris, C. B. & Olson, D. B. (2000). Connectivity of marine populations: Open or closed? *Science* **287**, 857-859.

Cowen, R. K., Paris, C. B. & Srinivasan, A. (2006). Scaling of connectivity in marine populations. *Science* **311**, 522-527.

Craig, M. T., Eble, J. A., Robertson, D. R. & Bowen, B. W. (2007). High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). *Marine Ecology Progress Series* **334**, 245-254.

Craig, P., Choat, H., Axe, L. & Saucerman, S. (1997). Population biology and harvest of the coral reef surgeonfish *Acanthurus meatus* in American Samoa. *Fishery Bulletin* **95**, 680-693.

Craig, P. C. (1998). Temporal spawning patterns of several surgeonfishes and wrasses in American Samoa. *Pacific science* **52**, 35-39.

Crandall, E. D., Frey, M. A., Grosberg, R. K. & Barber, P. H. (2008a). Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. *Molecular Ecology* **17**, 611-626.

Crandall, E. D., Jones, M. E., Munoz, M. M., Akinronbi, B., Erdmann, M. V. & Barber, P. H. (2008b). Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle. *Molecular Ecology* **17**, 5276-5290.

Crandall, E. D., Treml, E. A., Liggins, L., Gleeson, L., Yasuda, N., Barber, P. H., Wörheide, G. & Riginos, C. (2014). Return of the ghosts of dispersal past: historical spread and contemporary gene flow in the blue sea star *Linckia laevigata*. *Bulletin of Marine Science* **90**, 399-425.

Crochelet, E., Roberts, J., Lagabrielle, E., Obura, D., Petit, M. & Chabanet, P. (2016). A model-based assessment of reef larvae dispersal in the Western Indian Ocean reveals regional connectivity patterns — Potential implications for conservation policies. *Regional Studies in Marine Science* **7**, 159-167.

Crossman, J. D., Choat, J. H. & Kendall, D. C. (2005). Nutritional ecology of nominally herbivorous fishes on coral reefs. *Marine Ecology Progress Series* **296**, 129-142.

Currey, L. M., Williams, A. J., Mapstone, B. D., Davies, C. R., Carlos, G., Welch, D. J., Simpfendorfer, C. A., Ballagh, A. C., Penny, A. L., Grandcourt, E. M., Mapleston, A., Wiebkin, A. S. & Bean, K. (2013). Comparative biology of tropical *Lethrinus* species (Lethrinidae): challenges for multi-species management. *Journal of Fish Biology* **82**, 764-788.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.

Dawson, M. N., Louie, K. D., Barlow, M., Jacobs, D. K. & Swift, C. C. (2002). Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. *Molecular Ecology* **11**, 1065-1075.

De Mitcheson Yvonne, S., Cornish, A., Domeier, M., Colin Patrick, L., Russell, M. & Lindeman Kenyon, C. (2008). A global baseline for spawning aggregations of reef fishes. *Conservation Biology* **22**, 1233-1244.

REFERENCES

DeBoer, T. S., Subia, M. D., Erdmann, M. V., Kovitvongsa, K. & Barber, P. H. (2008). Phylogeography and limited genetic connectivity in the endangered boring giant clam across the Coral Triangle. *Conservation Biology* **22**, 1255-1266.

DeWoody, J. A., Schupp, J., Kenefic, L., Busch, J., Murfitt, L. & Keim, P. (2004). Universal method for producing ROX-labeled size standards suitable for automated genotyping. *Biotechniques* **37**, 348-352.

Delrieu-Trottin, E., Mona, S., Maynard, J., Neglia, V., Veuille, M. & Planes, S. (2017). Population expansions dominate demographic histories of endemic and widespread Pacific reef fishes. *Scientific Reports* **7**, 40519.

DiBattista, J. D., Feldheim, K. A. & Bowen, B. W. (2011). Microsatellite DNA markers to resolve population structure and hybridization of two closely related surgeonfish species, *Acanthurus nigricans* and *Acanthurus leucosternon*. *Conservation Genetics Resources* **3**, 159-162.

DiBattista, J. D., Rocha, L. A., Craig, M. T., Feldheim, K. A. & Bowen, B. W. (2012). Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and Microsatellites. *Journal of Heredity* **103**, 617-629.

DiBattista, J. D., Travers, M. J., Moore, G. I., Evans, R. D., Newman, S. J., Feng, M., Moyle, S. D., Gorton, R. J., Saunders, T. & Berry, O. (2017). Seascape genomics reveals fine-scale patterns of dispersal for a reef fish along the ecologically divergent coast of Northwestern Australia. *Molecular Ecology* **26**, 6206-6223.

DiBattista, J. D., Whitney, J., Craig, M. T., Hobbs, J.-P. A., Rocha, L. A., Feldheim, K. A., Berumen, M. L. & Bowen, B. W. (2016). Surgeons and suture zones: Hybridization among four surgeonfish species in the Indo-Pacific with variable evolutionary outcomes. *Molecular Phylogenetics and Evolution* **101**, 203-215.

Domeier, M. L. & Colin, P. L. (1997). Tropical reef fish spawning aggregations: Defined and reviewed. *Bulletin of Marine Science* **60**, 698-726.

Dorenbosch, M., Pollux, B. J. A., Pustjens, A. Z., Rajagopal, S., Nagelkerken, I., van der Velde, G. & van der Staay, S. Y. M.-. (2006). Population structure of the Dory snapper, *Lutjanus fulviflamma*, in the western Indian Ocean revealed by means of AFLP fingerprinting. *Hydrobiologia* **568**, 43-53.

Drummond, A. J. & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

REFERENCES

Drummond, A. J., Rambaut, A., Shapiro, B. & Pybus, O. G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution* **22**, 1185-1192.

Duda Jr, F. T. & Palumbi, R. S. (1999). Population structure of the black tiger prawn, *Penaeus monodon*, among western Indian Ocean and western Pacific populations. *Marine Biology* **134**, 705-710.

Earl, D. A. & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359-361.

Eble, J. A., Rocha, L. A., Craig, M. T. & Bowen, B. W. (2011a). Not all larvae stay close to home: Insights into marine population connectivity with a focus on the brown surgeonfish (*Acanthurus nigrofuscus*). *Journal of Marine Biology* **2011**, 12.

Eble, J. A., Toonen, R. J. & Bowen, B. W. (2009). Endemism and dispersal: Comparative phylogeography of three surgeonfishes across the Hawaiian Archipelago. *Marine Biology* **156**, 689-698.

Eble, J. A., Toonen, R. J., Sorenson, L., Basch, L. V., Papastamatiou, Y. P. & Bowen, B. W. (2011b). Escaping paradise: Larval export from Hawaii in an Indo-Pacific reef fish, the Yellow Tang (*Zebrasoma flavescens*). *Marine Ecology Progress Series* **428**, 245-258.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792-1797.

Elliott, N. (1996). Allozyme and mitochondrial DNA analysis of the tropical saddle-tail sea perch, *Lutjanus malabaricus* (Schneider), from Australian waters. *Marine and Freshwater Research* **47**, 869-876.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* **14**, 2611-2620.

Excoffier, L. & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567.

Excoffier, L., Smouse, P. E. & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.

Faurby, S. & Barber, P. H. (2012). Theoretical limits to the correlation between pelagic larval duration and population genetic structure. *Molecular Ecology* **21**, 3419-3432.

REFERENCES

Féral, J.-P. (2002). How useful are the genetic markers in attempts to understand and manage marine biodiversity? *Journal of Experimental Marine Biology and Ecology* **268**, 121-145.

Fisher, R. & Hogan, J. D. (2007). Morphological predictors of swimming speed: a case study of pre-settlement juvenile coral reef fishes. *Journal of Experimental Biology* **210**, 2436.

Fisher, R., Leis, J. M., Clark, D. L. & Wilson, S. K. (2005). Critical swimming speeds of late-stage coral reef fish larvae: variation within species, among species and between locations. *Marine Biology* **147**, 1201-1212.

Fitzpatrick, J. M., Carlon, D. B., Lippe, C. & Robertson, D. R. (2011). The West Pacific diversity hotspot as a source or sink for new species? Population genetic insights from the Indo-Pacific parrotfish *Scarus rubroviolaceus*. *Molecular Ecology* **20**, 219-234.

Froese, R. & Binohlan, C. (2000). Empirical relationships to estimate asymptotic length, length at first maturity and length at maximum yield per recruit in fishes, with a simple method to evaluate length frequency data. *Journal of Fish Biology* **56**, 758-773.

Froese R, Pauly D, editors: FishBase; 2018: Available: www.fishbase.org. Accessed 30 January 2018.

Fu, Y.-X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**, 915-925.

Gaither, M. R., Bernal, M. A., Coleman, R. R., Bowen, B. W., Jones, S. A., Simison, W. B. & Rocha, L. A. (2015). Genomic signatures of geographic isolation and natural selection in coral reef fishes. *Molecular Ecology* **24**, 1543-1557.

Gaither, M. R., Bowen, B. W., Bordenave, T. R., Rocha, L. A., Newman, S. J., Gomez, J. A., van Herwerden, L. & Craig, M. T. (2011a). Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the indo-pacific barrier with contemporary overlap in the coral triangle. *BMC Evolutionary Biology* **11**, 189.

Gaither, M. R., Jones, S. A., Kelley, C., Newman, S. J., Sorenson, L. & Bowen, B. W. (2011b). High connectivity in the deepwater snapper *Pristipomoides filamentosus* (Lutjanidae) across the Indo-Pacific with isolation of the Hawaiian Archipelago. *PLoS ONE* **6**, e28913.

Gaither, M. R. & Rocha, L. A. (2013). Origins of species richness in the Indo-Malay-Philippine biodiversity hotspot: evidence for the centre of overlap hypothesis. *Journal of Biogeography* **40**, 1638-1648.

Gaither, M. R., Toonen, R. J., Robertson, D. R., Planes, S. & Bowen, B. W. (2010). Genetic evaluation of marine biogeographical barriers: perspectives from two widespread Indo-Pacific snappers (*Lutjanus kasmira* and *Lutjanus fulvus*). *Journal of Biogeography* **37**, 133-147.

Gardner, T. A., Côté, I. M., Gill, J. A., Grant, A. & Watkinson, A. R. (2003). Long-term region-wide declines in Caribbean Corals. *Science* **301**, 958-960.

Gerhardinger, L. C., Hostim-Silva, M., Medeiros, R. P., Matarezi, J., Bertoncini, Á. A., Freitas, M. O. & Ferreira, B. P. (2009). Fishers' resource mapping and goliath grouper *Epinephelus itajara* (Serranidae) conservation in Brazil. *Neotropical Ichthyology* **7**, 93-102.

Gerlach, G., Atema, J., Kingsford, M. J., Black, K. P. & Miller-Sims, V. (2007). Smelling home can prevent dispersal of reef fish larvae. *Proceedings of the National Academy of Sciences* **104**, 858-863.

Glez-Pena, D., Gomez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola F. & Posada, D. (2010). ALTER: program-oriented conversion of DNA and protein alignments. *Nucleic Acids Research* **38**, W14-8.

Goode, M. G. & Rodrigo, A. G. (2007). SQUINT: a multiple alignment program and editor. *Bioinformatics* **23**, 1553-1555.

Goudet, J. (1995). FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. *Journal of Heredity* **86**, 485-486.

Grandcourt, E., Al Abdessalaam, T. Z., Francis, F. & Al Shamsi, A. (2010). Age-based life history parameters and status assessments of by-catch species (*Lethrinus borbonicus*, *Lethrinus microdon*, *Pomacanthus maculosus* and *Scolopsis taeniatus*) in the southern Arabian Gulf. *Journal of Applied Ichthyology* **26**, 381-389.

Grant, W. & Bowen, B. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* **89**, 415-426.

Green, A. L., Maypa, A. P., Almany, G. R., Rhodes, K. L., Weeks, R., Abesamis, R. A., Gleason, M. G., Mumby, P. J. & White, A. T. (2015). Larval dispersal and movement patterns of coral reef fishes, and implications for marine reserve network design. *Biological Reviews* **90**, 1215-1247.

Grilo, C., Chircop, A. & Guerreiro, J. (2012). Prospects for transboundary marine protected areas in East Africa. *Ocean Development & International Law* **43**, 243-266.

Grüss, A., Kaplan, D. M. & Robinson, J. (2014). Evaluation of the effectiveness of marine reserves for transient spawning aggregations in data-limited situations. *ICES Journal of Marine Science* **71**, 435-449.

Guerreiro, J., Chircop, A., Grilo, C., Viras, A., Ribeiro, R. & van der Elst, R. (2010). Establishing a transboundary network of marine protected areas: Diplomatic and management options for the east African context. *Marine Policy* **34**, 896-910.

Gulland, J. A. (1971). The Fish Resources of the Ocean. West Byfleet, Surrey, England: Fishing News (Books) Ltd.

Gust, N., Choat, J. & Ackerman, J. (2002). Demographic plasticity in tropical reef fishes. *Marine Biology* **140**, 1039-1051.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95-98.

Hartup, J. A., Marshell, A., Stevens, G., Kottermair, M. & Carlson, P. (2013). *Manta alfredi* target multispecies surgeonfish spawning aggregations. *Coral Reefs* **32**, 367-367.

Hauser, L., Adcock, G. J., Smith, P. J., Bernal Ramírez, J. H. & Carvalho, G. R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences* **99**, 11742-11747.

Healey, A. J. E., Gouws, G., Fennessy, S. T., Kuguru, B., Sauer, W. H. H., Shaw, P. W., McKeown, N. J. & Handling editor, W. S. G. (2018a). Genetic analysis reveals harvested *Lethrinus nebulosus* in the Southwest Indian Ocean comprise two cryptic species. *ICES Journal of Marine Science* **75**, 1465-1472.

Healey, A. J. E., McKeown, N. J., Taylor, A. L., Provan, J., Sauer, W., Gouws, G. & Shaw, P. W. (2018b). Cryptic species and parallel genetic structuring in Lethrinid fish: Implications for conservation and management in the southwest Indian Ocean. *Ecology and Evolution* **8**, 2182-2195.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution* **59**, 1633-1638.

Hellberg, M. E., Burton, R. S., Neigel, J. E. & Palumbi, S. R. (2002). Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science* **70**, 273-290.

Hernawan, U. E., van Dijk, K.-j., Kendrick, G. A., Feng, M., Biffin, E., Lavery, P. S. & McMahon, K. (2017). Historical processes and contemporary ocean currents drive genetic structure in the seagrass *Thalassia hemprichii* in the Indo-Australian Archipelago. *Molecular Ecology* **26**, 1008-1021.

Hicks, C. C. & McClanahan, T. R. (2012). Assessing gear modifications needed to optimize yields in a heavily exploited, multi-species, seagrass and coral reef fishery. *PLoS ONE* **7**, e36022.

Hilborn, R. & Walters, C. J. (2001). Quantitative fisheries stock assessment: Choice, Dynamics and Uncertainty: Routledge, Chapman & Hall, Inc. 570p.

Hogan, J. D., Thiessen, R. J. & Heath, D. D. (2010). Variability in connectivity indicated by chaotic genetic patchiness within and among populations of a marine fish. *Marine Ecology Progress Series* **417**, 263-275.

Holsinger, K. E. & Weir, B. S. (2009). Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. *Nature Reviews Genetics* **10**, 639-650.

Horne, J. B. (2014). Thinking outside the barrier: neutral and adaptive divergence in Indo-Pacific coral reef faunas. *Evolutionary Ecology* **28**, 991-1002.

Horne, J. B., van Herwerden, L., Choat, J. H. & Robertson, D. R. (2008). High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners. *Molecular Phylogenetics and Evolution* **49**, 629-638.

Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754-755.

Hughes, T. P., Barnes, M. L., Bellwood, D. R., Cinner, J. E., Cumming, G. S., Jackson, J. B. C., Kleypas, J., van de Leemput, I. A., Lough, J. M., Morrison, T. H., Palumbi, S. R., van Nes, E. H. & Scheffer, M. (2017a). Coral reefs in the Anthropocene. *Nature* **546**, 82-90.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., Babcock, R. C., Beger, M., Bellwood, D. R., Berkelmans, R., Bridge, T. C., Butler, I. R., Byrne, M., Cantin, N. E., Comeau, S., Connolly, S. R., Cumming, G. S., Dalton, S. J., Diaz-Pulido, G., Eakin, C. M., Figueira, W. F., Gilmour, J. P., Harrison, H. B., Heron, S. F., Hoey, A. S., Hobbs, J.-P. A., Hoogenboom, M. O., Kennedy, E. V., Kuo, C.-y., Lough, J. M., Lowe, R. J., Liu, G., McCulloch, M. T., Malcolm, H. A., McWilliam, M. J., Pandolfi, J. M., Pears, R. J., Pratchett, M. S., Schoepf, V., Simpson, T., Skirving, W. J., Sommer, B., Torda, G., Wachenfeld, D. R., Willis, B. L. & Wilson, S. K. (2017b). Global warming and recurrent mass bleaching of corals. *Nature* **543**, 373-377.

Hui, M., Kraemer, W. E., Seidel, C., Nuryanto, A., Joshi, A. & Kochzius, M. (2016). Comparative genetic population structure of three endangered giant clams (Cardiidae: *Tridacna* species) throughout the Indo-West Pacific: implications for divergence, connectivity and conservation. *Journal of Molluscan Studies* **82**, 403-414.

Hutchinson, W. F., Oosterhout, C. v., Rogers, S. I. & Carvalho, G. R. (2003). Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus*

morhua). *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**, 2125-2132.

Hutchison, D. W. & Templeton, A. R. (1999). Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**, 1898-1914.

Huyghe, F. & Kochzius, M. (2017). Highly restricted gene flow between disjunct populations of the skunk clownfish (*Amphiprion akallopisos*) in the Indian Ocean. *Marine Ecology* **38**, e12357.

Imron, Jeffrey, B., Hale, P., Degnan, B. M. & Degnan, S. M. (2007). Pleistocene isolation and recent gene flow in *Haliotis asinina*, an Indo-Pacific vetigastropod with limited dispersal capacity. *Molecular Ecology* **16**, 289-304.

Jackson, A. M., Semmens, B. X., Sadovy de Mitcheson, Y., Nemeth, R. S., Heppell, S. A., Bush, P. G., Aguilar-Perera, A., Claydon, J. A. B., Calosso, M. C., Sealey, K. S., Schärer, M. T. & Bernardi, G. (2014). Population structure and phylogeography in nassau grouper (*Epinephelus striatus*), a mass-aggregating marine fish. *PLoS ONE* **9**, e97508.

Johnson, M. S. & Black, R. (1984). Pattern beneath the chaos: The effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution* **38**, 1371-1383.

Jombart, T., Devillard, S. & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11**, 94.

Jones, G. P., Almany, G. R., Russ, G. R., Sale, P. F., Steneck, R. S., van Oppen, M. J. H. & Willis, B. L. (2009). Larval retention and connectivity among populations of corals and reef fishes: History, advances and challenges. *Coral Reefs* **28**, 307-325.

Jones, G. P., Milicich, M. J., Emslie, M. J. & Lunow, C. (1999). Self-recruitment in a coral reef fish population. *Nature* **402**, 802-804.

Jost, L. O. U. (2008). GST and its relatives do not measure differentiation. *Molecular Ecology* **17**, 4015-4026.

Katoh, K., Misawa, K., Kuma K. & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**, 3059-3066.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647-1649.

Kelly, R. P. & Palumbi, S. R. (2010). Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. *PLoS ONE* **5**, e8594.

Keyghobadi, N. (2007). The genetic implications of habitat fragmentation for animals. *Canadian Journal of Zoology* **85**, 1049-1064.

Kinlan, B. P. & Gaines, S. D. (2003). Propagule dispersal in marine and terrestrial environments: A community perspective. *Ecology* **84**, 2007-2020.

Klanten, O. S., Choat, J. H. & van Herwerden, L. (2007). Extreme genetic diversity and temporal rather than spatial partitioning in a widely distributed coral reef fish. *Marine Biology* **150**, 659-670.

Knowlton, N. (1992). Thresholds and Multiple Stable States in Coral Reef Community Dynamics1. *American Zoologist* **32**, 674-682.

Kochzius, M. & Nuryanto, A. (2008). Strong genetic population structure in the boring giant clam, *Tridacna crocea*, across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. *Molecular Ecology* **17**, 3775-3787.

Kochzius, M., Seidel, C., Hauschild, J., Kirchhoff, S., Mester, P., Meyer-Wachsmuth, I., Nuryanto, A. & Timm, J. (2009). Genetic population structures of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina*. *Marine Ecology Progress Series* **396**, 211-220.

Kochzius, M., Söller, R., Khalaf, M. A. & Blohm, D. (2003). Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **28**, 396-403.

Kotiaho, J. S., Kaitala, V., Komonen, A. & Päivinen, J. (2005). Predicting the risk of extinction from shared ecological characteristics. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 1963-1967.

Kuiter, R. H. & Debelius, H. (2001). Surgeonfishes, rabbitfishes and their relatives. A comprehensive guide to Acanthuroidei (marine fish families). TMC Publishing, Chorleywood.

Lambeck, K. & Chappell, J. (2001). Sea level change through the last glacial cycle. *Science* **292**, 679-686.

Lambeck, K., Yokoyama, Y. & Purcell, T. (2002). Into and out of the Last Glacial Maximum: sea-level change during Oxygen Isotope Stages 3 and 2. *Quaternary Science Reviews* **21**, 343-360.

Larmuseau, M. H. D., Raeymaekers, J. A. M., Hellemans, B., Van Houdt, J. K. J. & Volckaert, F. A. M. (2010). Mito-nuclear discordance in the degree of population differentiation in a marine goby. *Heredity* **105**, 532-542.

Lavery, S., Moritz, C. & Fielder, D. R. (1996). Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. *Molecular Ecology* **5**, 557-570.

Leis, M. J. & Carson-Ewart, M. B. (1997). In situ swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. *Marine Ecology Progress Series* **159**, 165-174.

Leis, M. J., Herwerden, L. V. & Patterson, H. M. (2011). Estimating connectivity in marine fish populations: What works best? *Gibson, R.N., Atkinson, R.J.A., and Gordon, J.D.M., (eds.) Oceanography and Marine Biology: An Annual Review. Oceanography and Marine Biology: An Annual Review* **49**, CRC Press, Boca Raton, FL, USA, PP. 193-234.

Lessios, H. A. (2008). The great American schism: Divergence of marine organisms after the rise of the central American Isthmus. *Annual Review of Ecology, Evolution, and Systematics* **39**, 63-91.

Lessios, H. A., Kane, J. & Robertson, D. R. (2003). Phylogeography of the pantropical sea urchin *Tripneustes*: Contrasting patterns of population structure between oceans. *Evolution* **57**, 2026-2036.

Lessios, H. A., Kessing, B. D. & Pearse, J. S. (2001). Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution* **55**, 955-975.

Lessios, H. A., Kessing, B. D., Robertson, D. R. & Paulay, G. (1999). Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. *Evolution* **53**, 806-817.

Lessios, H. A. & Robertson, D. R. (2006). Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proceedings of the Royal Society B Biological Sciences* **273**, 2201-2208.

Levin, N., Beger, M., Maina, J., McClanahan, T. & Kark, S. (2018). Evaluating the potential for transboundary management of marine biodiversity in the Western Indian Ocean. *Australasian Journal of Environmental Management* **25**, 62-85.

Liggins, L., Treml, E. A., Possingham, H. P. & Riginos, C. (2016). Seascape features, rather than dispersal traits, predict spatial genetic patterns in co-distributed reef fishes. *Journal of Biogeography* **43**, 256-267.

Liu, S. Y. V., Dai, C. F., Allen, G. R. & Erdmann, M. V. (2012). Phylogeography of the neon damselfish *Pomacentrus coelestis* indicates a cryptic species and different species origins in the West Pacific Ocean. *Marine Ecology Progress Series* **458**, 155-167.

Lobel, P. S. & Robinson, A. R. (1988). Larval fishes and zooplankton in a cyclonic eddy in Hawaiian waters. *Journal of Plankton Research* **10**, 1209-1223.

Lowe, W. H. & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology* **19**, 3038-3051.

Ludt, W. B., Bernal, M. A., Bowen, B. W. & Rocha, L. A. (2012). Living in the past: Phylogeography and population histories of Indo-Pacific wrasses (Genus *Halichoeres*) in shallow lagoons versus outer reef slopes. *PLoS ONE* **7**, e38042.

Ludt, W. B. & Rocha, L. A. (2014). Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. *Journal of Biogeography* **42**, 25-38.

Lumpkin, R. & Johnson, G. C. (2013). Global ocean surface velocities from drifters: Mean, variance, El Niño–Southern Oscillation response, and seasonal cycle. *Journal of Geophysical Research: Oceans* **118**, 2992-3006.

Mangi, S. C. & Roberts, C. M. (2006). Quantifying the environmental impacts of artisanal fishing gear on Kenya's coral reef ecosystems. *Marine Pollution Bulletin* **52**, 1646-1660.

Mangi, S. C., Roberts, C. M. & Rodwell, L. D. (2007). Financial comparisons of fishing gear used in Kenya's coral reef lagoons. *Ambio* **36**, 671-676.

Marandel, F., Lorance, P., Andrello, M., Charrier, G., Le Cam, S., Lehuta, S. & Trenkel, V. M. (2017). Insights from genetic and demographic connectivity for the management of rays and skates. *Canadian Journal of Fisheries and Aquatic Sciences* **75**, 1291-1302.

Marie, A. D., van Herwerden, L., Choat, J. H. & Hobbs, J.-P. A. (2007). Hybridization of reef fishes at the Indo-Pacific biogeographic barrier: a case study. *Coral Reefs* **26**, 841-850.

Marko, P. B. (2004). 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. *Molecular Ecology* **13**, 597-611.

Marshell, A. & Mumby, P. J. (2015). The role of surgeonfish (Acanthuridae) in maintaining algal turf biomass on coral reefs. *Journal of Experimental Marine Biology and Ecology* **473**, 152-160.

Mayorga-Adame, C. G., Batchelder, H. P. & Spitz, Y. H. (2017). Modeling larval connectivity of coral reef organisms in the Kenya-Tanzania region. *Frontiers in Marine Science* **4**.

Mazor, T., Possingham, H. P. & Kark, S. (2013). Collaboration among countries in marine conservation can achieve substantial efficiencies. *Diversity and Distributions* **19**, 1380-1393.

McClanahan, T. R. (2015). Biogeography versus resource management: how do they compare when prioritizing the management of coral reef fish in the south-western Indian Ocean? *Journal of Biogeography* **42**, 2414-2426.

McClanahan, T. R., Graham, N. A. J., Calnan, J. M. & MacNeil, M. A. (2007). Toward pristine biomass: Reef fish recovery in coral reef marine protected areas in Kenya. *Ecological Applications* **17**, 1055-1067.

McClanahan, T. R. & Hicks, C. C. (2011). Changes in life history and ecological characteristics of coral reef fish catch composition with increasing fishery management. *Fisheries Management and Ecology* **18**, 50-60.

McClanahan, T. R., Kaunda-Arara, B. & Omukoto, J. O. (2010). Composition and diversity of fish and fish catches in closures and open access fisheries of Kenya. *Fisheries Management and Ecology* **17**, 63-76.

McClanahan, T. R. & Mangi, S. (2001). The effect of a closed area and beach seine exclusion on coral reef fish catches. *Fisheries Management and Ecology* **8**, 107-121.

McClanahan, T. R. & Mangi, S. C. (2004). Gear-based management of a tropical artisanal fishery based on species selectivity and capture size. *Fisheries Management and Ecology* **11**, 51-60.

McClanahan, T. R., Muthiga, N. A., Kamukuru, A. T., Machano, H. & Kiambo, R. W. (1999). The effects of marine parks and fishing on coral reefs of northern Tanzania. *Biological Conservation* **89**, 161-182.

McCormick, I. M. (1999). Delayed metamorphosis of a tropical reef fish (*Acanthurus triostegus*): a field experiment. *Marine Ecology Progress Series* **176**, 25-38.

McMillan, W. O. & Palumbi, S. R. (1995). Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. *Proceedings: Biological Sciences* **260**, 229-236.

Mildenberger, T. K., Taylor, M. H. & Wolff, M. (2017). TropFishR: an R package for fisheries analysis with length-frequency data. *Methods in Ecology and Evolution* **8**, 1520-1527.

Mirams, A. G. K., Treml, E. A., Shields, J. L., Liggins, L. & Riginos, C. (2011). Vicariance and dispersal across an intermittent barrier: population genetic structure of marine animals across the Torres Strait land bridge. *Coral Reefs* **30**, 937-949.

Mitarai, S., Siegel, D. A., Watson, J. R., Dong, C. & McWilliams, J. C. (2009). Quantifying connectivity in the coastal ocean with application to the Southern California Bight. *Journal of Geophysical Research: Oceans* **114**, C10026.

Mora, C. & Sale, P. F. (2002). Are populations of coral reef fish open or closed? *Trends in Ecology & Evolution* **17**, 422-428.

Mumby, P. J., Harborne, A. R., Williams, J., Kappel, C. V., Brumbaugh, D. R., Micheli, F., Holmes, K. E., Dahlgren, C. P., Paris, C. B. & Blackwell, P. G. (2007). Trophic cascade facilitates coral recruitment in a marine reserve. *Proceedings of the National Academy of Sciences* **104**, 8362-8367.

Muths, D., Gouws, G., Mwale, M., Tessier, E. & Bourjea, J. (2012). Genetic connectivity of the reef fish *Lutjanus kasmira* at the scale of the western Indian Ocean. *Canadian Journal of Fisheries and Aquatic Sciences* **69**, 842-853.

Muths, D., Tessier, E. & Bourjea, J. (2015). Genetic structure of the reef grouper *Epinephelus merra* in the West Indian Ocean appears congruent with biogeographic and oceanographic boundaries. *Marine Ecology* **36**, 447-461.

Muths, D., Tessier, E., Gouws, G., Craig, M., Mwale, M., Mwaluma, J., Mwandya, A. & Bourjea, J. (2011). Restricted dispersal of the reef fish *Myripristis berndti* at the scale of the SW Indian Ocean. *Marine Ecology Progress Series* **443**, 167-180.

Nakajima, Y., Matsuki, Y., Lian, C., Fortes, M. D., Uy, W. H., Campos, W. L., Nakaoka, M. & Nadaoka, K. (2014). The Kuroshio current influences genetic diversity and population genetic structure of a tropical seagrass, *Enhalus acoroides*. *Molecular Ecology* **23**, 6029-6044.

Neumann, R. M., Guy, C. S., & Willis, D. W. (2012). Length, weight, and associated indices. Fisheries techniques, 3rd edition American Fisheries Society, Bethesda, Maryland. 2012:637–676.

Nolasco, R., Gomes, I., Peteiro, L., Albuquerque, R., Luna, T., Dubert, J., Swearer, S. E. & Queiroga, H. (2018). Independent estimates of marine population connectivity are more concordant when accounting for uncertainties in larval origins. *Scientific Reports* **8**, 2641.

Nuryanto, A. & Kochzius, M. (2009). Highly restricted gene flow and deep evolutionary lineages in the giant clam *Tridacna maxima*. *Coral Reefs* **28**, 607-619.

O'Donnell, J. L., Beldade, R., Mills, S. C., Williams, H. E. & Bernardi, G. (2017). Life history, larval dispersal, and connectivity in coral reef fish among the Scattered Islands of the Mozambique Channel. *Coral Reefs* **36**, 223-232.

Obura, D. (2012). The diversity and biogeography of Western Indian Ocean reef-building corals. *PLoS ONE* **7**, e45013.

Obura, D. O. (2001). Participatory monitoring of shallow tropical marine fisheries by artisanal fishers in Diani, Kenya. *Bulletin of Marine Science* **69**, 777-791.

Obura, D., Smits, M., Chaudhry, T., McPhillips, J., Beal D. & Astier, C. (2017). Reviving the Western Indian Ocean economy: Actions for a sustainable future. *WWF International, Gland*.

Okemwa, G. M., Kaunda-Arara, B., Kimani, E. N. & Ogutu, B. (2016). Catch composition and sustainability of the marine aquarium fishery in Kenya. *Fisheries Research* **183**, 19-31.

Osio, G. C., Orio, A. & Millar, C. P. (2015). Assessing the vulnerability of Mediterranean demersal stocks and predicting exploitation status of un-assessed stocks. *Fisheries Research* **171**, 110-121.

Otwoma, L. M. & Kochzius, M. (2016). Genetic population structure of the coral reef sea star *Linckia laevigata* in the Western Indian Ocean and Indo-West Pacific. *PLoS ONE* **11**, e0165552.

Otwoma, L. M., Reuter, H., Timm, J. & Meyer, A. (2018). Genetic connectivity in a herbivorous coral reef fish (*Acanthurus leucosternon* Bennet, 1833) in the Eastern African region. *Hydrobiologia* **806**, 237-250.

Palumbi, S. R. (2003). Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* **13**, 146-158.

Papadopoulou, A. & Knowles, L. L. (2016). Toward a paradigm shift in comparative phylogeography driven by trait-based hypotheses. *Proceedings of the National Academy of Sciences* **113**, 8018-8024.

Pauly, D. (1980). On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. *ICES Journal of Marine Science* **39**, 175-192.

Pauly, D. (1984). Fish population dynamics in tropical waters: a manual for use with programmable calculators. *ICLARM Studies and Reviews* **8**, 325.

Pauly, D., Christensen, V., Dalsgaard, J., Froese, R. & Torres, F. (1998). Fishing down marine food webs. *Science* **279**, 860-863.

Peakall, R. & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**, 2537-2539.

Pellissier, L., Leprieur, F., Parravicini, V., Cowman, P. F., Kulbicki, M., Litsios, G., Olsen, S. M., Wisz, M. S., Bellwood, D. R. & Mouillot, D. (2014). Quaternary coral reef refugia preserved fish diversity. *Science* **344**, 1016-1019.

Pinsky, M. L., Saenz-Agudelo, P., Salles, O. C., Almany, G. R., Bode, M., Berumen, M. L., Andréfouët, S., Thorrold, S. R., Jones, G. P. & Planes, S. (2017). Marine dispersal scales are congruent over evolutionary and ecological time. *Current Biology* **27**, 149-154.

Planes, S. (1993). Genetic differentiation in relation to restricted larval dispersal of the convict surgeonfish *Acanthurus triostegus* in French Polynesia. *Marine Ecology Progress Series* **98**, 237-246.

Planes, S. & Fauvelot, C. (2002). Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. *Evolution* **56**, 378-399.

Planes, S., Jones, G. P. & Thorrold, S. R. (2009). Larval dispersal connects fish populations in a network of marine protected areas. *Proceedings of the National Academy of Sciences* **106**, 5693-5697.

Planes, S., Parroni, M. & Chauvet, C. (1998). Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia. *Marine Biology* **130**, 361-368.

Poortvliet, M., Longo, G. C., Selkoe, K., Barber, P. H., White, C., Caselle, J. E., Perez-Matus, A., Gaines, S. D. & Bernardi, G. (2013). Phylogeography of the California sheephead, *Semicossyphus pulcher*: the role of deep reefs as stepping stones and pathways to antitropicality. *Ecology and Evolution* **3**, 4558-4571.

Portnoy, D. S., Hollenbeck, C. M., Renshaw, M. A., Cummings, N. J. & Gold, J. R. (2012). Does mating behaviour affect connectivity in marine fishes? Comparative population genetics of two protogynous groupers (Family Serranidae). *Molecular Ecology* **22**, 301-313.

Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253-1256.

Prince, J., Victor, S., Kloulchad, V. & Hordyk, A. (2015). Length based SPR assessment of eleven Indo-Pacific coral reef fish populations in Palau. *Fisheries Research* **171**, 42-58.

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959.

Puebla, O., Bermingham, E. & Guichard, F. (2009). Estimating dispersal from genetic isolation by distance in a coral reef fish (*Hypoplectrus puella*). *Ecology* **90**, 3087-3098.

Puebla, O., Bermingham, E. & McMillan, W. O. (2012). On the spatial scale of dispersal in coral reef fishes. *Molecular Ecology* **21**, 5675-5688.

Puritz, J. B., Keever, C. C., Addison, J. A., Barbosa, S. S., Byrne, M., Hart, M. W., Grosberg, R. K. & Toonen, R. J. (2017). Life history predicts past and present population connectivity in two sympatric sea stars. *Ecology and Evolution* **7**, 3916-3930.

Puritz, J. B. & Toonen, R. J. (2011). Coastal pollution limits pelagic larval dispersal. *Nature Communications* **2**, 226.

Pusack, T. J., Christie, M. R., Johnson, D. W., Stallings, C. D. & Hixon, M. A. (2014). Spatial and temporal patterns of larval dispersal in a coral-reef fish metapopulation: evidence of variable reproductive success. *Molecular Ecology* **23**, 3396-3408.

Ragionieri, L., Cannicci, S., Schubart, C. D. & Fratini, S. (2010). Gene flow and demographic history of the mangrove crab *Neosarmatium meinerti*: A case study from the western Indian Ocean. *Estuarine, Coastal and Shelf Science* **86**, 179-188.

Randall, E. J. (1956). A revision of the surgeonfish genus *Acanthurus*. *Pacific science* **10**, 159-235.

Randall, E. J. (2002). Surgeonfishes of Hawaii and the world. Mutual Publishing and Bishop Museum Press, Hawaii.

Ratsimbazafy, H. A. & Kochzius, M. (2018). Restricted gene flow among western Indian Ocean populations of the mangrove whelk *Terebralia palustris* (Linnaeus, 1767) (Caenogastropoda: Potamididae). *Journal of Molluscan Studies* **84**, 163-169.

Raymond, M. & Rousset, F. (1995). GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Reece, J. S., Bowen, B. W., Smith, D. G. & Larson, A. (2010). Molecular phylogenetics of moray eels (Muraenidae) demonstrates multiple origins of a shell-crushing jaw (Gymnomuraena, Echidna) and multiple colonizations of the Atlantic Ocean. *Molecular Phylogenetics and Evolution* **57**, 829-835.

Rehren, J., Wolff, M. & Jiddawi, N. (2018). Fisheries assessment of Chwaka Bay (Zanzibar) – following a holistic approach. *Journal of Applied Ichthyology* **34**, 117-128.

Reid, D. G., Lal, K., Mackenzie-Dodds, J., Kaligis, F., Littlewood, D. T. J. & Williams, S. T. (2006). Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. *Journal of Biogeography* **33**, 990-1006.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Ridgway, T. & Sampayo, E. M. (2005). Population genetic status of the Western Indian Ocean: What do we know? *West Indian Ocean Journal of Marine Science* **4**, 1-10.

Riginos, C., Buckley, Y. M., Blomberg, S. P. & Treml, E. A. (2014). Dispersal capacity predicts both population genetic structure and species richness in reef fishes. *The American Naturalist* **184**, 52-64.

Riginos, C., Douglas, K. E., Jin, Y., Shanahan, D. F. & Treml, E. A. (2011). Effects of geography and life history traits on genetic differentiation in benthic marine fishes. *Ecography* **34**, 566-575.

Riginos, C. & Liggins, L. (2013). Seascape genetics: Populations, individuals, and genes marooned and adrift. *Geography Compass* **7**, 197-216.

Roberts, C. M. (1997). Connectivity and management of caribbean coral reefs. *Science* **278**, 1454-1457.

Robertson, D. R., Polunin, N. V. C. & Leighton, K. (1979). The behavioral ecology of three Indian Ocean surgeonfishes (*Acanthurus lineatus*, *A. leucosternon* and *Zebrasoma scopas*): their feeding strategies, and social and mating systems. *Environmental Biology of Fishes* **4**, 125-170.

Robinson, J., Cinner, J. E. & Graham, N. A. J. (2014). The influence of fisher knowledge on the susceptibility of reef fish aggregations to fishing. *PLoS ONE* **9**, e91296.

Robinson, J., Graham, N. A. J., Cinner, J. E., Almany, G. R. & Waldie, P. (2015). Fish and fisher behaviour influence the vulnerability of groupers (Epinephelidae) to fishing at a multispecies spawning aggregation site. *Coral Reefs* **34**, 371-382.

Rocha, L. A., Bass, A. L., Robertson, D. R. & Bowen, B. W. (2002). Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Molecular Ecology* **11**, 243-251.

Rogers, A. R. (1995). Genetic evidence for a Pleistocene population explosion. *Evolution* **49**, 608-615.

Rogers, A. R. & Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9**, 552-569.

Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106.

Sadovy de Mitcheson, Y., Craig, M. T., Bertoncini, A. A., Carpenter, K. E., Cheung, W. W. L., Choat, J. H., Cornish, A. S., Fennessy, S. T., Ferreira, B. P., Heemstra, P. C., Liu, M., Myers, R. F., Pollard, D. A., Rhodes, K. L., Rocha, L. A., Russell, B. C., Samoilys, M. A. & Sanciangco, J. (2013). Fishing groupers towards extinction: A global assessment of threats and extinction risks in a billion dollar fishery. *Fish and Fisheries* **14**, 119-136.

Saenz-Agudelo, P., Jones, G. P., Thorrold, S. R. & Planes, S. (2011). Connectivity dominates larval replenishment in a coastal reef fish metapopulation. *Proceedings of the Royal Society B: Biological Sciences* **278**, 2954-2961.

Sala, E., Aburto-Oropeza, O., Paredes, G. & Thompson, G. (2003). Spawning aggregations and reproductive behavior of reef fishes in the Gulf of California. *Bulletin of Marine Science* **72**, 103-121.

Sale, P. F., Cowen, R. K., Danilowicz, B. S., Jones, G. P., Kritzer, J. P., Lindeman, K. C., Planes, S., Polunin, N. V. C., Russ, G. R., Sadovy, Y. J. & Steneck, R. S. (2005). Critical science gaps impede use of no-take fishery reserves. *Trends in Ecology & Evolution* **20**, 74-80.

Samoilys, M. A., Osuka, K., Maina, G. W. & Obura, D. O. (2017). Artisanal fisheries on Kenya's coral reefs: Decadal trends reveal management needs. *Fisheries Research* **186**, 177-191.

Schneider, S. & Excoffier, L. (1999). Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: Application to human mitochondrial DNA. *Genetics* **152**, 1079-1089.

Schott, F. A. & McCreary Jr, J. P. (2001). The monsoon circulation of the Indian Ocean. *Progress in Oceanography* **51**, 1-123.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**, 233-234.

Schultz, L. P. & Woods, L. P. (1948). *Acanthurus triostegus marquesensis*, a new subspecies of surgeonfish, family Acanthuridae, with notes on related forms. *Journal of the Washington Academy of Sciences* **38**:248-251.

Scrucca, L. (2013). GA: A package for genetic algorithms in R. *Journal of Statistical Software* **53**, 1-37.

Selkoe, K. A., D'Aloia, C. C., Crandall, E. D., Iacchei, M., Liggins, L., Puritz, J. B., von der Heyden, S. & Toonen, R. J. (2016). A decade of seascape genetics: contributions to basic and applied marine connectivity. *Marine Ecology Progress Series* **554**, 1-19.

Selkoe, K. A., Gaggiotti, O. E., ToBo, L., Bowen, B. W. & Toonen, R. J. (2014). Emergent patterns of population genetic structure for a coral reef community. *Molecular Ecology* **23**, 3064-3079.

Selkoe, K. A., Gaines, S. D., Caselle, J. E. & Warner, R. R. (2006). Current shifts and kin aggregation explain genetic patchiness in fish recruits. *Ecology* **87**, 3082-3094.

Selkoe, K. A. & Toonen, R. J. (2011). Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. *Marine Ecology Progress Series* **436**, 291-305.

Selwyn, J. D., Hogan, J. D., Downey-Wall, A. M., Gurski, L. M., Portnoy, D. S. & Heath, D. D. (2016). Kin-aggregations explain chaotic genetic patchiness, a commonly observed genetic pattern, in a marine fish. *PLoS ONE* **11**, e0153381.

Shaw, P. W., Hendrickson, L., McKeown, N. J., Stonier, T., Naud, M. J. & Sauer, W. H. H. (2010). Discrete spawning aggregations of loliginid squid do not represent genetically distinct populations. *Marine Ecology Progress Series* **408**, 117-127.

Sheppard, C. (2000). *Coral reefs of the Indian Ocean their ecology and conservation. In*: McClanahan, T.R., Sheppard, C.R.C. and Obura, D.O. (eds.) *Coral Reefs of the Indian Ocean their ecology and Conservation*. Oxford University Press.525 pp.ISBN 0-19-512596-7.

Silva, I. C., Mesquita, N. & Paula, J. (2010a). Genetic and morphological differentiation of the mangrove crab *Perisesarma guttatum* (Brachyura: Sesarmidae) along an East African latitudinal gradient. *Biological Journal of the Linnean Society* **99**, 28-46.

Silva, I. C., Mesquita, N. & Paula, J. (2010b). Lack of population structure in the fiddler crab *Uca annulipes* along an East African latitudinal gradient: genetic and morphometric evidence. *Marine Biology* **157**, 1113-1126.

Song, C. B., Near, T. J. & Page, L. M. (1998). Phylogenetic relations among percid fishes as inferred from mitochondrial cytochromeb DNA sequence data. *Molecular Phylogenetics and Evolution* **10**, 343-353.

Sorenson, L., Santini, F., Carnevale, G. & Alfaro, M. E. (2013). A multi-locus timetree of surgeonfishes (Acanthuridae, Percomorpha), with revised family taxonomy. *Molecular Phylogenetics and Evolution* **68**, 150-160.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, C. A. & Robertson, J. (2007). Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *BioScience* **57**, 573-583.

Stobutzki, I. C. & Bellwood, D. R. (1997). Sustained swimming abilities of the late pelagic stages of coral reef fishes. *Marine Ecology Progress Series* **149**, 35-41.

Streets, T. H. (1877). Ichthyology. In: Contributions to the natural history of the Hawaiian and Fanning islands and Lower California, made in connection with the United States North Pacific surveying expedition, 1873-75. *Bulletin of the United States National Museum* **1**: 43-102.

Sunnucks, P. & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution* **13**, 510-524.

Swallow, J. C., Schott, F. & Fieux, M. (1991). Structure and transport of the East African Coastal Current. *Journal of Geophysical Research: Oceans* **96**, 22245-22257.

Taberlet, P., Meyer, A. & Bouvetv, J. (1992). Unusual mitochondrial DNA polymorphism in two local populations of blue tit *Parus caeruleus*. *Molecular Ecology* **1**, 27-36.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585-595.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725-2729.

Taylor, B. M., Houk, P., Russ, G. R. & Choat, J. H. (2014). Life histories predict vulnerability to overexploitation in parrotfishes. *Coral Reefs* **33**, 869-878.

Taylor, M. H. & Mildenberger, T. K. (2017). Extending electronic length frequency analysis in R. *Fisheries Management and Ecology* **24**, 330-338.

Teske, P. R., Papadopoulos, I., Zardi, G. I., McQuaid, C. D., Edkins, M. T., Griffiths, C. L. & Barker, N. P. (2007). Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: Planktonic, abbreviated and direct development. *Marine Biology* **152**, 697-711.

Then, A. Y., Hoenig, J. M., Hall, N. G., & Hewitt, D. A. (2015). Evaluating the predictive performance of empirical estimators of natural mortality rate using information on over 200 fish species. *ICES Journal of Marine Science* **72**, 82-92.

Thorrold, S. R., Jones, G. P., Hellberg, M. E., Burton, R. S., Swearer, S. E., Neigel, J. E., Morgan, S. G. & Warner, R. R. (2002). Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bulletin of Marine Science* **70**, 291-308.

Thorrold, S. R., Jones, G. P., Planes, S. & Hare, J. A. (2006). Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. *Canadian Journal of Fisheries and Aquatic Sciences* **63**, 1193-1197.

Thresher, R. E. (1984). *Reproduction in reef fishes*: Tfh Publications Incorporated.

Timm, J., Figiel, M. & Kochzius, M. (2008). Contrasting patterns in species boundaries and evolution of anemonefishes (Amphiprioninae, Pomacentridae) in the centre of marine biodiversity. *Molecular Phylogenetics and Evolution* **49**, 268-276.

Timm, J. & Kochzius, M. (2008). Geological history and oceanography of the Indo-Malay Archipelago shape the genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*). *Molecular Ecology* **17**, 3999-4014.

Tuda, P. M., Wolff, M. & Breckwoldt, A. (2016). Size structure and gear selectivity of target species in the multispecies multigear fishery of the Kenyan South Coast. *Ocean & Coastal Management* **130**, 95-106.

Turner, T. F., Wares, J. P. & Gold, J. R. (2002). Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, Estuarine-dependent marine fish (*Sciaenops ocellatus*). *Genetics* **162**, 1329-1339.

Tyler, J. C. & Micklich, N. R. (2011). A new genus and species of surgeon fish (Perciformes, Acanthuridae) from the Oligocene of Kanton Glarus, Switzerland. *Swiss Journal of Paleontology* **130**, 203-216.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. (2004). micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Villesen, P. (2007). FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes* **7**, 965-968.

Visram, S., Yang, M.-C., Pillay, R. M., Said, S., Henriksson, O., Grahn, M. & Chen, C. A. (2010). Genetic connectivity and historical demography of the blue barred parrotfish (*Scarus ghobban*) in the western Indian Ocean. *Marine Biology* **157**, 1475-1487.

Vogler, C., Benzie, J., Barber, P. H., Erdmann, M. V., Ambariyanto, Sheppard, C., Tenggardjaja, K., Gérard, K. & Wörheide, G. (2012). Phylogeography of the crown-of-thorns starfish in the Indian Ocean. *PLoS ONE* **7**, e43499.

Voris, H. K. (2000). Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* **27**, 1153-1167.

Warner, R. R. & Cowen, R. K. (2002). Local retention of production in marine populations: Evidence, mechanisms, and consequences. *Bulletin of Marine Science* **70**, 245-249.

Weber, A. A. T., Mérigot, B., Valière, S. & Chenuil, A. (2015). Influence of the larval phase on connectivity: strong differences in the genetic structure of brooders and broadcasters in the *Ophioderma longicauda* species complex. *Molecular Ecology* **24**, 6080-6094.

Weersing, K. & Toonen, R. J. (2009). Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series* **393**, 1-12.

Wetherall, J. A. (1986). A new method for estimating growth and mortality parameters from length frequency data. *Fishbyte* **4**, 12-14.

White, C., Selkoe, K. A., Watson, J., Siegel, D. A., Zacherl, D. C. & Toonen, R. J. (2010). Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B: Biological Sciences* **277**, 1685-1694.

Whiteman, E. A. & Côté, I. M. (2004). Monogamy in marine fishes. *Biological Reviews* **79**, 351-375.

REFERENCES

Williams, S. T. & Benzie, J. A. H. (1998). Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within the Indo-West Pacific defined by color morphs, mtDNA, and allozyme data. *Evolution* **52**, 87-99.

Williams, S. T., Jara, J., Gomez, E. & Knowlton, N. (2002). The marine Indo-West Pacific break: Contrasting the Resolving Power of Mitochondrial and Nuclear Genes1. *Integrative and Comparative Biology* **42**, 941-952.

Wilson, S. K., Graham, N. A. J., Pratchett, M. S., Jones, G. P. & Polunin, N. V. C. (2006). Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? *Global Change Biology* **12**, 2220-2234.

Wismer, S., Hoey, A. S. & Bellwood, D. R. (2009). Cross-shelf benthic community structure on the Great Barrier Reef: relationships between macroalgal cover and herbivore biomass. *Marine Ecology Progress Series* **376**, 45-54.

Wren, J. L. K., Kobayashi, D. R., Jia, Y. & Toonen, R. J. (2016). Modeled population connectivity across the Hawaiian archipelago. *PLoS ONE* **11**, e0167626.

WWF, 2006.The Eastern Africa coastal forests ecoregion. Strategic framework for conservation: 2005 – 2025. WWF-EARPO, Nairobi.

WWF, 2004. The Eastern African Marine Ecoregion Vision: A large scale conservation approach to the management of biodiversity. WWF: Dar es Salaam, Tanzania. 53 pp.

Xiang, Y., Gubian, S., Suomela, B. & Hoeng, J. (2013). Generalized simulated annealing for efficient global optimization: The GenSA package for R. *The R Journal* **5**, 13-28.

Yasuda, N., Nagai, S., Hamaguchi, M., Okaji, K. E. N., GÉRard, K. & Nadaoka, K. (2009). Gene flow of *Acanthaster planci* (L.) in relation to ocean currents revealed by microsatellite analysis. *Molecular Ecology* **18**, 1574-1590.

Zatcoff, M. S., Ball, A. O. & Sedberry, G. R. (2004). Population genetic analysis of red grouper, *Epinephelus morio*, and scamp, *Mycteroperca phenax*, from the southeastern U.S. Atlantic and Gulf of Mexico. *Marine Biology* **144**, 769-777.

ACKNOWLEDGEMENTS

First, I am very grateful to the organizations that provided the funding for this Ph.D. research, including the Leibniz Centre For Tropical Marine Research, Bremen (ZMT), German Academic Exchange Service (DAAD), and the Ministry of Higher Education, Science, and Technology Kenya (MoHEST). I wish also to acknowledge the support of Bremen International Graduate School for Marine Sciences (GLOMAR) to attend the third international workshop of the Diversity of the Indo-Pacific Network (DIPnet) at Stellenbosch, South Africa.

Certainly, this work would not have been possible without the support of my supervisors PD. Dr. Hauke Reuter and Dr. Achim Meyer, who provided scientific advice and encouragement during the execution of this Ph.D. I would also like to acknowledge the other two members in my Ph.D. committee, Professor Matthias Wolff and Dr. Janne Timm who generously gave input on my work. Many thanks to the examination committee: Prof. Dr. Wilhelm Hagen, Prof. Dr. Martin Zimmer, Rebecca Borges, and Anna Feuring.

Special thanks are also extended to the fishermen and field assistants at the following landing sites along the Eastern African coastline: Kiunga, Malindi, Kuruwitu, Mombasa, Msambweni, Shimoni, Tanga, Kunduchi, Mtwara, and Kilindi. In particular, I wish to acknowledge Mr. Daniel Ocharo, Mr. Joshua Omweri, Mr. Abubakar Athman, Mr. January Ndagala, Mr. Shaban Chikambo, and Mr. Patroba Matiku for providing logistical support during fieldwork. I am indebted to Dr. Michelle Gaither, Dr. Joseph DiBattista, and Dr. Joshua Copus of the Hawaii Institute of Marine Biology for providing samples from Seychelles, Diego Garcia, Cocos Keeling, and Christmas Island. I would also like to thank Professor Dr. Marc Kochzius and Hajaniaina Ratsimbazafy of the Vrije Universiteit Brussel for assisting in sample collections in Madagascar.

I want to extend my appreciation to the DAAD African section in particular to Mr. Cay Etzold, MS. Caroline Wiegand, Mr. Martin Nawade, and Ms. Anja Bengelstorff for providing me with

personal support before and after settlement in Germany. I would also like to thank the Studentenwerk Bremen office and in particular Frau Heike Niemeyer for helping me to get accommodation during my Ph.D. stay in Bremen. Appreciations are also to extended to Die Friese Jugend und Jugendkulturzentrum Bremen for helping me to repair my bicycle on several occasions. I am indebted to ZMT technicians Sonja Peters, Stefanie Brohl, and Constanze von Waldthausen for their support during laboratory analysis.

The Ph.D. journey would not have been possible without the company of great colleagues at ZMT Bremen including Fabian Werner, Seth Mensa, Paul Tuda, Manuel Jänig, Rebecca Borges, Michael Murunga, Jialin Zhang and many others not listed here. Finally, my wife Flora deserves extraordinary thanks for taking care of our son Leroy, while I was busy with my Ph.D. project.

APPENDIX

Supplementary materials for chapter 2

Supplementary Table 2.1 Pairwise D_{EST} values among populations of *Acanthurus leucosternon* in Eastern Africa. Sample codes are presented in Table 1.

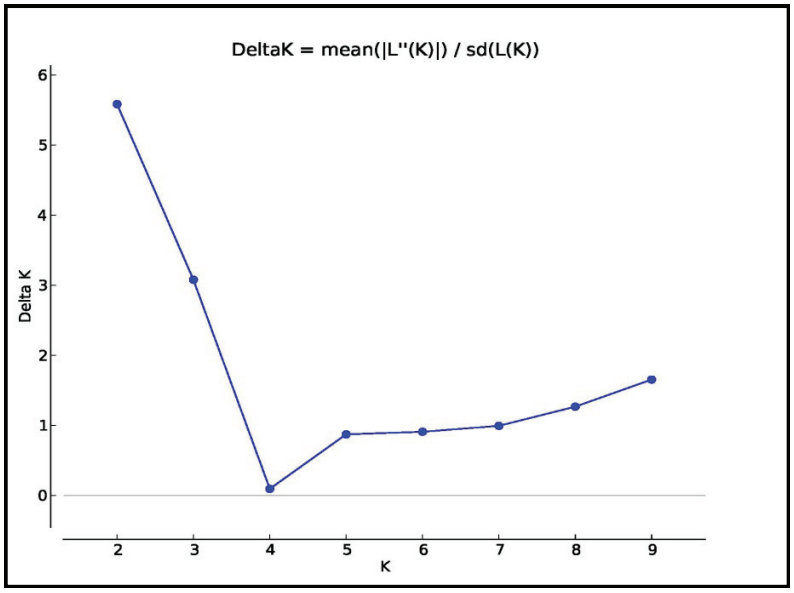
	ML	DS	KL	MT	MO	KR	KM	TA	KU
DS	0.019^{ns}								
KL	-0.001^{ns}	0.057^{ns}							
MT	-0.008^{ns}	0.044^{ns}	0.019^{ns}						
MO	0.003^{ns}	0.078^{ns}	0.024^{ns}	0.021^{ns}					
KR	0.043^{ns}	0.061^{ns}	0.049^{ns}	0.042^{ns}	0.070^{ns}				
KM	-0.017^{ns}	0.030^{ns}	0.003^{ns}	0.020^{ns}	0.035^{ns}	0.035^{ns}			
TA	0.037^{ns}	0.029^{ns}	0.019^{ns}	0.019^{ns}	0.035^{ns}	0.023^{ns}	0.027^{ns}		
KU	0.030^{ns}	0.024^{ns}	0.049^{ns}	0.013^{ns}	0.003^{ns}	0.059^{ns}	0.043^{ns}	0.054^{ns}	
MS	0.004^{ns}	-0.002^{ns}	0.012^{ns}	0.023^{ns}	0.020^{ns}	0.028^{ns}	-0.020^{ns}	-0.002^{ns}	0.056^{ns}

After Bonferroni correction, $P < 0.05$*, ns = not significant

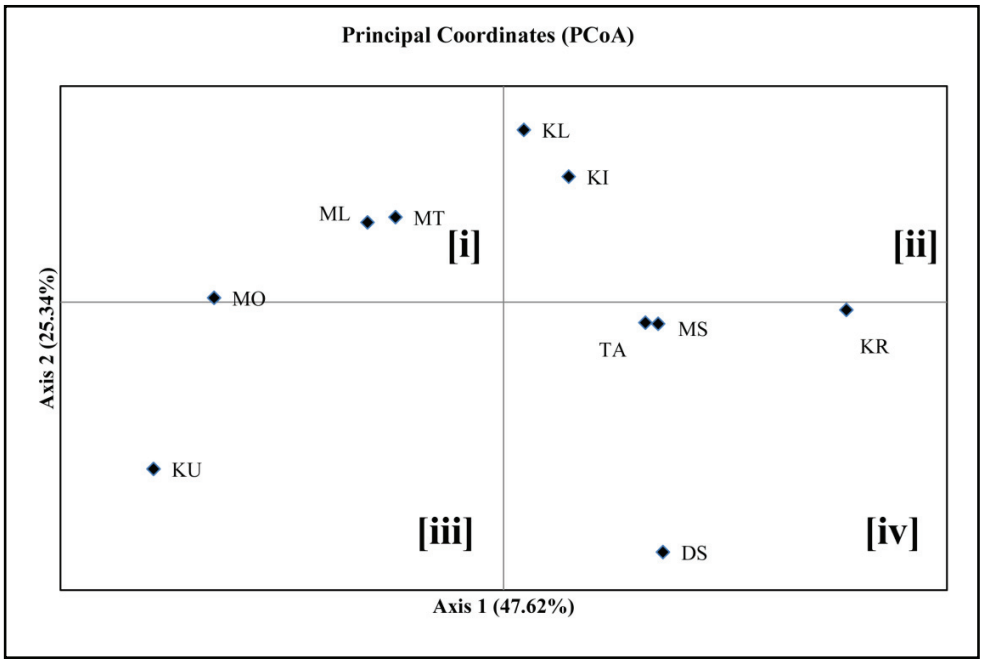
Supplementary Table 2.2 Pairwise Φ_{ST} values among populations of *Acanthurus leucosternon* in Eastern Africa. Sample codes are presented in Table 3.

	KU	DS	KL	MH
KU	0.000			
DS	-0.017^{ns}	0.000		
KL	-0.031^{ns}	-0.018^{ns}	0.000	
MH	-0.019^{ns}	-0.022^{ns}	-0.019^{ns}	0.000

ns= not significant



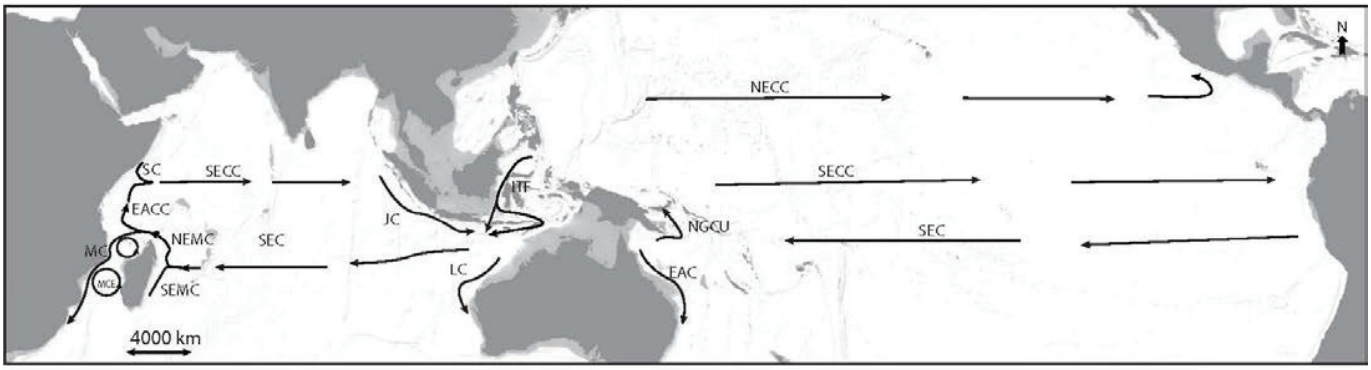
Supplementary Figure 2.1 Plot of Delta K against the K (clusters), the highest value of Delta K indicates the likely number of cluster (K=2) for Eastern African *A. leucosternon* populations.



Supplementary Figure 2.2 Principle coordinates analysis (PCoA) for *Acanthurus leucosternon* using unbiased Nei genetic distance based on 10 loci. The percentage variation of the PCoA is represented by axis 1 (47.62%) and 2 (25.34%). For sample sites, abbreviation see Table 1.

APPENDIX

Supplementary materials for chapter 3



Supplementary Figure 3.1 Map of Indo-Pacific with prominent ocean currents. Abbreviations: SC, Somali current; SECC, South Equatorial Counter Current; EACC, East African Coast Current; NEMC, Northeast Madagascar Current; MCE, Mozambique Channel Eddies; MC, Mozambique Current; ITF, Indonesian Throughflow; LC, Leeuwin Current; EAC, East Australian Current; NGCU, New Guinea Under Current; NECC, North Equatorial Counter Current; and JC, Java Current.

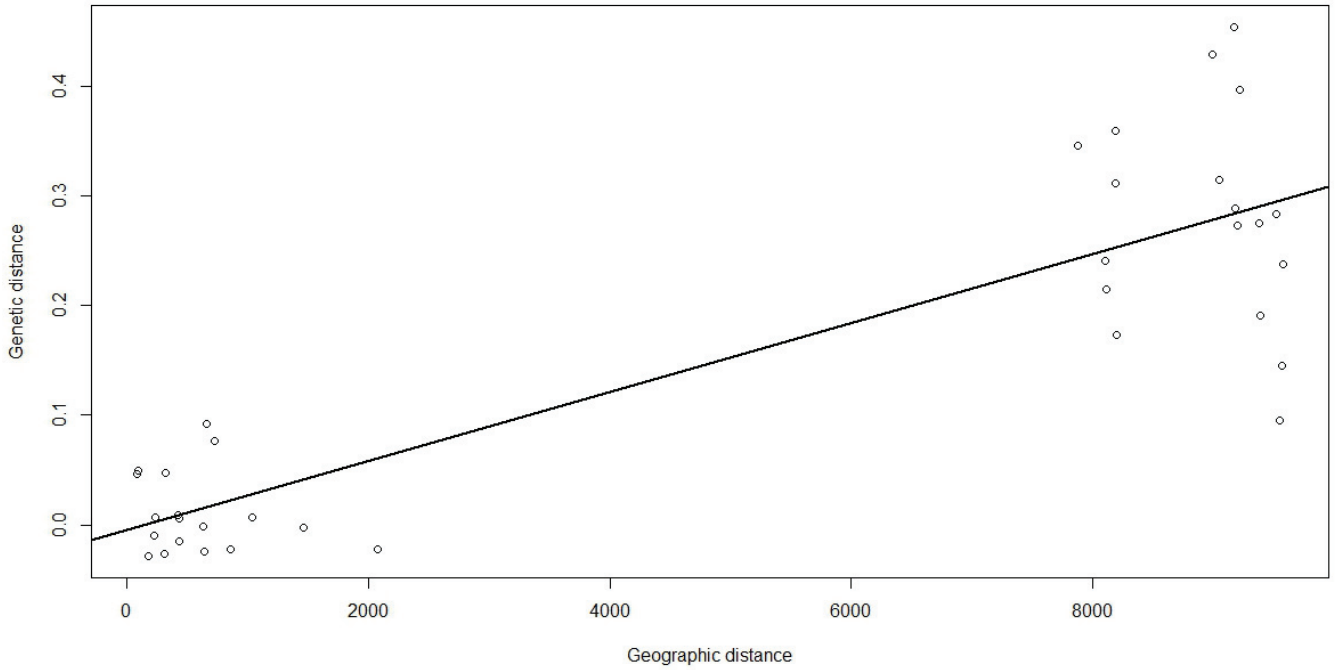
Supplementary materials for chapter 4

Supplementary Table 4.1 Pairwise F_{ST} values among populations of *Acanthurus leucosternon* in the Western Indian Ocean. Sample codes are presented in Table 1.

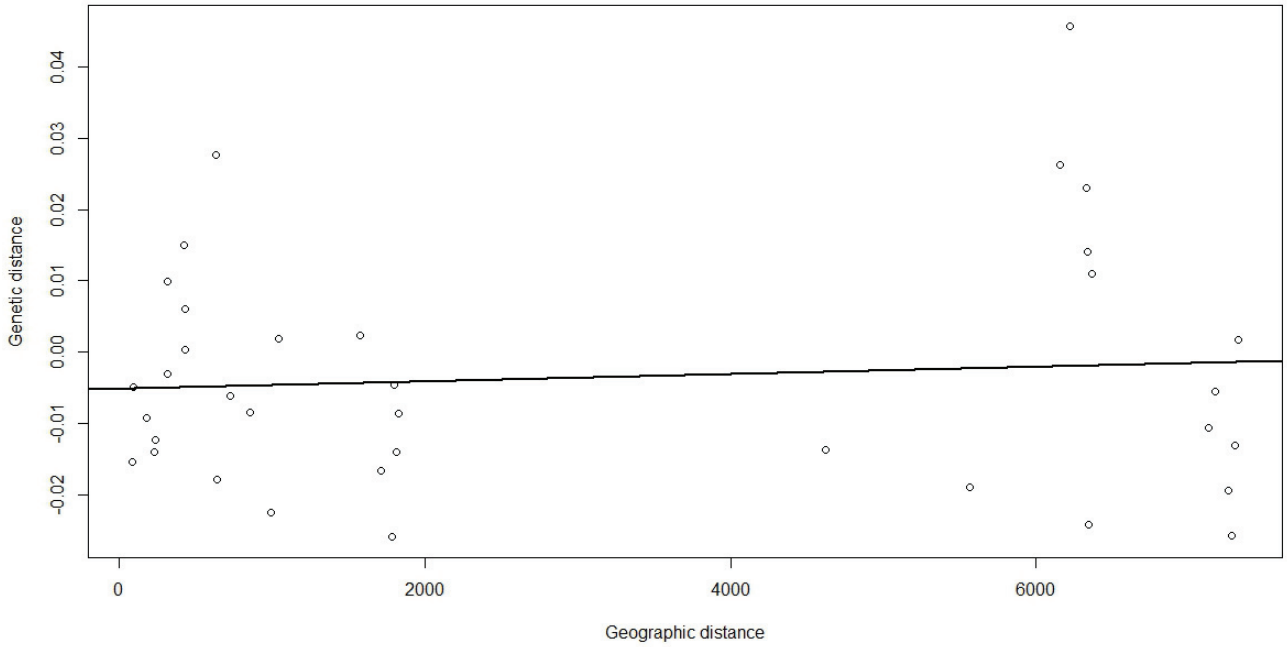
	KU	ML	KR	MO	MS	KM	TA	DS	MT
KU		0.003485	0.007748	0.000325	0.006316	0.004949	0.006608	0.002667	0.001632
ML	0.002873		0.004807	0.000298	0.000459	-0.001854	0.004091	0.002094	-0.0009
KR	0.008264	0.004438		0.008075	0.002855	0.003811	0.001448	0.007166	0.00489
MO	-0.000059	0.000352	0.00826		0.001906	0.003672	0.003607	0.007762	0.002204
MS	0.006055	0.000728	0.002389	0.002303		-0.002366	-0.001327	-0.00022	0.002471
KM	0.004729	-0.00127	0.003695	0.003694	-0.00172		0.002907	0.003315	0.002321
TA	0.006335	0.002805	0.002155	0.002085	-0.00221	0.001554		0.001812	0.001833
DS	0.00366	0.002328	0.005699	0.00729	-0.00021	0.00408	0.00224		0.004956
MT	0.001399	-0.000849	0.004837	0.002574	0.002614	0.002542	0.002037	0.005204	

Supplementary Table 4.2 Pairwise F_{ST} values among populations of *Acanthurus triostegus* in the Western Indian Ocean. Sample codes are presented in Table 1.

	KU	ML	KR	MO	MS	KM	TA	DS	MT
KU		0.005974	0.012695	0.001408	0.007225	0.006242	0.007315	0.0043	0.004488
ML	0.006481		**0.003049***	0.005846	-0.002731	0.005405	-0.006485	0.003172	0.008028
KR	0.012684	0.002094		0.012618	0.001034	0.003153	-0.001443	0.007133	0.005052
MO	0.001665	0.005528	0.012428		0.002278	0.004866	0.003428	0.000965	0.004059
MS	0.007934	-0.003245	0.000839	0.002749		-0.002758	-0.005795	0.004	0.003307
KM	0.0072	0.005794	0.003371	0.005217	-0.002494		-0.000071	-0.00088	0.004455
TA	0.008674	-0.004168	0.000649	0.003325	-0.004769	0.001013		0.002371	0.002742
DS	0.004658	0.002931	0.006838	0.001298	0.003945	-0.00052	0.003192		-0.000393
MT	0.004668	0.008027	0.004821	0.004175	0.003451	0.004764	0.003448	-0.00031	



Supplementary Figure 4.1 A scatter plot of the correlation between the geographic distance (km) and pairwise Φ_{ST} estimates for the 9 sampling locations of *A. triostegus* in the Indian Ocean ($r^2 = 0.75$ $P < 0.0001$).



Supplementary Figure 4.2 A scatter plot of the correlation between the geographic distance (km) and pairwise Φ_{ST} estimates for the 9 sampling locations for *A. leucosternon* in the Indian Ocean ($r^2 = 0.19$, P = 0.5

List of papers and author contribution

Paper 1

Otwoma, L. M., Reuter, H., Timm, J. & Meyer, A. (2018). Genetic connectivity in a herbivorous coral reef fish (*Acanthurus leucosternon* Bennet, 1833) in the Eastern African region. *Hydrobiologia* **806**, 237-250.

Contributions by first author (LMO): concept and design- 90%, data acquisition- 90%, data analysis and interpretation-80%, and manuscript writing and revisions - 80%

Paper 2

Otwoma, L.M., Diemel, V., Reuter, H., Kochzius, M., Meyer, A., (2018). Genetic population structure of the convict surgeonfish *Acanthurus triostegus*: A phylogeographic reassessment across its range. *Journal of Fish Biology* (in press). Doi:10.1111/jfb.13686

Contributions by first author (LMO): concept and design- 80%, data acquisition- 70%, data analysis and interpretation-90%, and manuscript writing and revisions - 90%

Paper 3

Otwoma, L.M., Reuter, H., Meyer, A., (2018). Comparative phylogeography of *Acanthurus* species in the Indian Ocean: Genetic population structure of sympatric sister species with divergent mating behaviour. (In preparation)

Contributions by first author (LMO): concept and design- 90%, data acquisition- 90%, data analysis and interpretation-90%, and manuscript writing and revisions - 90%

Paper 4

Otwoma, L.M., Reuter, H., (2018). Response to exploitation and life history characteristics of two *Acanthurus* species with divergent mating behaviour along the Kenyan coastline. (In preparation)

Contributions by first author (LMO) : concept and design- 90%, data acquisition- 80%, data analysis and interpretation-100%, and manuscript writing and revisions - 90%

Versicherung an Eides Statt

Ich, **Levy, Otwoma**; Anschrift, **Luisental 29C , Bremen 28359**; Matr.-Nr. **3026440**

versichere an Eides Statt durch meine Unterschrift, dass ich die vorstehende Arbeit selbständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe.

Ich versichere an Eides Statt, dass ich die vorgenannten Angaben nach bestem Wissen und Gewissen gemacht habe und dass die Angaben der Wahrheit entsprechen und ich nichts verschwiegen habe.

Die Strafbarkeit einer falschen eidesstattlichen Versicherung ist mir bekannt, namentlich die Strafandrohung gemäß § 156 StGB bis zu drei Jahren Freiheitsstrafe oder Geldstrafe bei vorsätzlicher Begehung der Tat bzw. gemäß § 161 Abs. 1 StGB bis zu einem Jahr Freiheitsstrafe oder Geldstrafe bei fahrlässiger Begehung.

Bremen, 12.9.2018
Ort, Datum

_____
Unterschrift