Gorgonian Responses to Environmental Change on Coral Reefs in SE Sulawesi, Indonesia

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Te Whare Wānanga o te Ūpoko o te Ika a Māui

Dedicated entirely to my family, Mike, Penny and Giles Rowley,

whose love knows no bounds.

ABSTRACT

Gorgonian corals (Cnidaria: Anthozoa: Octocorallia) are conspicuous, diverse and often dominant components of benthic marine environments. Intra- & interspecific morphological variability in gorgonians are influenced by environmental factors such as light, sedimentation and flow rates. Yet, little is known about the responses of gorgonian taxa to environmental parameters particularly in Indonesia, despite their high regional abundance and diversity. With a burgeoning human population and subsequent marine resource exploitation, reefs throughout the Indonesian archipelago are under rapid decline and often destroyed. Conservation surveys are however, underway with a tendency to overlook gorgonian taxa primarily due to unresolved taxonomic assignment leading to difficulties in field identification.

The aims of this study were to: 1) characterise gorgonian diversity and ecology across a gradient of habitat quality within the Wakatobi Marine National Park (WMNP), SE Sulawesi, Indonesia, 2) assess morphological and genetic variability between morphotypes of the ubiquitous zooxanthellate isidid *Isis hippuris* Linnaeus 1758 from healthy and degraded reefs, 3) determine if *I. hippuris* morphotypes are environmentally induced (plastic) or genetically derived through reciprocal transplant experiments (RTEs) between contrasting reefs and thus, 4) identify mechanisms of plasticity capacity or divergence through phenotypic trait integration in response to environmental change.

Ecological surveys revealed considerable gorgonian diversity with a total of 197 species and morphotypes from 42 genera, and 12 families within the suborders Calcaxonia and Holaxonia and the group Scleraxonia, with current estimates of over 21 new species and 28 new species records for the region. Gorgonian abundance and diversity increased with reef health and bathymetry. However, a clear loss of gorgonian diversity existed with increased sedimentation and reduced light due to anthropogenic disturbance. In particular, two distinct *I. hippuris* morphotypes were highly abundant between environmental clines: short-branched multi/planar colonies on healthy reefs, and long-branched bushy colonies on degraded reefs. Comparative morphological and molecular analyses using ITS2 sequence and predicted secondary structure, further corroborated haplotype differences relative to morphotypes between environments. However, unsatisfactory assignment of *I. hippuris* morphotypes to previously described alternatives (*Isis reticulata* Nutting 1910, *Isis minorbrachyblasta* Zou, Huang & Wang 1991) questions the validity to such taxonomic assignments. Phylogenetic analyses also confirm that

the polyphyletic nature of the Isididae lies in its type species *I. hippuris*, being unrelated to the rest of its family members.

A one-year RTE revealed three key results, that: 1) reduced survivorship of healthy reef morphotypes on degraded reefs implied the onset of lineage segregation through immigrant inviability, 2) prominent phenotypic traits were at the morphological and bio-optical levels revealing high phenotypic plasticity in healthy clones, and relative insensitivity to environmental change in degraded reef morphotypes, indicative of local adaptation leading to incipient ecological divergence, and 3) photoacclimation at the bio-optical level was not attributed to endosymbiont diversity or shuffling, with all test colonies possessing a novel clade D1a *Symbiodinium*.

While it is clear that gorgonian taxa within the WMNP are of exceptional diversity and abundance, responses to environmental perturbation highlight three pertinent, testable ideas. Firstly, increased species richness specifically with depth in azooxanthellate taxa, invite tests of deep-reef refugia previously established through geological change. Secondly, ecological assessment targets research on informative taxa for focused systematics and mechanisms of phenotypic divergence. Thirdly, exploring intrinsic and extrinsic interactions that define the host-symbiont relationship and differential biological success using physiological and next generation sequencing approaches. These objectives would provide considerable insight into the evolutionary processes to environmental change, accelerated by anthropogenic encroachment.

Taken together, this work signifies that gorgonian corals within the WMNP are of foremost diversity and concern, exhibiting informative ecological and mechanistic responses to environmental perturbation. This evidence elicits tests of deep-reef refugia, priority systematics, mechanisms of ecological divergence and physiological assessment. Such tests inevitably expand our understanding of the intrinsic and extrinsic associations of gorgonian taxa to environmental change from an historical and predictive perspective yielding benefits to conservation assessment and management.

DISCLAIMER & AUTHORSHIP STATEMENT

This thesis was conceived, conducted - including all field, laboratory and data analyses - and written by the author. Notable exceptions are Dr. Xavier Pochon who guided and assisted in molecular work and analyses. Miss Francesca Koethe made light microscope images for *Isis hippuris* Linnaeus 1758 sclerite measurements. All chapters are modified versions of a series of manuscripts to be submitted for publication on thesis submission, therefore some content overlap will exist between chapters.

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LIST OF ABBREVIATIONS

***$a_{\text{chl } a}$** – Chlorophyll *a* specific absorption coefficient

AMNH – American Museum of Natural History

BNHM – British Natural History Museum

BPBM – Bernice Pauahi Bishop Museum (USA)

CAP - Canonical analysis of principal coordinates

Chl *a* – Chlorophyll *a*

COX1 – Mitochondrial-encoding cytochrome oxidase subunit1 region

F – Fluorescence

Fm' – Maximum yield of chlorophyll fluorescence in a light-adapted state/ambient light

ΔF/Fm' – Light-adapted quantum yield of PSII (below)

ITS – Internally transcribed spacer regions of ribosomal DNA

NIWA – National Institute of Water and Atmospheric Research (New Zealand)

nMDS – Non-metric multidimensional scaling

NMNH – National Museum of Natural History (formerly the USNM, USA; see below)

PAM – Pulse amplitude modulation

PAR – Photosynthetically active radiation

PERMANOVA – Permutational ANOVA (analyses of variance)

psbA – Chloroplast plastid-coding *psbA* minicircle which encodes the D1 protein of photosystem II

PSII – Photosystem II (two)

RTEs – Reciprocal Transplant Experiments

SIMPER – Similarity percentages

USNM – United States National Museum (now the NMNH, USA; see above)

$\boldsymbol{V_E}$ – Environmental variance

$\boldsymbol{V_{error}}$ – Residual Components

$\boldsymbol{V_G}$ – Genetic variance

$\boldsymbol{V_{G,A}}$ – Additive genetic variance

$\boldsymbol{V_{G,D}}$ – Dominant genetic variance

$\boldsymbol{V_{GxE}}$ – Genetic and environmental interaction variance

$\boldsymbol{V_{GI}}$ – Epistatic variance

$\boldsymbol{V_P}$ – Phenotypic variance

GLOSSARY

Absorbance (*D*) – Measure of the capacity of e.g., chlorophyll to absorb light of a specific wavelength

Absorption/Absorptance (*A*) – Light fraction absorbed

Adaptation – The products of natural selection by which an organism may become better suited to its environment for a specific function(s)

Adaptive – Having the capacity for adaptation (above)

Adaptive plasticity – Where plasticity is considered beneficial and maintained by selection

Additive genetic variance ($V_{G,A}$) – Deviation from the population mean phenotype due to additive allele effects (substituting one allele for another) at a given locus or the multiple loci of a polygenic trait (below)

Anastomose – Forming a network (in the context of colony morphology)

Anthocodia – The distal part of a polyp, bearing the mouth and tentacles; can be retracted within the calyx, stem, branch or cortex

Anthostele (Calyx) – The proximal, rigid part of polyps in some octocoral species, often stiffened by sclerites. In some cases the anthocodia is withdrawn into the anthostele

Arborescent – Tree-like colonies possessing a stem

Axial sheath – Part of the colonial coenenchyme immediately surrounding the axis, usually delimited by the longitudinal stem canals and characterised by sclerites commonly different in form from those of the overlying coenenchyme

Axis – Central, longitudinal supporting structure which can consist of either, 1) an inner central horny (gorgonin) chord with or without calcareous material (loculi), 2) scleritic inner medulla and outer coenenchyme, or 3) non-scleritic central axis which may be hollow or

cross-chambered

Axis cortex (**Cortex)** – Tissue layer surrounding the central part of the axis; either the coenenchyme surrounding the medulla or the horny layer surrounding the central chord

Bushy – Colonies with abundant branches typically in all directions

Canal (Longitudinal) – Longitudinal partitions of the gastrovascular cavity of the polyp running longitudinally along the central axis, often nested within axial groves

Canalisation – The reduced sensitivity (*sensu* fixed) of a phenotype to changes or perturbations in the underlying genetic and nongenetic factors that determine its expression

Capstan – Sclerite: rod with two whorls of tubercules or wards and terminal tufts

Central chord (Core) – The central part of a Holaxonian axis, consisting solely of horny material or horny material with varying permeations of calcareous material (loculi). Can be hollow and cross-chambered in some families (see Medulla)

Club – Monaxial (single axis) sclerites enlarged at the head, and tapered at the opposite end, the handle

Coenenchyme – The colonial soft tissue between the polyps, consisting of the mesoglea usually containing sclerites and penetrated by the network of solenia and gastrodermal canals

Cortex – Tissue layer surrounding the central part of the axis; either the coenenchyme surrounding the medulla or the horny layer surrounding the central chord.

Cross – Stellate sclerite with four rays in a single plane

Crown (Collaret) – The ring of transversely placed, usually bow-shaped sclerites encircling the anthocodia below the tentacles

Diving-PAM – A diving-pulse amplitude modulation fluorometer measuring the effective quantum yield (Φ) of photochemical energy conversion during photosynthesis

Dominant genetic variance ($V_{G,D}$) – Deviation from the population mean phenotype due to interactions between alleles at the same locus

Dumb-bell – Sclerite with two nearly spherical, warty heads and a distinct waist that is longer than in the double sphere

Encrusting – Colony morphology consisting of a thick fleshy layer covering the substrate

Environmental variance (V_E) – Phenotypic variance among individual members of a population due to environmental effects

Epigenetic - Resulting from external rather than genetic influences, whereby modification in gene expression/function are independent of the DNA sequence. Epigenetic influences can become heritable and fixed (through genetic assimilation) over time

Epistatic interaction variance (V_{GI}) – Genetic variance due to epistasis (below)

Epistasis – The expression of a gene is modified by the presence of one or more 'modifier genes' from different loci.

Evolutionary capacitance – The storage and release of genetic information (variation), typically leading to fixation through genetic assimilation as a consequence of epigenetic heritability

Flabellate – Fan-shaped (arborescent)

Gastric cavity (Gastrovascular cavity; Coelenteron) – Interior space of a polyp

Gastrodermal canals – Wide, endodermal-lined canal connected with the narrower solenia and originating from them

Genetic accommodation – A process where a phenotype is originally produced in response to either a mutational or environmental stimulus (genetic assimilation in the latter; below)

Genetic assimilation – A process where a phenotype originally produced in response to environmental change later becomes genetically encoded typically through epigenetic

heritability. *Sensu stricto* process of phenotypic evolution by genetic accommodation (above)

Genetic variance (V_G) – Phenotypic variance among individual members of a population due to genetic effects

Genetic and environmental interaction variance (V_{GxE}) – Genotype by environment interaction whereby genotypes differentially respond to their environment

Gonads – Reproductive cells along the septa within the gastrovascular cavity

Gorgonin – Horny proteinaceous material forming with calcareous (loculi and/or sclerites) material of the inner and/or outer layers of the central axis

Hermatypic – Reef-building corals, typically depositing aragonite structures contributing to or the basis of coral reef development e.g., most Scleractinian corals

Holobiont – Biological unit including the host and its microbial associate communities

Integration – Characters (phenotypic traits or modules) behaving as a unit with integration manifested as coordinated character change in ontogeny, phylogeny, space, time, magnitude or direction

Internode – Hard, calcareous segment of the jointed axis (e.g., Melithaeidae, Isididae)

Intraspecific variation – Any differences among individuals of a single species.

ITS2 cladal type – Genetic variant of the ITS2 region below that of a clade and currently unresolved taxonomically

Loculi (Loculus) – Calcified or fibre-filled space in the holoxonian axis, especially in Plexauridae, appearing crescentic or lenticular in cross section

Medulla – Central supporting structure of the Scleraxonia consisting of densely packed sclerites, gorgonin and occasional longitudinal canals. Surrounded by the coenenchyme (see

Axis, Central Chord)

Mesenteries (Septa/um) – Eight thin, radial and longitudinal, non-calcareous partitions joining the pharynx to the body wall and dividing the polyp gastrovascular cavity. Each septum bears a longitudinal retractor muscle

Mesenteries filaments (Septal filaments) – The thickened convoluted edges of the mesenteries (septum) below the pharynx. The two mesenteries opposite the siphonoglyph are long and heavily flagellated

Modularity – Degree to which a system's components can be separated and recombined; thus organisms are considered to consist of phenotypic modules

Monophyletic – A group of organisms descended from a common evolutionary ancestor or ancestral group, particularly one that is not shared with any other group

Multiplanar - Branched colonies in which the branches grow in several planes

Neck zone (Introvert) – Soft, thin-walled basal section of the anthocodia below the tentacles bearing little or no sclerites. Permits introversion of the anthocodia into the anthostele (calyx)

Node – The flexible horny (gorgonin) segment of a jointed axis (see internode)

Oral disc – Area of the polyp immediately surrounding the mouth and formed by the inner basal parts of the tentacles

Pharynx – Tubular section of the digestive system connecting the mouth and the gastrovascular cavity; possesses one or two flagellated grooves (siphonoglyphs)

Phenotype – The set of observable traits of an individual due to its interaction of its genotype with the environment

Phenotypic plasticity – Environmentally induced changes in an organism's phenotype within its lifetime; a specific form of intraspecific variation (see above).

Phenotypic variance (V_P) – Variance within a quantitative phenotypic trait (see additive, dominant, epistatic, genetic, environmental, and genetic and environmental interaction)

Polyphyletic - A group of organisms derived from more than one evolutionary ancestor or ancestral group, therefore not suitable for placing in the same taxon

PSII – Photosystem II (two); a photosystem reaction centres/protein complex that uses light energy for the splitting of water through oxidation in photosynthesis

Pinnules – The lateral, hollow processes arranged in two opposite rows along each tentacle

Planar – Arborescent branching colonies where branches generally grow in a single plane

Plasticity - Environment-dependent phenotype expression

Pleiotropy – The influence of a single gene on several seemingly unrelated phenotypic traits

Point(s) – Eight longitudinal rows of chevroned sclerites around the distal part of the anthocodia, superposing the crown if present

Polygenic – Phenotypic trait controlled or the product of two or more genes and its environment (see additive genetic variance)

Polyp (Zooid) – Any individual within a (octocoral) colony, which may be monomorphic (possess single polyp type) or consist of more than one type e.g., autozooids and siphonozooids (e.g., Coralliidae)

Polyphenic trait (Polyphenism) – A trait which gives rise to multiple, distinct phenotypes from a single genotype due to differential environmental conditions

Radiates – Sclerites with symmetrically radiating processes in a single or multiple planes

Reaction norms (Norms of reaction) – A single genotypes phenotypic expression as a function of environmental variation.

Reticulate – Branching colonies where branches anastomose forming net-like structures

Sclerite – Calcareous skeletal element present in or on the Octocoral soft tissue matrix or axial composition

Septa (Septum, Mesenteries) - Eight thin, radial and longitudinal, non-calcareous partitions joining the pharynx to the body wall and dividing the polyp gastrovascular cavity. Each septum bears a longitudinal retractor muscle

Siphonoglyph (Sulcus) – Strongly ciliated groove extending down one side of the pharynx

Solenia (Solenium) – Narrow endodermal canal lined with gastrodermis within the coenenchyme, forming a network by interconnecting the gastric cavities of the polyps and larger canals

Spicule – Skeletal element of non-cnidarian taxa (e.g., Porifera), often confused with sclerite

Spindles – Monaxial (single axis) sclerites that are straight or curved and pointed at both ends

Symbiodinium Freudenthal 1962 (**Zooxanthellae)** – Unicellular dinoflagellate free-living or endosymbiotic alga. The largest group of endosymbiotic dinoflagellates

Synapomorphic – Of a character trait that evolves once in the common ancestor (and not in its ancestors) of two or more lineages, themselves possibly exhibiting further modified versions of that trait

Residual components [V_{error}] – Phenotypic variance accounted for by developmental noise, bet-hedging, behavioural or other unaccountable factors

Zooxanthellar/e (*Symbiodinium*) – Colloquial name for *Symbiodinium* Freudenthal 1962

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CHAPTER 1: GORGONIAN RESPONSES TO ENVIRONMENTAL CHANGE: PLASTICITY VS. ADAPTATION?

1.1 INTRODUCTION

Gorgonian corals (Cnidaria: Anthozoa: Octocorallia) are conspicuous, diverse and often dominant components of intertidal and subtidal benthic marine environments; most notably tropical shallow reef, deep sea, and mesophotic habitats (Wirshing et al. 2005, McFadden et al. 2010a, Cerrano et al. 2010). Gorgonians are modular, suspension feeding colonial sessile organisms defined primarily by a semi-rigid scleroproteinaceous (gorgonin) axis with varying amounts of calcification (Bayer 1961, Grasshoff 1999, Sánchez et al. 2003a). Originally classified under the order Gorgonacea (now taxonomically obsolete), Bayer (1981) included gorgonians within the Octocorallia order Alcyonacea on the basis of intermediate forms obscuring any definitive morphological boundaries. Thus, gorgonians remain in a state of taxonomic confusion despite being of ecological (Fabricius & De'ath 2004), commercial (Grigg 2002), climatic (Thresher et al. 2010), evolutionary (Sánchez 2004), pharmaceutical (Bayer et al. 1974, Bordeleau et al. 2006, Susilaningsih et al. 2010), and conservation importance (Dayton 2003, Linares et al. 2008). Many gorgonians have been used as conservation 'flagship' species (Tinsely 2005, Linares et al. 2008, Cerrano et al. 2010), being ecologically diverse, long-lived engineering taxa that maintain habitat heterogeneity and provide secondary space to other organisms, thereby enhancing ecosystem function (Cerrano et al. 2010, Mumby et al. 2010). Irrespective of their ecological diversity and global distribution, the greatest paucity of information however, exists in the Indonesian Archipelago (Tomasik 2004), a surprising reflection particularly given that zooxanthellate gorgonians are often one of the primary space occupants of tropical reefs (McFadden et al. 2006).

Gorgonian responses to their environment are intriguingly complex. Their population demographics are principally driven, together or in part, by environmental factors including substrate, light, temperature, sedimentation, salinity, current regime and flow rates (Bayer 1981, Fabricius & Alderslade 2001). In addition, biotic factors such as competition, predation, symbioses, reproduction, settlement and developmental properties provide local scale refinement. These factors have been shown to induce intra- and inter-specific morphological variability (West 1997, 1998, Linares et al. 2008), habitat selection and colony orientation (Grigg 1972, Sánchez et al. 2003a). Anatomical and behavioural adaptations, which include polyp expansion, chemical or nematocyst complement, colony dynamics, branching morphology, sclerite type and morphology, as well as photoacclimation, zooxanthellae density

and content, are all responses to reef life. Yet, what stands this group apart from other marine metazoans, with the exception of perhaps Porifera (Bayer 1961, Shearer et al. 2002), is the enormous degree of variability as yet largely unexplored. Whether such variability is the result of phenotypic plasticity, therefore adaptive, or an adaptation potentially leading to incipient speciation remains to be seen. Nevertheless, to investigate evolutionary processes and create effective conservation strategies, it is essential to define species and species boundaries. However, such taxonomic resolution is often confounded by considerable phenotypic variability, cryptic and sibling taxa (Knowlton 1992), and lack of gorgonian research primarily due to difficulties in field identification (Fabricius & Alderslade 2001). In addition, molecular markers such as mitochondrial DNA (mtDNA) used to delineate species and/or taxonomic groups are highly conserved in Cnidaria revealing little or no taxonomic variation (France et al. 1996, Shearer et al. 2002). Thus, the 'Species Problem' is exemplified and of fascinating complexity in gorgonians. Nonetheless, with a modular, clonal nature and advancements in molecular markers (e.g. Conception et al. 2008, McFadden et al. 2010a), gorgonians provide an innovative platform from which to study the evolution of environmentally plastic or dependent characters (Gotthard & Nylin 1995) and subsequent modes of speciation.

1.2 GORGONIAN ANATOMY

Colonial, polypoid and sessile, gorgonian octocorals are characterised by polyps bearing eight pinnate tentacles, eight mesenteries dividing the gastrovascular cavity, and nematocyst (collectively 'cnidae') complement (Figure 1.1: Bayer 1961, Berntson 1998). What defines a gorgonian coral specifically is the division of the coenenchyme - the tissue between and containing the polyps, gastrovascular canals, sclerites and solenia (see Figure 1.1) - into the outer cortex and inner axial medulla (as in the Scleraxonians Figure 1.3a) or a central axis with or without a central cortex (Figure 1.3c,b). This tissue division may or may not give rise to the archetype arborescent morphology of a gorgonian and places emphasis on structural – functional optima of resource acquisition in particular environments. For example, encrusting zooxanthellate taxa in high light and hydrodynamic regimes, and tall flexible arborescent colonies perpendicular to the prevailing water currents. Ultimately it is the central or inner axial layer consisting of varying levels of calcareous and/or gorgonin (proteinaceous) material that lend gorgonians their name.

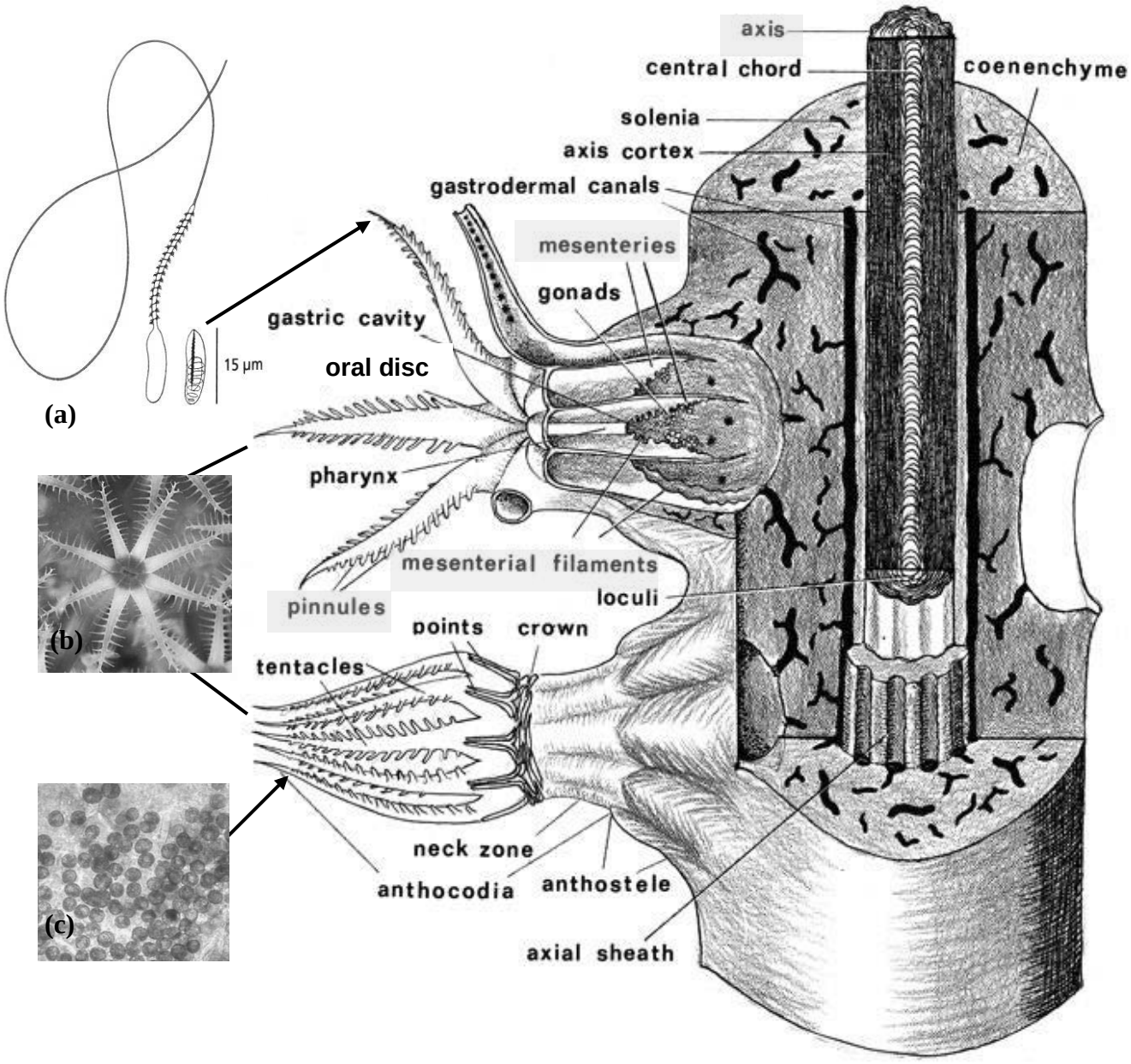


Figure 1.1 Schematic diagram of gorgonian anatomy modified from Bayer et al. (1983), with (a) Octocoral nematocyst capsule (modified from Grasshoff & Bargibant 2001), (b) polyp overhead view (taken by Eco-Divers 2008), and (c) endosymbiont dinoflagellates (zooxanthellae) within the polyp gastrodermis (source Hoegh-Guldberg). Yellow highlighted traits are particularly characteristic of gorgonian corals. Glossary of terms provided on page xvi.

The coenenchyme growth leads to the continual addition of polyps that may retract entirely into the coenenchyme (Figure 1.2a), into low or high calyces (Figure 1.2b,c) or contract through hydrostatic deflation into low mounds or tall scleritic polyp structures (Figure 1.2d,e). The latter two cases do not reinforce the anthostele and thus are not termed calyces (Stachowitsch 1992).

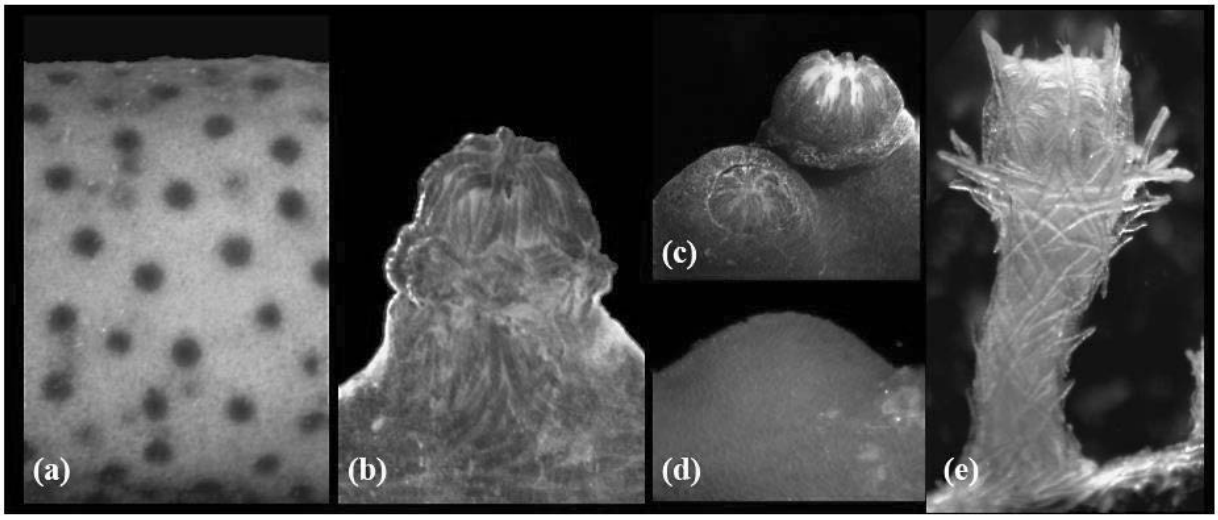


Figure 1.2 Gorgonian polyp morphology. Retractile polyps of (a) *Isis hippuris* Linnaeus 1758 completely into the coenenchyme, (b) *Astrogorgia* Verrill 1868 into a high calyx, and (c) in to a low calyx plus partial expansion. Non-retractile but contractile polyps of (d) *Verrucella* Milne-Edwards & Haime 1857, and (e) *Acanthogorgia* Gray 1857 (photography by Rowley 2009).

Gorgonian polyps consist of three thin layers: the mesoglea, a gelatinous fibrous matrix also containing amoeboid and scleroblast cells, sandwiched between the outer epidermis and inner gastro- or endodermis cellular layer lining the mesenteries, pharynx, gastric cavity and tentacles (Stachowitsch 1992, Fabricius & Alderslade 2001). The eight mesenteries divide and increase the surface area of the gastric cavity through the pharynx to the eight pinnate tentacles, themselves mesentery extensions. The free inner edge of each mesentery below the pharynx is thickly lined (mesentery filament) and varies in function with two flanking the longitudinal siphonglyph or sulcus, which through ciliary action beats water through the polyp into the solenia or canals to the rest of the colony. In zooxanthellate species the dinoflagellate endosymbionts are present within the gastrodermal cells or within vacuoles in the gastric cavity (Fabricius & Alderslade 2001).

Nematocysts are only produced by cnidarians as key mechanisms of defence and offence (Fautin 2009). Gorgonian nematocysts are typically in the tentacles, if present at all, with octocoral cnidae somewhat pitiful compared to that of other cnidarians (e.g., Hydrozoa, Scyphozoa, Cubozoa) consisting of a single type (Figure 1.1a) out of the thirty currently recognised for this phylum (Fautin 2009). Irrespective, gorgonians have continued to persist since at least the Lower Ordovician (Lindstrom 1978, Bengtson 1981, Cope 2005) despite their relative simplicity. It is not unreasonable to suggest that their current poor cnida content, diversity and

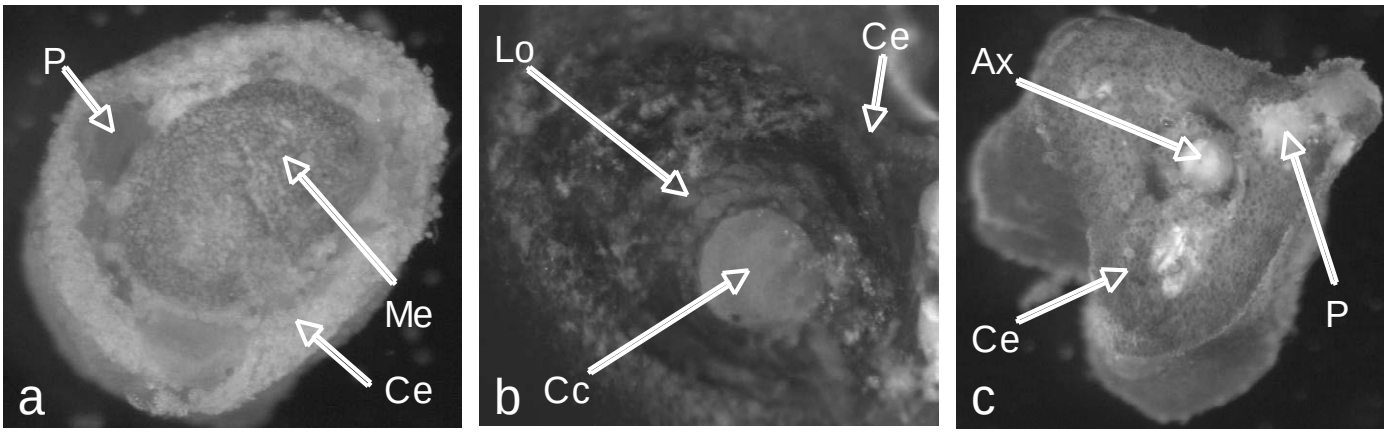


Figure 1.3. Gorgonian comparative axis cross-section structure. (a) Scleraxonian axis cross-section showing coenenchyme, polyp and central medulla (*Annella reticulata* Ellis & Solander 1786, 6 mm). (b) Holaxonian axis cross-section showing loculus, central core and coenenchyme (*Astrogorgia cf. dumbea* Grasshoff 1999, 7 mm). (c) Calcaxonian axis cross-section showing coenenchyme, axis and polyp (*Viminella* sp. Gray 1870, 4 mm). Ax = Axis, Cc = Central core, Ce = Coenenchyme, Lo = Loculus, Me = Medulla, P = Polyp. Images: Wakatobi Marine National Park, Indonesia, 5 – 15 m depth (Rowley) 2009, 2010.

potency may be due to increasing redundancy over time through a greater chemical defense battery, which may or may not have evolved as a consequence of strong associations with their microbiota.

1.3 GORGONIAN CLASSIFICATION

Gorgonians (sea fans and sea whips) are within the subclass Octocorallia (or Alcyonaria), which take a basal position within Anthozoa, itself basal within the phylum Cnidaria (Bridge et al. 1992). Octocorallia are a clearly defined monophyletic group based on both their molecular and anatomical characters (France et al. 1996, Bayer 1961, Berntson et al. 1998, 2001, McFaddenet al. 2010a).

Gorgonian taxonomy began almost four centuries ago, being originally described and classified as ‘marine plants’ (Rumphius 1741, Bayer 1959, Grasshoff 2001), with eventual classification based on colony morphology and the shape and arrangement of calcareous sclerites found within the polyps and coenenchyme (Kükenthal 1919, Bayer 1981, Grasshoff 2001, Vargas et al. 2010). Bayer (1961, 1981) further confirmed gorgonian delineation on the basis of axis mineralogy (Figure 1.3a-c). Therefore, through comparative morphology gorgonians currently comprise the suborders, Holaxonians and Calcaxonians Grasshoff 1999 and the group Scleraxonians Studer 1887 within the order Alcyonacea (Bayer 1981, Figure 1.4). The Scleraxonian ‘group’ however,

is not strictly a suborder due to several intermediate forms and the likelihood of families evolving from numerous separate evolutionary lines (Fabricius & Alderslade 2001). Nonetheless, Scleraxonians typically possess a scleritic inner medulla with varying low levels of gorgonin. The Holaxonians have, with one exception (Keroeididae Kinoshita 1910), a whole horny axis supported by non-scleritic calcareous material. The relatively recent (Grasshoff 1999) Calcaxonians are delineated by a solid non-scleritic calcite or aragonite axis, which may alternate or fuse with gorgonin fibers (Bayer 1981, Fabricius & Alderslade 2001; Figure 1.3). However, hollow axial members exist (Watling & France 2011, Alderslade & McFadden 2012)!

Curiously, molecular studies confirm Octocorallia monophyly but lack phylogenetic support for such morphology-based sub-ordinal groupings within the Alcyonacea (reviewed by McFadden et al. 2010a, Figure 1.4). Sánchez et al. (2003a) using partial 16S mitochondrial rDNA, complete 18S nuclear rDNA sequences (from France et al. 1996 & Berntson et al. 2001 respectively), INDELS (insertions-deletions) and predicted secondary structure of the 1su-rRNA (16S) revealed two main branching gorgonian clades; Calcaxonia and Alcyoniina-Holaxonia, with Scleraxonians present in both groups. Moreover, McFadden et al. (2006) using the mitochondrial protein-coding regions, *msh1* (now *mtMutS*) and ND2 on *all* Octocorallia sub-groups further supported the findings of Berntson et al. (2001), revealing three well-supported clades (*cf.* Figure 1.4). Gorgonian phylogeny therefore, typically recognises two groups, with further separation of deep and shallow water clades within Calcaxonians (McFadden et al. 2006, Pante et al. 2012), and Scleraxonians being polyphyletic (Sanchez et al. 2003a). Furthermore, mapping morphological characters onto molecular-derived phylogenies revealed synapomorphic and homoplasious characters, for example axial structure and surface sclerite morphology respectively in Caribbean Holaxonians (Sanchez et al. 2003b). Such phylogenetic research, though informative, fails to satisfactorily resolve at the subordinal or family-level (McFadden et al. 2010a) due to such considerable overlap and lack of reliable morphological characters (Wirshing et al. 2005), further confounded by remarkably slow evolving mitochondria in the Anthozoa (Shearer et al. 2005). However, Octocoral mitochondria contain *msh1*, a homolog of the bacterial mismatch repair gene MutS (Pont-Kingdon et al. 1995, 1998), synapomorphic for the subclass and twice as variable as other traditionally used mitochondrial markers (France & Hoover 2001, van der Ham et al. 2009, McFadden et al. 2010a). Yet, levels of *msh1* inter- and intraspecific variation have been shown to differ between genera (McFadden et al. 2010b), and combined use of *msh1*, mitochondrial and/or nuclear genes seldom leads to increased taxonomic resolution (e.g., Sánchez et al. 2003b, Wirshing et al. 2005, Herrera et al. 2010). Furthermore, studies utilising

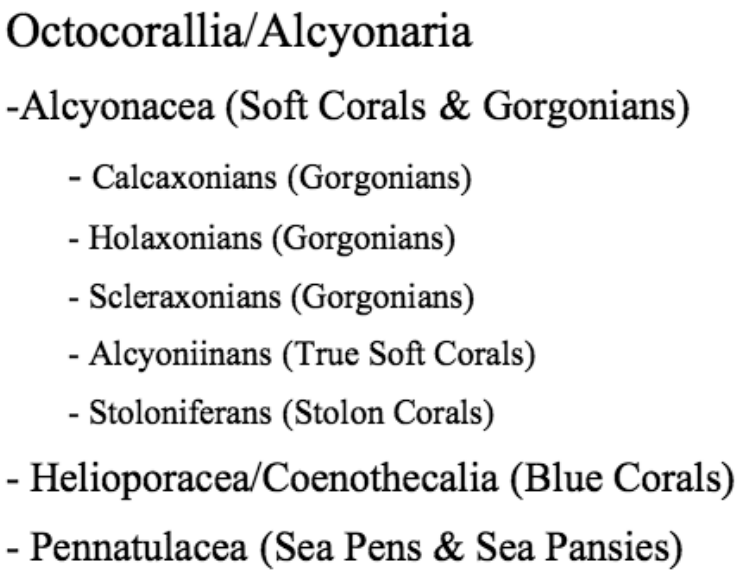


Figure 1.4 Octocoral phylogeny based on morphological characters currently in use based on Bayer (1981) and Fabricius & Alderslade (2001).

nuclear rDNA internal transcribed spacers (ITS) reveal conflicting results both between and within gorgonian species (Aguilar & Sánchez 2007, Sánchez et al. 2007, Dueñas & Sánchez 2009, Gutiérrez-Rodríguez et al. 2009, *cf.* Calderón et al. 2006). Such multicopy markers (tandem repeats of transcription units within a cell) are subject to intragenomic variation (variability between such tandem repeats), controversial in the validity of the results (*cf.* Coleman 2003, 2007, 2009). The predicted RNA secondary structure is more conserved and therefore, generally considered more phylogenetically informative particularly in the cases of Caribbean and deep-sea gorgonians (Aguilar & Sánchez 2007, Sánchez et al. 2007, Sánchez & Dorado 2008, Dueñas & Sánchez 2009). Nonetheless, Concepcion et al. (2008) revealed cryptic species delineation using the single-copy marker SRP54 (>33% variation *cf.* <10% in mtDNA ND2 & 6) within *Carijoa riisei* Duchassaing & Michelotti 1860, thus informative intraspecific variation without the caveats of intragenomic variation. In contrast, Watling & France (2011) discovered large numbers of indels (sequence insertions and deletions) rendered the sequences inoperable for phylogenetic analyses in the Keratoisidinae.

As with morphological characteristics the choice and utility of molecular marker(s) appears subjective relative to their resolution efficiency (Wirshing et al. 2005) and target taxon. What is clear is that gorgonians, and octocorals generally, lack reliable phylogenetic hypotheses and much work is yet to be completed (Sánchez et al. 2003a). A combination of specific markers still may provide sufficient resolution to make inferences on both phylogenetic and evolutionary

principles, even though not being as informative as sequencing the entire genome. Thus, for the purpose of this review Bayer's somewhat tenuous three-group (suborders Holaxonia and Calcaxonia, and Scleraxonian group) system, currently utilised by most octocoral taxonomists (e.g., Fabricius & Aldersade 2001, Daly et al. 2007; Figure 1.3 & 4), will be referred to with reference to further studies where appropriate.

1.4 MORPHOLOGY

Gorgonian morphological variability, whether environmentally plastic, genetically derived or the product of genotype-by-environment interaction(s), display a variety of forms within and between habitats. Colony morphology can be arborescent, flabellate/fan-shaped, spiraled, planar, pinnate, tangled/untangled bushes, lyrate, candelabra, reticulate, encrusting, lobular, or a combination of such forms (Bayer et al. 1983, Fabricius & Alderslade 2001). Colonies display nested modularity from branched (or unbranched): e.g., reticulate, alternate, pinnate, irregular, dichotomous, monopodal, fistulate, or a combination; polyps (modules *sensu stricto*) typically retracted or contracted (Figure 1.2a - e); and supra-modular traits e.g., intercalice distance, calice diameter and branch length (Sánchez et al. 2007, Prada et al. 2008). There is considerable variability within such traits, apparently independent of common ancestry, with no complete understanding of developmental or evolutionary processes (Sánchez 2004, France 2007, Sánchez et al. 2007). However, such phenotypic variation among gorgonian individuals is essentially the raw material of natural selection (Sánchez & Lasker 2003, Pigliucci 2005).

Anthozoans possess developmental genes within the putative Hox1, Hox2, and Hox 9+ gene families giving inference to a rudimentary "Hox code" (Ryan et al. 2007). Possession of such highly conserved gene clusters indicates a greater complexity in form and development for Cnidaria, providing insight into Hox evolution; Hox genes previously considered a bilaterian invention (Ryan et al. 2007). However, knowledge of morphogen gradients regulating cnidarian morphogenesis is poor, yet crucial in developing our understanding on how morphogenesis is controlled by genome-by-environment interactions (Kaandorp & Kübler 2001). Nonetheless, advances in developmental biology in combination with integrative investigations - *in vivo, in vitro* and *in silico* - are beginning to provide valuable insights into the evolution of both developmentally constrained and environmentally dependent gorgonian characters, especially with regard to branching structure (Sánchez et al. 2004, 2007, Brown 2007).

Branching systems are open networks, thus linked between any two points and not closed circuits (Kaandorp & Kübler 2001). Such networks are present in all complex systems from

rivers (Horton 1945) to the mammalian nervous system with various indices assigned to branches for morphometric comparisons (reviewed by Kaandorp & Kübler 2001). Understanding complex gorgonian architecture and developmental processes has lead to numerous comparative morphometeric studies often concluding the potential dependence of growth form on genetic and/or environmental parameters (e.g., Kim et al. 2004, Sánchez et al. 2004, Linares et al. 2008, Prada et al. 2008). Branching gorgonians develop in a sub-apical process, resulting in mother-daughter relationships: the primary (mother) branch producing secondary 'daughter' branches at fixed distances/internodes (Sánchez et al. 2004). The mother-daughter ratio (*c*) indicates the relationship between colony form and growth (= branching), with colony shape maintained when (*c*) is constant irrespective of gorgonian species (Sánchez et al. 2004). This pattern is particularly constant in alternate branching taxa and is suggested to indicate physiological developmental constraints or canalization (Sánchez et al. 2004). Furthermore, determinate growth (through self-organized criticality) follows a scaling power law relative to mother branch size frequency distribution. Sánchez (2004) goes on to review differences in colony size and growth patterns whilst (*c*) is constant, perhaps an example of heterochrony – differences in colony size and shape due to changes in timing or rate of developmental events (Gould 1977). In addition, colony growth will slow asymptotically as the maximum number of mother branches is reached (Sánchez 2004). Interestingly, compensatory growth experiments in the Japanese scleraxonian, *Melithaea flabellifera* Kükenthal 1908 revealed that optimal size and branch density were determinate by maintaining colony form through irregular and heterogeneous growth (Matsumoto 2004), a compensatory thus determinate pattern also reported in other gorgonians (Sánchez & Lasker 2003, Kim et al. 2004). Quantification of gorgonian branching networks has further revealed an emergent level of module integration at the colony level (internode distance and branch length; Sánchez & Lasker 2003). Patterns of morphological trait integration are independent of polyp iteration; a few changes at the polyp level having no significant affect on colony architecture (Sánchez & Lasker 2003, Sánchez 2004). Nevertheless, character trait inter-dependence may be the product of heritable pleiotropy (multiple phenotypic traits due to a single gene), linkage disequilibrium, or concerted evolution operating on traits for a specific function (Sánchez & Lasker 2003). Moreover, convergent evolution (homoplasy) in gorgonian colony architectures, first proposed by Bayer (1953), is both phylogenetically corroborated (Sánchez et al. 2003b, Wirshing et al. 2005, Aguilar & Sánchez 2007, Cairns & Bayer 2009) and frequently observed between closely related species (Sánchez 2004, Watling et al. 2012).

Heterogeneity in form exists due to the feedback between growth, and micro- and macro-physical environments (Kaandorp & Kübler 2001). Thus, branching, growth and form are continuously undergoing physiological adjustments relative to environmental change (Velimo 1975, Matsumoto 2004, Roark et al. 2006). However, gorgonians have a slow growth rate (Grigg 1974, Noé & Dullo 2006, Tracey et al. 2007), such that investigations of eco-phenotypic and genetic effects using reciprocal transplants principally focus on microstructure variation (sclerite; e.g., West et al. 1993, West 1997) and specific genetic markers (Prada et al. 2008, Gutiérrez-Rodríguez et al. 2009). Furthermore, gorgonian transplant experiments at opposite ends of an environmental gradient such as depth, frequently revealed phenotypic plasticity, thus environmentally induced traits (Bayer 1961, Brazeau et al. 1991, West et al. 1993, West 1997, Kim & Lasker 1997, Kim et al. 2004, Skoufas 2006). Such phenotypic expression aligned with differences in genetic markers, but these markers (e.g. *msh1*, ITS – caveats discussed above) are not specific for the phenotypic trait(s) observed and are limited in their ability to investigate environmental challenge responses. Interestingly, detection of branching initiation in the gorgonian *Pseudopterogorgia* [now *Antillogorgia*] *elisabethae* Bayer 1961 using the Hox marker *anthox* revealed differences in gene expression within and between branch locations, yet failed to give sufficient resolution as a marker for branch initiation (Brown 2007). Thus, much work on the expression of genes specific to phenotypic plasticity has yet to be conducted whether from the genome or of epigenomic origin.

The interplay between gorgonian developmental gene expression and resource supply is unknown, however use of simulation models and morphometric analyses may infer resource allocation structure and surplus, further triggering phenotypic plasticity events. Colony architecture determines its own morphological trajectory in marine hydroids. Sheet and runner-like morphologies influence polyp pumping activity that in turn reduces or increases gastrovascular flow rate and relative cellular oxidation states in developing hydroids (Blackstone & Buss 1993). The resulting internal tensions trigger developmental gene expression (e.g., Cnox). Whether such colonial expansion thresholds exist in gorgonians remains to be seen, however, determinate growth appears to be 1) evident (e.g. Matsumoto 2004, Sánchez & Lasker 2003, Sánchez et al. 2004, Lasker et al. 2008), and 2) most likely influenced during early life stages (Cossins et al. 2006).

1.5 ECOLOGY

Ecologically diverse, gorgonians are important components of marine habitats from the deep sea to the tropical intertidal (Fabricius & Alderslade 2001, McFadden et al. 2006). Gorgonian

abundance, diversity and distribution depend upon environmental factors such as substrate type, light, temperature, sedimentation, current regime and flow rates (Garrabou et al. 2001), which have been shown to induce intra- and interspecific morphological variability both within and between individual colonies (van Oppen et al. 2005). Yet little is known of the ecology, biology and variable phenotypic responses of gorgonian taxa relative to environmental parameters, particularly in both the deep sea (Parrish 2007) and the Indo-Pacific (van Oppen et al. 2005).

Gorgonian ecology often reflects reproductive strategies and/or changes along environmental gradients relative to individual species tolerances (Fabricius & Alderslade 2001). Gorgonians reproduce both sexually and asexually, with a variety of strategies having differing effects on population growth (Lasker 1990, 1996, 2006, Lasker et al. 1988 1996), even including parthenogenesis (Brazeau & Lasker 1998). Most research has been conducted on Caribbean and Mediterranean taxa, however, the majority being internal brooders (sperm cast, *sensu* Pemberton et al. 2003) with short pelagic larval duration before settlement. Nevertheless, staggered or neap tide spawning events, planktonic larval displacement by water currents, and chemotaxis through conspecific or coralline algal exudates (Fabricius & Alderslade 2001) may further influence local distribution, abundance and survival. In addition, gorgonians are subject to considerable endemism (Grasshoff & Bargibant 2001, Piccianno & Ferrier-Pagès 2007) with just a single shallow water gorgonian (*Acabaria bicolor* Nutting 1908) in the Hawaiian Islands and American Samoa (Fenner pers. comm., 2010). Nonetheless, shallow water gorgonians are highly abundant and predominantly zooxanthellate in the Caribbean compared to the Indo-Pacific, by far the most diverse, yet little researched taxa (Grasshoff & Bargibant 2001).

Most gorgonian species are restricted to relatively small areas, such as islands (e.g., Aldabra, Bayer 1996; New Caledonia, Grasshoff & Bargibant 2001), with evolutionary processes often constrained by dispersal ability in terms of life history and biogeography. With a low range-size frequency distribution (~4 million km^2 to > 10 million km^2 for low and high range-size dispersing taxa respectively, as a proxy taken from Hughes et al. 2002) and endemism suggested central to the Indo-Pacific, mid-range dispersers may create the potential for the isolation of populations (mid-domain effect: see Colwell & Lees 2000) or subpopulations thus, vicariance with large dispersing, pandemic taxa having a decreased probability of speciation due to a greater ability to populate wider areas relative to habitat availability (Brown 2014, but see Hughes et al. 2002, Connolly et al. 2003, Colwell et al. 2005,). However, the mid-domain effect – suggesting an increased overlap of species ranges at the centre of a ‘domain’ leading to a peak of species richness – appears to be positively correlated with prevailing ocean currents revealing

high species richness at periferal Indo-Pacific locations in numerous invertebrate groups (Connolly et al. 2003, Budd & Pandolfi 2010). Furthermore, increasing evidence reveals little correlation with short dispersion time and endemism for many taxonomic groups within the Coral Triangle, lending question to dispersal ability and reproductive mode as effective indicators of biodiversity particularly in the face of habitat availability as present in dense archepelago's such as Indonesia. In other words, if there's always somewhere to settle then dispersal ability is of less importance. Nevertheless, with only little knowledge of gorgonian species distribution, developmental strategies and taxonomy *per se*, it is not clear how such hypotheses extend to this group, diminishing their potential utility as a conservation indicator group for effective management strategies.

Gorgonians show considerable phenotypic plasticity likely as an important factor contributing to their broad distribution as a group, which may further lead to genetic accommodation and/or assimilation and divergence (West-Eberhard 2003, 2005). However, gorgonian responses to environmental parameters vary across taxa, with zooxanthellate taxa absent in highly polluted areas, and tolerant azooxanthellate taxa often having a high susceptibility to fungal infections, colonization by fouling organisms, and a high partial mortality (Fabricius & Alderslade 2001). Complex habitats provide more vertical relief, colonizable area, and greater microhabitat variability than soft benthic substrata (Etnoyer et al. 2010). Yet even in the presence of suitable substratum, most gorgonians are absent in areas of high turbidity due to the physical impairment of settlement, feeding, reproduction and growth. Habitats characterised by low wave action, high turbidity and sedimentation rates, favour encrusting *Briareum* spp. (Fabricius & Alderslade 2001), likely due to morphological and behavioural pre-adaptations such as phenotypic and photoacclimatory plasticity, colony dynamics, polyp density and size, reproductive strategy and recruitment survival (Stafford-Smith 1993, Anthony 2000). However, turbid habitats are marginal for zooxanthellate gorgonians, with no evidence of hard coral community replacement. Unsurprisingly, zooxanthellate gorgonian taxa follow similar depth ranges to scleractinia, however their relative reliance on endosymbiont photosynthetic carbon appears to be species specific (Sorokin 1991). Furthermore, high water motion and localized upwelling provide elevated nutrients for primary productivity and enhanced food availability (Jokiel 1978, Reed 1983, Sebens 1984). Arborescent branching gorgonians orientate themselves perpendicular to the predominant water current in order to maximize food capture (Grigg 1972, Fabricius & Alderslade 2001). Pristine, high hydrodynamic conditions facilitate the largest colonies, but can also limit colony size (Linares et al. 2008) and enhance relative gorgonian diversity. Such patterns may be attributed to intermediate disturbance levels, maintaining relative species

diversity within a reef community (Connell 1978, Ostrander et al. 2000). Moreover, scaling effects may also lead to determinate growth and size whereby colony size reaches its functional capacity. Taken together, sedimentation, light and water flow appear major factors controlling local gorgonian populations (Meesters et al 2001, Linares et al. 2008).

Gorgonians are ecologically diverse, long-lived, slow growing engineering species (species which "modify, maintain and create habitats." Jones et al. 1994) with growth rates as little as 14 μm yr^{-1} (Roark et al. 2006), making them effective bioarchives (Risk et al. 2007, Williams & Grottoli 2010) yet vulnerable to disturbance, which can dramatically affect whole communities (Linares et al. 2008). Deep sea and Mediterranean gorgonian reefs are particularly vulnerable to the commercial harvesting of precious corals (*Corallium* spp.; Grigg 2001, Santangelo et al. 1993) and bottom trawling (Watling & Norse 1998, Hall-Spencer et al. 2002). Furthermore, susceptibility to bleaching events and disease outbreaks has increased dramatically, having profound effects on gorgonian taxa in tropical (Smith et al. 1996, Geiser et al. 1998) and temperate regions (Cerrano et al. 2000, Garrabou et al. 2001, Hall-Spencer et al. 2007), likely due to global climate change. Gorgonians are therefore conservation indicator taxa, providing both habitat and refugia for numerous organisms including commercially important juvenile and adult fish species (Hall-Spencer et al. 2002). Understanding patterns of gorgonian ecology, physiology and morphological variation through cross-disciplinary approaches will be increasingly important in management and remedial conservation efforts.

Due to their longevity and architectural diversity numerous marine organisms are associated with gorgonians, commonly exhibiting novel phenotypic adaptations. Most notably are morphological mimics such as the charismatic pigmy seahorse taxa e.g., *Hippocampus denise* Lourie & Randall 2003, facultative to various Scleraxonian and Holaxonian hosts (Lourie & Randall 2003). Unlike seahorses however, associates such as the gastropod *Cyphoma gibbosum* Linnaeus 1758 predate upon the polyps of their host, now the principle predator of Atlantic shallow water gorgonians as a result of predator release from over fishing (Birkepile & Hay 2007). Furthermore, *C. gibbosum* has recently been traced as a likely vector of the fungus *Aspergillus sydowii* (Bainier & Sartory) Thom & Church 1926, which decimated populations of the Caribbean gorgonian *Gorgonia* spp. (Smith & Weil 2004). However, compared to hermatypic (hard) corals gorgonians have few specialist predators (Puglisis et al. 2000), probably the result of secondary metabolite production in response to competition (Kim & Lasker 1997, Van Oppen et al. 2005), predation and changes in environmental stimuli (West et al. 1993, Hoover et al. 2008). Interestingly, transcriptome analyses have revealed an induced

chemical response to predation stress by *Chaetodon* spp. on the soft coral *Sinularia polydactyla* Eherenberg 1834 (Hoover et al. 2008). Such metabolic responses are thought to increase in both variability and abundance at lower latitudes likely due to increased predation and competition (Puglisi et al. 2000).

Irrespective of their battery of chemical defenses, and alloimmunity (see Salter-Cid & Bigger 1991), certain gorgonian taxa are particularly susceptible to fouling, often as a consequence of mechanical damage, pollution or predation reducing reproductive output, further leading to a colonization cascade of opportunistic fouling organisms commonly resulting in host mortality (Gerhart 1990, Weinbauer & Velimirov 1996). Fouling extent has been attributed to increases in temperature or light (Drohan et al. 2005), as is also the case with disease (Cerrano et al. 2000, Harvell et al. 2001). Nevertheless, associate organisms have also been shown to remove sediment and consume boring and fouling larvae (Goh et al. 1999). Such symbioses are thought to be advantageous, gorgonians having reduced mucus secretary cells compared to other Cnidaria (Fabricius & Alderslade 2001).

1.6 TROPHIC ECOLOGY

Thus far, it is evident that gorgonians are modular organisms, with determinate colony growth, form and size (Lasker et al. 2003) due to the iterative addition of polyps and branches, and within colony canalisation providing effective resource allocation structure (Sánchez & Lasker 2003). Furthermore, colony form can depend on feeding strategy and the same genotype can show different allocation patterns in different environments, consistent with the 'partitioning' hypothesis (Poorter & Nagel 2000, Weiner 2004).

Growth form and resource allocation also change to counter the effects of environmental factors such as sedimentation (Riegal & Branch 1996) and depth (West 1997). Moreover, sedimentation has profound effects on coral metabolism by decreasing photosynthetic productivity in zooxanthellate gorgonian taxa, and increasing respiration (carbon-loss) by 95-100% through increased mucus production (Riegl & Branch 1995). The long-term effects of such expenditure on gorgonians are unknown, with virtually nothing known about gorgonian coral symbioses in the Indo-Pacific.

Most gorgonians are colonial suspension feeding heterotrophs, predominantly capturing suspended particulate organic matter (POM; Tsounis et al. 2005, Picciano & Ferrier-Pagès 2007), as well as dissolved organic matter (DOM) and zooplankton (Fabricius & Klumpp 1995,

Fabricius & Alderslade 2001). Certain shallow water taxa particularly in the Caribbean have a moderate dependence on phototrophy, harbouring symbiotic zooxanthellae within the gastrodermal tissue (Fabricius & Alderslade 2001), with a concomitant plasticity in growth form relative to the environment (Kaandorp & Kübler 2001, Prada et al. 2008). Phenotypic variability relative to their surrounding habitat such as colouration (Sánchez et al. 2007), branching dynamics (e.g., Matsumoto 2004, Sánchez 2004), colony surface area, polyp density and inter-calice distance (West et al. 1993, Prada et al. 2008) have been shown to enhance food capture and efficiency. Most importantly is the extension of polyps - the primary feeding apparatus that can also bear photosynthetic endosymbionts (*Symbiodinium* Freudenthal 1962) in zooxanthellate taxa - into the water column whereby colony growth and form are interdependent on resource availability. Differential resource allocation patterns (hetero/phototrophic capacity) can vary relative to the environment in the same genotype, with or without morphological change (Sebens 1997, Poorter & Nagel 2000, Weiner 2004).

Assessing differential resource allocation patterns at the species level particularly in contrasting environments may be informative of the mechanisms of phenotypic variability within and between taxa, and ultimately tractable responses to environmental change. Energy apportionment relative to food acquisition and transfer from the environments and/or symbiont can be measured using stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). However, gorgonian research has focused on the relative isotopic signatures of the calcareous axis and skeletal elements (sclerites) usually as bioarchives (*sensu* Williams & Grottoli 2010, Risk et al. 2002). For example, azooxanthellate gorgonian skeletal $\delta^{13}C$ increased as nitrogen $\delta^{15}N$ decreased with increasing depth, further correlating with suspended POM values (Williams & Grottoli 2010). Feeding experiments on *Corallium rubrum* Linnaeus 1758 also revealed a preference for autotrophic flagellates that increased with temperature (Picciano & Ferrier-Pagès 2007). Yet experiments on numerous shallow water taxa within the Great Barrier Reef revealed that the heterotrophic food source was species-specific irrespective of zooxanthellate status (Sorokin 1991). Nevertheless, until recently (Baker et al. 2011) nothing was known of the isotopic ratios of gorgonian soft tissues, primarily due to the unsuccessful separation of sclerites giving erroneous $\delta^{13}C$ signatures (Grottoli pers. comms. 2010). Interestingly Baker et al. (2011) revealed using Caribbean zooxanthellate gorgonians, that light primarily affected $\delta^{15}N$ fractionation even though the values were minimal; this still has bearing on differentiating the effects of pollution on coral reef taxa. Comparative analyses of soft tissue and endosymbiont isotopic ratios in alignment with the surrounding environment would further elucidate the relationship between host, symbiont and their intrinsic and extrinsic energy transfer role(s) in

benthic – pelagic coupling. Moreover, exploring how endosymbiont (i.e., *Symbiodinium*) types influence the physiological performance of the holobiont to environmental change would greatly enhance our understanding of what drives host-symbiont associations.

The photosynthetic dinoflagellates within the genus *Symbiodinium* associate with numerous coral reef invertebrates, most notably the hermatypic (reef building) Scleractinian corals. Diversity within the genus *Symbiodinium* is under continual investigation, but is hampered by a lack of morphological distinction between sub-generic clades (A-I; Pochon & Gates 2010) with differences identified at the genetic level (e.g., LaJeunesse 2002, Pochon et al. 2006, 2012). Technological advances continually reveal novel and cryptic variation (e.g., Silverstein et al. 2012) increasingly associated with photophysiological tolerance (Jones et al. 2008, LaJeunesse et al. 2008, Hennige et al. 2009, but see Abrego et al. 2008) and the complex interplay between host and symbiont. Zooxanthellate gorgonians are major reef components on Caribbean reefs, but only a few taxa are present in the Indo-Pacific. Most show symbiont specificity (Goulet et al. 2008) with *Briareum* Gray 1859 and *Isis hippuris* Linnaeus 1758 known to harbour the putatively stress tolerant clade D *Symbiodinium* (van Oppen et al. 2005). Interestingly, both transplant and laboratory experiments on the gorgonian *Briareum* sp. reveal symbiont 'switching' in response to environmental parameters in the Caribbean (Lewis & Coffroth 2004); a proposed adaptive response borne from the nonselective *Symbiodinium* acquisition by juvenile hosts (Coffroth et al. 2001). Thus, specific *Symbiodinium* spp. in different host species across unique physical-environmental conditions may be linked to abiotic regime. Furthermore, algal clade selection by either symbiont 'switching' (exogenously) or symbionts 'shuffling' within a host coral colony is controversial (Baker 2003, Goulet 2006, Apprill & Gates 2009), as either mechanism assumes that the coral species can host multiple algal genotypes, sequentially or simultaneously. Increasing evidence suggests such a phenomenon (Baker et al. 2004) as a mechanism of survival over the numerous climate and sea level fluctuations, with relatively little extinction in scleractinian corals alone over the last 220 MY (Veron, 1995). Yet many species host only a single *Symbiodinium* clade regardless of environmental conditions or transplantation experiments e.g., *Fungia* [now *Lobactis*] *scutaria* Lamarck 1801 retained its original zooxanthellae type (C1b) for 35 yrs after transplantation from the Indo-Pacific to the Caribbean (LaJeunesse et al. 2005). Mechanisms of endosymbiont acquisition and diversity however, remain to be elucidated in Indo-Pacific zooxanthellate gorgonians, particularly within the Coral Triangle.

Intriguingly, molecular and histological evidence revealed two previously described azooxanthellate gorgonian species, *Junceella fragilis* Ridley 1884 in the Philippines and *Euplexaura nuttingi* Kükenthal 1919 on the Great Barrier Reef, as possessing clade G of the symbiotic zooxanthellae *Symbiodinium* (van Oppen *et al*. 2005, Williams et al. 2010). Individuals were found in shallow turbid waters and lacked host pigmentation in contrast to their brightly coloured deeper azooxanthellate counterparts. Such evidence may represent differential phenotypic expression of a genotype under varying environmental conditions (West et al. 1993), in addition to raising questions on the obligate nature of *Symbiodinium* with such taxa. The significance of such a discovery, and if enhanced fitness through mixotrophy in turbid environments is adaptive plasticity or plasticity as an adaptation, remains to be elucidated. Yet a low reliance on photosynthetic gain increases the likelihood of survival under high temperature and/or irradiance stress. Interestingly, the cnidarian-algal symbiosis has been shown to be maintained by altering the expression of existing genes involved in vital cellular processes, and is thus not due to 'symbioses-specific' genes (Rodriguez-Lanetty et al. 2006, 2008).

1.7 PHENOTYPIC PLASTICITY

Central to evolutionary theory is the historical connectivity of all life within and between the environment, and the ability for biological change irrespective of scale. Whether at the population, species, individual, phenotypic trait or molecular level, evolution is considered inherent - the decent of biological variation through natural selection (Darwin 1859) or the non-adaptive influences of genetic drift (Hurst 2009). Such biological variation in response to environmental heterogeneity reinforces survival and reproductive success, particularly in sessile taxa when subject to novel environments. Variability at the phenotypic level (morphological, behavioural, and/or physiological) is conditionally expressed relative to environmental cue(s), within a single generation. In other words, phenotypic plasticity depicts multiple phenotypes from a single genotype in response to environmental variation (Pfenning et al. 2010). Thus, such phenotypic variability occurs within the lifespan of an individual as a consequence of high plasticity capacity or the release of cryptic genetic variability through environmental stress (evolutionary capacitance breakdown; Rice 2008). With such variation, particularly at the phenotypic level, how can one delimit a species and, therefore, differentiate between plasticity capacity and divergent taxa? Moreover, does phenotypic plasticity provide the foundation for novel species through the selection of complex traits that enhance fitness and overall reproductive success, or does it simply obscure selection and species boundaries? Controversy continues to exist with regards the roll of phenotypic plasticity in diversification and speciation (Pfenning et al. 2010), but first, how does it arise and how, if at all, can it be assessed?

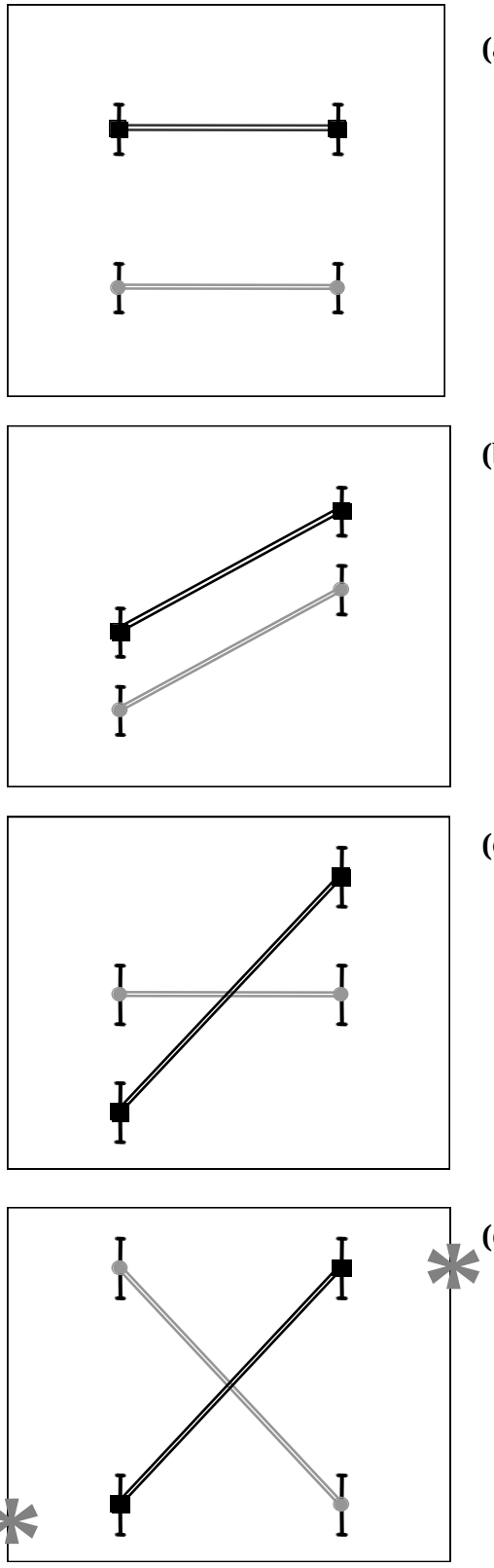
Phenotypic plasticity in response to environmental heterogeneity may be adaptive or genetically derived (Gotthard & Nylin 1995, Hoogenboom et al. 2008). Plasticity itself can be the result of adaptation, with selection acting on a trait or an organism's ability to be plastic (adaptive plasticity). Intrinsic (development, life history, physiological, or genetic) and extrinsic factors (substrate, light, temperature, sedimentation, competition, predation, and hydrodynamics), alone or in concert, control phenotypic plasticity, the interaction of which can be visualized through reaction norms (Figure 1.5; Gotthard & Nylin 1995). Reaction norms, a set of phenotypic expressions of a single genotype over an environmental range/gradient (Stearns et al. 1991), visualize the plasticity capacity of a genotype. Through reciprocal transplantation of contrasting phenotypes between opposing environments over an appropriate timeframe, reaction norms can reveal inducible plastic or fixed traits, with concomittent fitness through survivability (Prada & Hellberg 2013). Phenotypic trait variance (*Vp*) in the singular or plural may be due to genetic (V_G), environmental (V_E) or interactive effects (V_{GxE}) where genotypes differentially respond to their environment, and can be expressed as:

$$Vp = V_G + V_E + V_{GxE} + V_{error} \quad (1)$$

where V_{error} accounts for developmental, bet-hedging, behavioural or other unaccountable noise. If selection acts on more favourable genotypes this causes a shift in the average environmental effect on a population leading to adaptive plasticity as an adaptation (Figure 1.5d; DeWitt & Scheiner 2004). Thus the genetic effects of phenotypic variance, or not as the case may be (i.e., canalisation), underpin plasticity capacity and can be depicted as:

$$V_G = V_{G,A} + V_{G,D} + V_{G,I} \quad (2)$$

where additive ($V_{G,A}$), dominant ($V_{G,D}$) and epistatic ($V_{G,I}$) genetic effects are the result of the relative heritability contribution of allele frequencies to the observed phenotype (Hagemann et al. 1999). Both additive (polygenic) and dominant genes act on specific loci, whereas an epistatic interaction effect is the result of modifier gene(s) at different loci (Johnson 1976, Byers 2008). The quantification of additive variation (average effect of substituting one allele for another) within a given population for e.g., functionally important phenotypic trait(s) reveals the action of incipient divergent selection or genetic drift and ultimately gene flow (Carlon et al.



(a) V_G Genetic variance: consistent genetic differences between morphotypes with no phenotypic plasticity.

(b) V_G Genetic variance and V_E Environmental variance: consistent phenotypic plasticity and additive genetic differences between morphotypes with no interaction effect, therefore suggesting two different taxa.

(c) V_E Environmental variance and V_{GxE} Genetic and environmental interaction variance: environmental main effect with genetic interaction variance, therefore morphotypes are not genetically differentiated and their response to the environment is plastic.

(d) V_{GxE} Genetic and environmental interaction variance: morphotypes have similarly yet opposing reactions to the environment, thus different taxa yet equally as plastic. Adaptive optima (asterisks) indicate that the square morphotype/taxon would likely be selected for; adaptive plasticity as an adaptation.

Figure 1.5 Reaction norm means of a hypothetical scenario of two morphotypes showing (a) V_G genetic variance, (b) V_G genetic and V_E environmental variance, (c) V_E environmental variance and V_{GxE} interaction variance, and (d) V_{GxE} interaction variance (modified from DeWitt & Scheiner 2004).

2011, Bird et al. 2012). Furthermore, patterns of covariance between phenotypic traits particularly in contrasting environments, can be indicative of functional trade-offs leading to divergence, however differentiation between adaptive (canalised) and developmentally plastic (epigenetic) influences on the observed phenotype can be inscrutable (DeWitt & Scheiner 2004). Thus, reaction norms as a product of reciprocal transplant experiements (RTEs) reveal only the source of variation, not the mechanism. Differentiation of plasticity at the mechanistic level would require both field and molecular analyses assessing genetic inheritance and epigenetic developmental effects. Nonetheless, significant population subdivision, particularly with the simultaneous expression of novel phenotypic traits (adaptive radiation; West-Eberhand 2005), is indicative of local adaptation, and thus population and species divergence (Kawecki & Ebert 2004, Pfenning et al. 2010). As divergent selection acts against intermediate genotypes, the environment is in fact the selective agent acting on plastic phenotypic traits and the ability to be plastic in the first place (Gilbert & Epel 2009). Thus plasticity can be a diversifying factor, with any number of possible trait combinations that may lead to the production of a novel phenotype, enhancing individual and/or population fitness in a particular environment (Santelices 1999, Magwene 2001b). Intrinsic and extrinsic factors will, therefore, shape species range size (Hughes et al. 2002), essentially reflecting processes of speciation, extinction, resilience to environmental change and overall species diversity and ecosystem functioning.

Fitness enhancement through an individual's (genotype) ability to adapt in heterogeneous environments is particularly important in sessile marine organisms, due to their inability to relocate to another environment. Selection, therefore, acts on plasticity capacity, with modular colonial invertebrates being arguably the most pliable to their physical environment (Kaandorp & Kübler 2001). Modular and colony growth and form are intrinsically linked to optimise resource acquisition, reproduction and minimize metabolic costs. Fundamental however, is the understanding of interactions between, and relative contributions of, the genome and physical environment to morphogenesis, which in most cases is unknown (Gutiérrez-Rodríguez et al. 2009).

An organism's response to its environment involves numerous biological mechanisms with population dynamics closely tied to resource allocation success, thus environmental regime (Weiner 2004). Multivariate phenotypic traits provide the observed phenotype at any one time which may be a consequence of plasticity capacity, strict adaptation thus canalization (genotypic), a combination of the two on various traits, or unique hybridization through repetitive introgression (Ladner & Palumbi 2012). How to differentiate such phenotypes as

actual and informative species in the context of biodiversity assessment for conservation management, can be an onerous task. Arguably one of the greatest challenges is species determination on shallow reefs within the Coral Triangle, exhibiting fitness enhancement through intense competition and the fixation of mutation through free ionization energy (Nei 2007) at low latitudes ('evolutionary rate hypothesis'; Rohde 1992, but see Weir & Schluter 2007). The Coral Triangle is a recognized "biodiversity hotspot", characterized by high species numbers and endemism, extending from the Philippines to the Solomon Islands (Carpenter & Springer 2005, Hoeksema 2007, Veron et al. 2009, Gaither & Rocha 2013). The Indonesian archipelago, within the Coral Triangle, is likely one of the greatest areas of marine biodiversity due to geological age, highest number of islands per unit of geographic area, and ecoclimatic stability lending greater time for evolutionary processes. The origins of such biodiversity are, however, controversial (Gaston et al. 1998, Chown & Gaston 2000, Bowen et al. 2013) and likely taxon-specific, mechanistic convergence relative to individual fitness response, with latitude itself being just a correlate for the mechanism(s) concerned. Nevertheless, such immense biodiversity is being destroyed, in an area still relatively unexplored, resulting in rudimentary biodiversity assessments and theoretical postulation (e.g., Hughes et al. 2002, Carpenter & Springer 2005). Comparative research across contrasting (healthy *versus* exploited) environments may reveal evolutionary mechanisms from plasticity to divergence through evolutionary capacitance in high energy, biodiverse, yet exploited habitats; therefore elevating descriptive biodiversity assessments to realistic conservation measures, from necessity to emergency in the face of anthropogenically induced destruction.

1.8 RESEARCH PERSPECTIVE

Understanding patterns of gorgonian ecology, physiology and morphological variation through cross-disciplinary approaches is essential for characterizing species, communities and population resilience to environmental change - increasingly important in management and remedial conservation efforts. Gorgonians show considerable trait variability, independent of common ancestry with such phenotypic plasticity likely to be an important factor contributing to their broad distribution. Furthermore, fitness can be an individual's ability to change. Whether such plasticity in gorgonian taxa is ecologically driven, genetically derived or a combination of the two is continuously explored through genetic, ecological, physiological, and recent advances in genomic research. Gorgonians as modular colonial organisms therefore, provide dynamic models in which to study evolutionary mechanisms as a consequence of their environment. Nonetheless, plasticity is an emergent property of the genotype, therefore it is susceptible to natural selection (Pigliucci 2005).

Clearly, gorgonian corals represent a highly diverse model group, yet it is surprising that the most diverse of all gorgonian taxa exist within an area that is least understood, particularly the Indonesian archipelago (van Ofwegen 2004), arguably the most biodiverse region of the coral triangle (Carpenter & Springer 2005, Hoeksema 2007, Veron et al. 2009, 2011). What species are present and how do such seemingly delicate organisms respond to their environment, especially to increasing threats through anthropogenic encroachment? What species exhibit marked biological success, opportunistic in the face of environmental change, and is such success expressed through morphological variability? Would such morphological variability both within and between taxa lead to resource allocation change, mitigating the effects of environmental change? If so, what are the relative contributions of genetic variation (plasticity capacity as an adaptation) and eco-phenotypic plasticity (e.g., acclimation) to the observed phenotypic variation? These questions are addressed utilising cross-disciplinary approaches on gorgonian taxa within the Wakatobi Marine National Park (WMNP), SE Sulawesi, Indonesia – the second largest national park in the country. Investigations are set to assess (1) shallow-water gorgonian diversity, and (2) subsequently infer plasticity or adaptive evolution in observed gorgonian morphotypes across environmental clines of both natural and anthropogenic origin.

1.9 AIMS & OBJECTIVES

With the purpose of highlighting the importance of gorgonian corals in marine ecosystems, specifically within the Coral Triangle, this research aims to evaluate gorgonian responses to environmental change predominantly as a consequence of anthropogenic disturbance. Ultimately, through underlying evolutionary principles, a sequential investigation of gorgonian ecology, taxonomy, and phenotypic dynamics, this study aims to increase both our understanding and awareness of this group as key indicators of reef health, influential in environmental impact management strategies.

To address how gorgonian corals respond to environmental change within the WMNP, SE Sulawesi, Indonesia, this research, in order of each chapter, aims to:

1. Characterise gorgonian diversity, abundance and distribution patterns across gradients of habitat quality within the WMNP.

2. Identify biologically successful gorgonian species across such environmental clines.

3. Further identify predictor environmental variable(s) inferred to influence the ecological

structure of gorgonian assemblages.

The first three objectives are achieved in Chapter 2 through a stratified ecological survey measuring corresponding environmental variables within each site. It is hypothesized that gorgonian corals can tolerate only clear, moderate to fast flowing hydrodynamic reef environments, and are therefore markedly reduced or absent in opposing habitats.

4. Quantify observed morphological variability in the zooxanthellate gorgonian *Isis hippuris* Linnaeus 1758 across environmental gradients on reefs within the WMNP.

5. Determine whether such *I. hippuris* morphotypes are both phenotypically and genetically partitioned across contrasting reef environments.

6. Further ascertain if the observed *I. hippuris* morphotypes represent previously described species, new species or a single species with highly variant, integrated phenotypic traits.

These three objectives are addressed in Chapter 3 firstly, assessing the taxonomic history of *I. hippuris* in the context of the two distinct morphotypes found within the WMNP (Chapter 2); and secondly by using both anatomical and molecular morphometrics between such morphotypes on the contrasting reefs. It is hypothesized that such phenotypic variability is merely plasticity as an adaptation in a single species across environmental clines. Further insight is also given into the phylogenetic position of *I. hippuris* within the sub-Class Octocorallia.

7. Determine if *I. hippuris* morphotypes across environmental gradients are environmentally induced (plastic) or genetically derived (canalized/adaptation).

8. Assess differential physiological responses of the *I. hippuris* holobiont to environmental change.

9. Investigate host-algal endosymbiont specificity between morphotypes across and as a consequence of environmental change.

10. Determine integrated phenotypic traits which interact to delimit *I. hippuris* morphotypes suggesting mechanisms of divergence through phenotypic trait integration in response to environmental perturbation.

Objectives 7 to 10 are addressed in Chapter 4 using a combination of multi-trait (morphology, endosymbiont type, and physiological components) and environmental measurements from a one-year reciprocal transplant experiment between sites of contrasting reef health at comparable optical depths. It is hypothesized that light availability is a primary vector (causal) of *I. hippuris* morphotypes further driving integration among phenotypic traits. The quantum efficiency of the *I. hippuris* holobiont through the functional integration of optical traits was therefore assessed through a reciprocal transplant experiment (RTE) for evidence for the onset of light-induced directional selection or plasticity capacity.

11. Summarise both the importance and status of Indonesian gorgonian octocorals as a consequence of this research.

This final objective aims to juxtapose this study's findings with existing and proposed research, highlighting integral knowledge gaps in the second-most common coral reef component in a region of high yet insufficiently researched biodiversity. Furthermore, the phylogenetic implications of this research are considered in the context of the family Isididae and the significance of certain phenotypic traits, in particular the central axis, as objects of selection; inherent or convergent?

All chapters are to be, or are in the process of submission for publication with the exception of chapters 1 and 5 which will be merged for publication as a single review.

CHAPTER 2: ENVIRONMENTAL GRADIENTS STRUCTURE GORGONIAN ECOLOGY ON CORAL REEFS IN SE SULAWESI, INDONESIA.

ABSTRACT

Indonesian coral reefs are the epicentre of marine biodiversity, yet are under rapid anthropogenically-induced decline. Therefore, the necessity for ecological monitoring of high diversity taxa facilitating effective management and conservation is paramount. This study presents a unique and comprehensive survey of shallow-water (0-15 m) gorgonian assemblage composition and structure across a gradient of habitat quality within the Wakatobi Marine National Park (WMNP), SE Sulawesi, Indonesia. A total of 197 species and morphotypes from 41 genera and 12 families within the Calcaxonian, Holaxonian and Scleraxonian groups, are reported with current estimates of 21 new species and 28 new species records for the region. Results from this extensive survey confirm high local gorgonian abundance, diversity and species richness in the absence of anthropogenic influence and increasing with depth. Notably, morphological variants of the zooxanthellate species *Isis hippuris* Linnaeus 1758 and *Briareum* Blainville 1830 drive site and habitat assemblage differences across environmental gradients. Azooxanthellate taxa particularly within the Plexauridae drive species richness and diversity with depth. However, collinearities among 14 predictor variables explained only 30% of gorgonian assemblage structure highlighting benthic characteristics, water flow and natural light as primary ecological drivers. Thus non-independence between zooxanthellate (S = 8, n = 1900) and azooxanthellate (S = 189, n = 1517) taxa partitioned distinct gorgonian communities into two trophic groups: autotrophs and heterotrophs respectively, with contrasting diversity and abundance patterns within and between study sites. Such trophic group partitioning and habitat specific morphotypes suggest resource allocation structure representing both alternate feeding strategies and acclimatory phenotypic responses to anthropogenic impacts on coral reefs. This study strongly supports the WMNP as an area of high regional gorgonian abundance and diversity with results undoubtedly propagating conservation and research benefits beyond those presented here.

Key words: Gorgonian corals · Indonesia · Ecology · Environmental Gradient · Coral Reefs

2.1 INTRODUCTION

The Indonesian Archipelago is central to marine biodiversity, likely consequential of geological and oceanographic processes influencing species diversification and persistence (Carpenter et al. 2011) at local and regional scales. Eastern Indonesian reefs are particularly diverse, with low climatic variability and strong seasonal upwellings, yet ecological assessments are sparse (Edinger et al. 2000, Tomascik et al. 2004). Increases in human population growth, continual marine resource exploitation through coral mining, cyanide, dynamite, and subsistence fisheries mean such biodiverse ecosystems are being destroyed before their components are discovered. Therefore, comparative assessment of coral reef communities relative to their environment, including the increasing assortment of anthropogenic influences, provides an essential resource for conservation management.

Gorgonian corals (Cnidaria: Anthozoa: Octocorallia) are conspicuous, diverse and often dominant components of benthic marine environments, notably tropical shallow reefs, deep-sea, and mesophotic habitats (Wirshing et al. 2005, Cerrano et al. 2010, McFadden et al. 2010a). Numerous gorgonians are conservation 'flagship' species (Tinsley 2005, Linares et al. 2008, Cerrano et al. 2010) being ecologically diverse, long-lived engineering taxa that maintain habitat heterogeneity and provide secondary space to other organisms (Buhl-Mortensen & Mortensen 2004, Buhl-Mortensen et al. 2010). Nevertheless, despite their ecological importance and diversity, the greatest paucity of information on gorgonians continues to exist within the Indonesian Archipelago (Tomascik et al. 2004).

Gorgonian corals are colonial suspension feeders primarily defined by a semi-rigid scleroproteinaceous (gorgonin) axis with varying amounts of calcification (Bayer 1961, Grasshoff 1999, Sánchez et al. 2003). Characteristic of the Octocorallia, their polyps bear eight pinnate tentacles, and eight mesenteries dividing the gastrovascular cavity (Bayer 1961, Berntson 1998). Originally classified under the order Gorgonacea (now taxonomically obsolete), gorgonians currently comprise the somewhat tenuous suborders, Holaxonia and Calcaxonia and the group Scleraxonians within the order Alcyonacea (Bayer 1981). Taxonomic efforts for Indo-Pacific gorgonians are however, confounded by widespread homoplasy, considerable morphological variability, cryptic and sibling taxa (Knowlton 1993). Classified as "poorly known" (van Ofwegen 2004), shallow water gorgonian taxonomy within Central Indonesia remains in a state of flux requiring resolute cross-disciplinary systematic, molecular and ecological approaches.

Gorgonian ecology reflects, together or in part, reproductive strategies and changes along environmental gradients relative to individual species tolerances (Fabricius & Alderslade 2001). Environmental factors such as substrate type, light, temperature, sedimentation, salinity, current regime and flow rate (Garrabou et al. 2001) influence gorgonian demography. Biotic factors further provide local-scale community refinement including competition, predation, symbioses, reproduction, settlement and developmental properties (Sánchez 2004). Such factors have been shown to induce intra- and inter-specific morphological variability (West 1997, 1998, Linares et al. 2008, Prada et al. 2008), habitat selection and colony orientation (Sánchez et al. 2003a). Nevertheless, gorgonians are typically synonymized with areas of low sedimentation and high water flow through strong currents and upwellings (Kinzie 1973, Birkeland 1974, Yoshioka & Yoshioka 1989, Sanchez et al. 1998), the largest planar arborescent colonies occurring in healthy reef environments (Meesters et al. 2001, Linares et al. 2008). Complex habitats provide more vertical relief, colonizable area, and microhabitat variability than soft benthic substrata (Etnoyer et al. 2010). Yet even in the presence of suitable substratum, most gorgonians have been shown to be absent in areas of high turbidity and sediment load likely due to the physical impairment of settlement, feeding, reproduction and growth (Bayer 1956, Anthony & Fabricius 2000). In contrast, high turbidity reefs in Singapore, for example, support healthy azooxanthellate gorgonian communities (Goh & Chou 1994). Reduced irradiance levels may therefore, provide competitive release (Rogers 1990) for azooxanthellate taxa; turbid habitats being marginal for zooxanthellate gorgonians, following similar depth ranges of Scleractinia with no evidence of hard coral community replacement (Fabricius & Alderslade 2001). Moreover, evidence for negative associations with other benthic space competitors appears absent in other areas (e.g., Yoshioka & Yoshioka 1989). However, resource partitioning theory predicts habitat specialists (Schoener 1974), as growth form and resource allocation plasticity counter the effects of environmental factors such as sedimentation (Riegl & Branch 1995) and depth (West 1997).

Colony growth and form are determinate through the iterative addition of polyps, branches, and within colony canalisation providing effective resource allocation structure (Sánchez & Lasker 2003), all of which depend on feeding strategy. Both azooxanthellate and zooxanthellate gorgonians show eco-phenotypic interactions which strongly correlate with depth (West et al. 1993) and size (Sebens 1982). Yet, whether such trophic division is a consequence of interspecific competition in oligotrophic coral reef systems is uncertain. The question remains, are strong interspecific competitive forces driving shallow water gorgonian ecology within the Indonesian archipelago?

Gorgonian distribution has been positively correlated with substrate availability and type (Goh & Chou 1994), localized overlapping of species range sizes (as a function of temperature) and benthic-pelagic coupling (Matsumoto et al. 2007). Yet little is known of both reproductive strategies and relative range sizes for most gorgonian species in the Indo-Pacific, likely having limited dispersal abilities and high endemism (Grasshoff & Bargibant 2001, Picciano & Ferrier-Pagès 2007). Moreover, larval recruitment plays a key role in gorgonian community structure (Yoshioka 1996), with staggered or neap tide spawning events (Benayahu & Loya 1981), planktonic larval displacement by water currents, and chemotaxis through conspecific or coralline algal exudates (Fabricius & Alderslade 2001) further influencing local distribution, abundance and survival.

Prominent drivers of gorgonian ecology, therefore remain unclear, usually describing regional differences (Singapore, Goh & Chou 1994; Caribbean, Sanchez et al. 1997; Guam, Paulay et al. 2003; Hong Kong, Fabricius & McCory 2006; Japan, Matsumoto et al. 2007). However, ecological factors that regulate species diversity, as well as consistency in species nomenclature, are of absolute research and conservation importance especially within the Indonesian archipelago, which is subject to continual overexploitation and habitat loss. Published surveys within Central Indonesia, such as the 'Siboga' (Versluys, 1902, 1906, Nutting 1910a-e, 1911, Stiasny 1937) and 'Snellius' (e.g., Stiasny 1940, Verseveldt 1966) expeditions sampled only deep water and Alcyoniidae taxa respectively, thus largely unrepresentative of shallow water gorgonians on Indonesian reefs. Annual rapid assessment surveys are increasingly conducted by conservation agencies (e.g., WWF, TNC) throughout the Indonesian archipelago, with a view for sustainable conservation management. Such surveys are rudimentary with sparse gorgonian taxonomic resolution. The disparity between gorgonian diversity and ecological assessment within Indonesia is, therefore primarily due to taxonomic uncertainty (Bayer 1981), with concomitant difficulties in field identification (Fabricius & Alderslade 2001) and dispersal patterns. Yet with continual habitat degradation across Indonesian coral reefs, will certain gorgonian species absorb significant magnitudes of such anthropogenic disturbance through succession and subsequent survival?

Little is known of gorgonian ecology within SE Sulawesi, Indonesia despite their high regional abundance and diversity. The aims of this study therefore, were (1) to characterise gorgonian assemblage composition and structure across a gradient of habitat quality within the WMNP, (2) to similarly assess gorgonian diversity and abundance between reef habitats as a function of

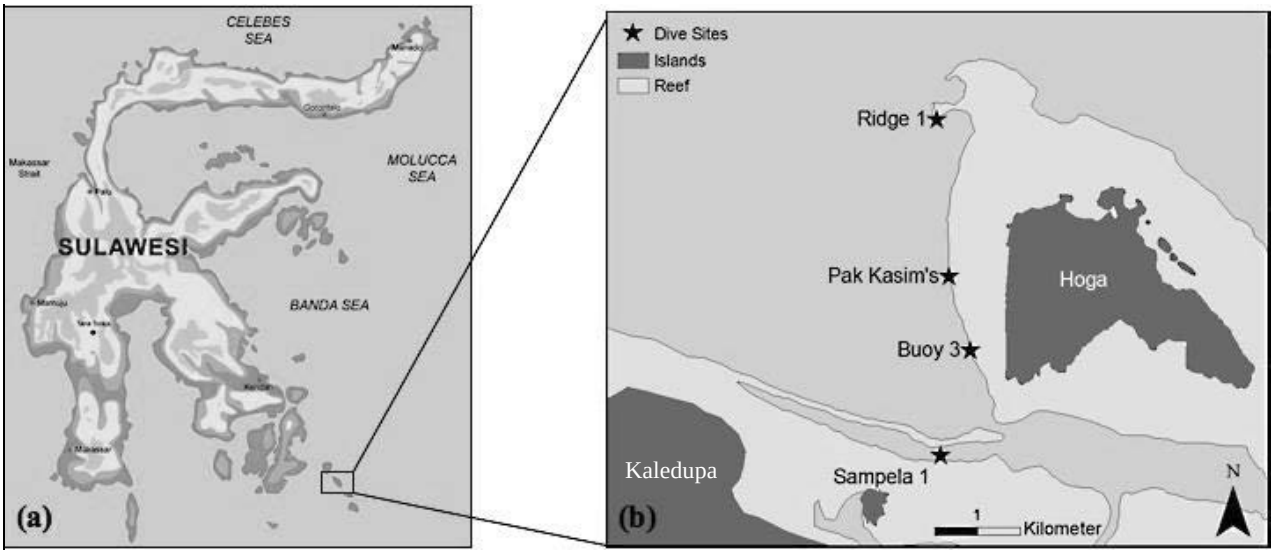


Figure 2.1. (a) Location map of the Wakatobi Marine National Park in S.E. Sulawesi, Indonesia. (b) Areas of study; Sampela, Buoy 3, Pak Kasim's and Ridge 1 off the islands of Kaledupa and Hoga respectively.

depth within each site, and (3) to identify potential environmental driver(s) of gorgonian assemblage structure.

2.2 METHODS

2.2.1 Study Area

The Wakatobi Marine National Park (Tukang Besi Islands) is a remote island group of ca. 13,900 km^2 in S.E. Sulawesi, Indonesia (Figure 2.1a). Established in 1996, the WMNP is the second largest marine park in Indonesia containing ca. 600 km^2 of the most biodiverse coral reefs in the world (Scaps & Denis 2007), with a low incidence of coral disease (0.57% see Haapkylä et al. 2007) and ENSO-induced bleaching events (Crabbe & Smith, 2003) likely due to local upwelling (Gieskes et al. 1988). Approximately 100,000 people live within the Wakatobi, resulting in extensive subsistence marine resource dependence and destructive commercial fisheries in populated areas. In this study four sites were selected around the islands of Kaledupa (ca. 17,000 people) and Hoga (<100 people, Figure 2.1b) relative to their variability in natural and anthropogenic disturbance. Sampela (impacted), an enclosed lagoon with an outer reef wall ca. 400 m from a Bajo (sea gypsy) village of ca. 1600 people, is subject to continuous exploitation through coral mining, fishing activities, and high sediment loading due to natural re-suspension, bioturbation through gleaning, and mangrove loss. Furthermore, community wastewater is continually released onto the reef (Haapkylä et al. 2007). Buoy 3 (intermediate I), ca. 500 m offshore, is a moderately sheltered fringing reef with a shear reef wall containing small cryptic overhang habitats. This site has an extended reef flat, which is subject to perpetual

'gleaning' of marine invertebrates by local inhabitants, in addition to recovering from coral mining and blast fishing since 2004. Pak Kasim's (intermediate II), ca. 500 m offshore, is an intermediate topographically complex fringing reef, also subject to coral mining and blast fishing on the reef flat and crest until 2004. Ridge 1 (healthy), ca. 1 km offshore, is an exposed reef ridge with strong water currents (Figure 2.1b) and upwelling with a small amount of blast fishing on the reef crest in 2004. The reef slope can also be shear, possessing cryptic overhang habitats. All sites have a pronounced reef flat (< 3 m [Ridge 1 being the exception as an offshore ridge, yet still having a shallow reef plateau ca. 3 m depth]), reef crest (3 - 6 m) and slope (> 6 m) with varying levels of sedimentation draining from the reef flats during spring tides.

2.2.2 Sample Collection

Gorgonian distribution and abundance. Gorgonian assemblage surveys were conducted between June and September 2009 using SCUBA, snorkeling and scaled digital image photography. Four 10 m belt (2 m either side) transects, laid ca. 20 m apart, ran parallel to the reef contour at each reef habitat (flat ≤ 3 m, crest ca. 6 m and slope ca. 12 m depth) within each site giving a total area surveyed of 1920 m^2. Individual colonies encountered along each transect, including beneath canopy structures (see Goatley & Bellwood 2011), were photographed using a Canon IXUS 900Ti, WP-DC7 u/w housing and INON UWL-105 AD x 0.51 lens. Each image was taken directly opposite and/or above each colony relative to colony morphology with a ruler aligned appropriately for scale. Voucher specimens (2 - 8 cm in length) were preserved in 96% EtOH for taxonomic clarification and stored at the Bernice P. Bishop Museum, Honolulu, USA (Accession number: 2014.005). Sclerites were dissolved from the surrounding tissue in 5% sodium hypochlorite solution and visualized using optical microscopy. Taxonomic identification followed Versluys (1902, 1906), Nutting (1910a-e, 1911), Stiasny (1937, 1940), Aurivillius (1931), Verseveldt (1966), van Ofwegen (1987), Grasshoff (1996, 1999), Grasshoff & Bargibant (2001), Fabricius & Alderslade (2001), and Bayer & Cairns (2004) with most colonies being identified to 'morphotypes' within genera due to the majority of gorgonian species within the Indo-Pacific being undescribed. However, individuals were grouped in accordance with Bayer's (1981) widely accepted three-group (suborders Holaxonia and Calcaxonia, and Scleraxonians group) system, family and genera therein.

Environmental Variables. Sites were characterized through the assessment of 14 (Table 2.1) environmental variables throughout the study period. Benthic characteristics were determined using transects as described for gorgonian surveys, and categorized according to English et al.

Table 2.1. Environmental characteristics of the four study sites in the Wakatobi Marine National Park, Indonesia. All values expressed as mean (± SE) with the exception of diurnal temperature range (˚C), light ($K_{d(PAR)}$) and sediment grain size (Φ). Abiotic: rock, rubble and sand; biotic: sponges, ascidians, algae (English et al. 1997).

Parameter Recorded	Mean value ± SE (where appropriate)			
Site	Sampela	Buoy 3	Pak Kasim's	Ridge 1
Latitude (S)	005˚ 29'01"	005˚ 28'38"	005˚ 27'57"	005˚ 26'57"
Longitude (E)	123˚45'08"	123˚45'47"	123˚45'18"	123˚45'38"
Temperature (˚C min-max)	25.61 – 29.36	24.69 – 29.25	26.59 – 30.457	24.06 – 28.07
Light ($K_{d(PAR)}$ min-max)	0.31 – 3.14	0.27 – 1.96	0.16 – 2.55	0.1 – 1.56
Salinity (PSU)	32.5 ± 0.45	33 ± 0.08	32.8 ± 0.52	32.6 ± 0.26
Flow (cm/s)	5.02 ± 2.18	4.17 ± 1.35	11.22 ± 2.55	30.54 ± 2.61
Chlorophyll-*a* (µg L^{1})	0.3 ± 0.01	0.27 ± 0.03	0.14 ± 0.01	0.35 ± 0.03
Turbidity (NTU)	4.38 ± 1.80	1.04 ± 0.53	0.54. ± 0.72	0.17 ± 0.33
Sedimentation (g d^{-1}, n = 12)	3.28 ± 0.26	1.52 ± 0.2	1.23 ± 0.13	1.16 ± 0.07
Sediment grain size (Φ, n = 12)	5 [31.25–62.5 µm]	1 [0.5–1 mm]	1 [0.5–1 mm]	1 [0.5–1 mm]
Rugosity Index (n = 12)	0.82 ± 0.04	0.79 ± 0.7	0.71 ± 0.03	0.61 ± 0.03
Hard Coral (%, n = 12)	5.33 ± 2.04	57.23 ± 4.6	36.72 ± 5.11	40.12 ± 3.1
Dead Coral/Rubble (%, n = 12)	38.34 ± 7.1	10.81 ± 3.61	12.21 ± 3.2	6.96 ± 1.27
Soft Coral (%, n = 12)	3.88 ± 1.42	9.84 ± 2.91	30.14 ± 4.85	38.98 ± 3.83
Biotic (%, n = 12)	4.31 ± 1.21	13.12 ± 4.43	4.26 ± 1.65	6.99 ± 1.44
Abiotic (%, n = 12)	48.14 ± 6.3	9.0 ± 3.13	16.67 ± 4.26	6.95 ± 1.9

(1997) utilizing the point (every 0.5 m) intercept transect method (Kingsford & Battershill 1998). Values are expressed as % cover (± SE). Rugosity (quantification of habitat complexity) was measured with a 7.30 m length chain laid over three replicate transects per habitat and calculated using the ratio of contoured surface distance to linear distance method (McCormick 1994).

Suspended sedimentation rates were assessed using four standard 1.0 litre sediment traps (English et al. 1997) deployed at each habitat within all sites for a 10 day period. Sediment and water were filtered (Whatman 0.2 µm pore size), dried at 60°C and weighed with rates expressed as g dry weight day^{-1}. Estimation of sediment grain diameter for all samples was determined using Retsch Technology® test sieves (aperture size range: 2.0, 1, 0.5, 0.125, 0.25, 0.063, <0.063 mm), logarithmically converted, expressed as *phi* (Φ) and classified under the Wentworth scale (Wentworth 1922). Water flow velocity was measured using a General Oceanics® flow meter with a low velocity rotor and custom made aluminum pipes for reef

placement and expressed as cm/s. Chlorophyll-*a* (as µg L^{-1}), salinity (PSU) and turbidity (NTU) were measured using RBR® XR-420 CTD data loggers. Temperature (˚C) and light ($K_{d(PAR)}$) were measured using HOBO® data loggers. The loggers were placed at each transect depth, recording every minute for up to 24 hours. Latitude and longitude were determined by a hand-held GPS meter (GARMIN eTrex®). All variables, with the exception of latitude and longitude, were entered into the statistical models as raw values. Values were edited visually with significant outliers removed.

2.2.3 Data Analyses

Data were analyzed using univariate (SPSS v18.0) and multivariate routines in the PRIMER-E v6.1.12 statistical package (Clarke & Gorley 2006), with PERMANOVA+ v1.02 extension (Anderson 2001). The first hypothesis was to characterise gorgonian diversity, abundance and distribution patterns across gradients of habitat quality within the WMNP. Gorgonian assemblage data were dispersion-weighted, a transformation procedure that accounts for the variance structure of individual species (Clarke et al. 2006). Differences in gorgonian assemblages were analysed according to a two-factor (site and habitat) crossed model with pairwise comparisons using 9999 permutations (PERMANOVA; Anderson 2001) based on a 'zero-adjusted' Bray-Curtis similarity matrix (Clarke et al. 2006b). Results were visualized using non-metric multidimensional scaling (nMDS) ordination comparable with a constrained canonical analysis of principal coordinates (CAP; Anderson & Willis 2003). Such comparisons reveal real group differences to the maximum variation between groups. The second hypothesis was to identify biologically successful gorgonian species across such environmental clines. In order to test this second hypothesis; prominent taxa contributing to dissimilarities among gorgonian assemblages were investigated using similarity percentages (SIMPER; Clarke 1993). The influence of dominant species revealed from the SIMPER analyses was further investigated using Pearson's product-moment correlations for each species with each canonical axes (Anderson & Willis 2003) and displayed as a vector overlay on CAP ordinations. Species diversity indices were used across sites and habitats including total number of species (S), the Hill numbers N1, N2 and modified ratio N21' (Peet 1974) to assess the influence of rare and dominant species, and taxonomic spread (equitability) respectively (Clarke & Gorley 2006). Zooxanthellate and azooxanthellate gorgonian distributions were tested for independence using the Wald–Wolfowitz (runs) test (SPSS v18.0; Wald & Wolfowitz 1943).

The final hypothesis was to identify predictor environmental variable(s) inferred to influence the ecological structure of gorgonian assemblages. This was investigated using the distance-based

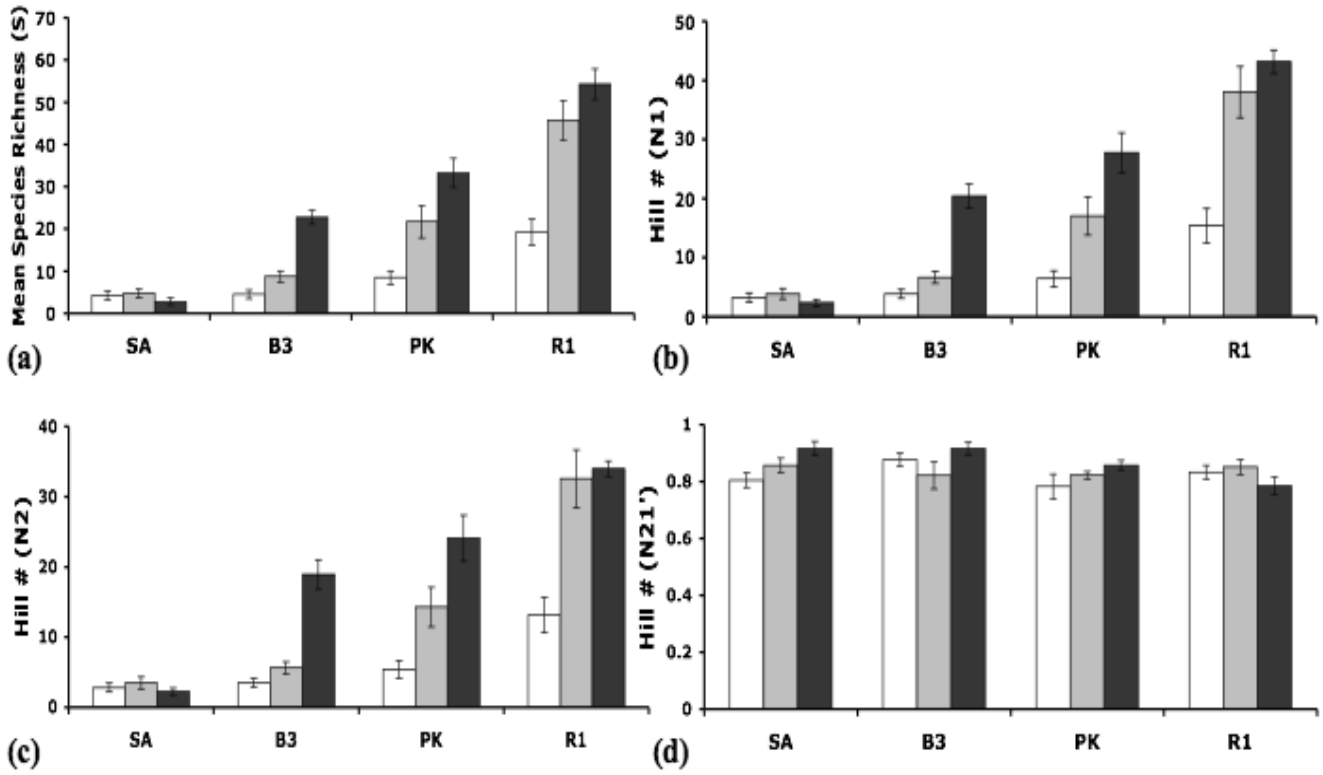


Figure 2.2. Gorgonian species richness (a), Hill's diversity indices N1 (b), N2 (c) and modified ratio for evenness N21' (d) (mean ± SE) across sites and habitats. Sa, Sampela; B3, Buoy 3; PK, Pak Kasim's; R1, Ridge 1. White bars, reef flat; grey bars, reef crest; black bars, reef slope.

forward selection analysis of linear models (DISTLM*forward*; McArdle & Anderson 2001) based on a Euclidean distance matrix. Variables were normalized and conditionally tested using 9999 permutations of the residuals under a reduced model (Anderson 2001). Results were visualized using the distance-based redundancy analysis ordination (dbRDA; McArdle and Anderson 2001).

2.3 RESULTS

2.3.1 Gorgonian Distribution and Abundance

A total of 3449 gorgonian colonies were documented in this study; 126, 445, 1165 and 1713 recorded at Sampela (impacted), Buoy 3 (intermediate I), Pak Kasim's (intermediate II) and Ridge 1 (healthy) respectively (Figure 2.2; Table 2.2). At present 197 gorgonian species and morphotypes from 37 genera, and 12 families within the suborders/group Calcaxonia, Holaxonia and Scleraxonia have been identified (Table 2.2). This list comprises 21 new species for the region, 28 new species records and another 115 as yet unidentified. Species richness, and diversity followed a typical pattern of increase from the impacted site Sampela to the pristine site Ridge 1, with 7 species at Sampela, 70 species at Buoy3, 80 species at Pak Kasim's and 130

Table 2.2. Gorgonian species inventory and abundance recorded in this study across sites in the WMNP. Z & AZ, zooxanthellate and azooxanthellate taxa as classified for statistical analyses. NS, new species; NR, new record for the area; NT, not present on transects but specimen collected; asterisk indicates species status or record unclear requiring further investigation.

Taxon	Z/AZ	NR/NS/ NT	Sampela	Buoy 3	Pak Kasim's	Ridge 1
[Group: Scleraxonians]						
Family: Anthothelidae Broch 1916						
Iciligorgia cf. *schrammi* Douchassaing 1870	AZ	*NT	-	-	-	-
Iciligorgia sp.1	AZ	*NT	-	-	-	-
Solenocaulon sp.1	AZ	*NT	-	-	-	-
Solenocaulon sp.2	AZ	*NT	-	-	-	-
Family: Briareidae Gray 1859						
Briareum excavatum Nutting 1911	Z	-	10	54	168	159
Briareum stechei Kükenthal 1908	Z	-	3	19	35	325
Briareum violaceum Roule 1908	Z	-	4	1	-	15
Family: Melithaeidae Gray 1870						
Acabaria cinquemiglia Grasshoff 1999	AZ	NR	-	2	1	1
Acabaria variabilis Hickson 1905	AZ	-	-	-	2	-
Acabaria sp.1	AZ	*	-	1	-	-
Acabaria sp.2	AZ	*	-	-	-	1
Acabaria sp.3 n.sp.	AZ	NS	-	-	-	2
Acabaria sp.4	AZ	*	-	-	1	8
Acabaria sp.5	AZ	*	-	-	-	6
Acabaria sp.6	AZ	*	-	-	-	2
Acabaria sp.7	AZ	*NT	-	-	-	-
Acabaria sp.8	AZ	*	-	-	-	1
Acabaria sp.9 n.sp.	AZ	NS	-	-	-	58
Acabaria sp.10 n.sp.	AZ	NS	-	-	1	5
Acabaria sp.11	AZ	*	-	-	-	1
Acabaria sp.12 n.sp.	AZ	NS	-	-	-	1
Acabaria sp.13	AZ	*	-	-	-	1
Acabaria sp.14	AZ	*	-	-	-	1
Acabaria sp.15	AZ	*	-	-	-	2
Acabaria sp.16 n.sp.	AZ	NS	-	-	-	2
Acabaria sp.17	AZ	*	-	-	-	1
Acabaria sp.18	AZ	*	-	4	4	12
Acabaria sp.18 n.sp.	AZ	NS	-	-	6	5
Acabaria sp.19	AZ	*	-	2	-	2
Acabaria sp.20	AZ	*	-	2	-	7
Acabaria sp.21	AZ	*	-	-	-	1
Acabaria sp.22	AZ	*	-	1	-	-
Acabaria sp.23	AZ	*	-	10	-	13
Acabaria sp.24 n.sp.	AZ	NS	-	1	-	-
Acabaria sp.25	AZ	*	-	-	-	1
Acabaria sp.26	AZ	*	-	-	-	4
Mopsella singularis Thomson 1916	AZ	-	-	-	-	8
Mopsella sp.1	AZ	*NT	-	-	-	-
Mopsella sp.2	AZ	*	-	-	-	1
Mopsella sp.3	AZ	*	-	-	-	4
Melithaea ochracea Linnaeus 1758	AZ	-	1	-	-	-
Melithaea squamata Nutting 1911	AZ	-	-	2	-	5
Melithaea sp.1	AZ	*	-	-	-	6
Melithaea sp.2	AZ	*	-	-	-	7
Melithaea sp.3	AZ	*	-	1	-	18

Taxon	Z/AZ	NR/NS/ NT	Sampela	Buoy 3	Pak Kasim's	Ridge 1
Melithaea sp.4	AZ	*	-	-	-	2
Melithaea sp.5	AZ	*	-	1	-	1
Melithaea sp.6	AZ	*	-	-	-	3
Melithaea sp.7	AZ	*NT	-	-	-	-
Melithaea sp.8	AZ	*	-	-	-	3
Melithaea sp.9	AZ	*	-	-	-	3
Family: Parisididae Aurivillius 1931						
Parisis sp.1	AZ	*	-	-	-	1
Family: Subergorgiidae Gray 1859						
Annella mollis Nutting 1910	AZ	-	-	-	-	3
Annella reticulata Ellis & Solander 1736	AZ	-	-	2	10	14
Annella sp.1 n.sp.	AZ	NS	-	9	-	17
Annella sp.2 n.sp.	AZ	NS	-	-	4	5
Subergorgia rubra Gray 1857	AZ	NT	-	-	-	-
Subergorgia suberosa Pallas 1766	AZ	NT	-	-	-	-
Subergorgia sp.1 n.sp.	AZ	NS/NT	-	-	-	-
[Suborder: Holaxonians]						
Family: Keroeididae Kinshita 1910						
Keroeides cf. *gracilis* Whitelegge 1897	AZ	*	-	1	-	-
Family: Gorgoniidae Lamouroux 1812						
Guaiagorgia sp.1	AZ	*NR/NT	-	-	-	-
Hicksonella princeps Nutting 1910	Z	*	-	-	-	1
Pinnigorgia sp.1	Z	*	-	-	2	1
Pseudopterogorgia sp.1	AZ	*	-	-	-	1
Rumphella aggregata Nutting 1910	Z	-	3	-	1	5
Rumphella antipathes Linnaeus 1758	Z	NR	-	-	-	1
Rumphella sp.1	Z	*	-	-	2	-
Family: Acanthogorgiidae Gray 1859						
Acanthogorgia cf. *isoyxa* Grasshoff 1999	AZ	NR	-	2	-	6
Acanthogorgia spinosa Hiles 1899	AZ	-	-	1	6	9
Acanthogorgia sp.1 n.sp.	AZ	NS	-	1	-	-
Acanthogorgia sp.2 n.sp.	AZ	NS	-	82	7	5
Acanthogorgia sp.3	AZ	*	-	-	-	1
Acanthogorgia sp.4	AZ	*	-	3	12	9
Acanthogorgia sp.5	AZ	*	-	1	14	8
Acanthogorgia sp.6 n.sp.	AZ	NS	-	-	3	12
Acanthogorgia sp.7	AZ	*	-	1	1	1
Acanthogorgia sp.8	AZ	*	-	-	1	-
Acanthogorgia sp.9	AZ	*	-	-	-	1
Acanthogorgia sp.10	AZ	*	-	-	1	1
Acanthogorgia sp.11	AZ	*	-	-	1	2
Anthogorgia sp.1	AZ	*NT	-	-	-	-
Muricella sp.1	AZ	*	-	2	2	-
Muricella sp.2	AZ	*NT	-	-	-	-
Family: Plexauridae Gray 1859						
Acanthomuricea sp.1	AZ	*	-	1	1	-
Astrogorgia bayeri van Ofwegen & Hoeksema 2001	AZ	NR/NT	-	-	-	-
Astrogorgia canala Grasshoff 1999	AZ	NR	-	-	6	11
Astrogorgia dumbea Grasshoff 1999	AZ	NR	-	-	1	3
Astrogorgia cf. *arborea* Thomson & Simpson 1909	AZ	*NT	-	-	-	-
Astrogorgia sp.1	AZ	*NT	-	-	-	-
Astrogorgia sp.2	AZ	*	-	1	1	-
Astrogorgia sp.3	AZ	*	-	-	-	4
Astrogorgia sp.6 n.sp.	AZ	NS	-	15	135	96
Astrogorgia sp.7	AZ	*	-	-	13	11
*Astrogorgi*a sp.8 n.sp.	AZ	NS	-	-	2	7
*Astrogorgi*a sp.9 n.sp.	AZ	NS	-	3	-	9

Taxon	Z/AZ	NR/NS/ NT	Sampela	Buoy 3	Pak Kasim's	Ridge 1
Astrogorgia sp.10	AZ	*	-	5	9	29
Astrogorgia sp.11	AZ	*	-	-	3	10
Astrogorgia sp.12	AZ	*	-	-	-	1
Astrogorgia sp.13	AZ	*NT	-	-	-	-
Astrogorgia sp.13	AZ	*	-	-	8	23
Astrogorgia sp.14	AZ	*NT	-	-	-	-
Astrogorgia sp.15 n.sp.	AZ	NS	-	-	1	6
Astrogorgia sp.16	AZ	*NT	-	-	-	-
Astrogorgia sp.17	AZ	*	-	1	24	14
Astrogorgia sp.18	AZ	*	-	20	19	43
Astrogorgia sp.19	AZ	*	1	-	-	-
Astrogorgia sp.20	AZ	*	-	1	19	22
Astrogorgia sp.21	AZ	*	-	-	4	1
Astrogorgia sp.22	AZ	*	-	-	1	1
Astrogorgia sp.23 n.sp.	AZ	NS	-	3	5	11
Bebryce hicksoni Thomson & Henderson 1905	AZ	-	-	3	1	3
Bebryce cf. *indica* Thomson 1905	AZ	*	-	6	16	59
Bebryce thomsoni Nutting 1910	AZ	-	-	-	1	3
Bebryce sp.1	AZ	*NT	-	-	-	-
Bebryce sp.2	AZ	*NT	-	-	-	-
Bebryce sp.3	AZ	*NT	-	-	-	-
Echinogorgia furfuracea Esper 1791	AZ	NR	-	-	-	2
Echinogorgia cf. *furfuracea* Esper 1791	AZ	*	-	1	3	3
Echinogorgia pseudosassapo Kölliker 1865	AZ	NR	-	-	1	-
Echinogorgia sp.1 n.sp.	AZ	NS	-	-	1	-
Echinogorgia sp.2. n.sp	AZ	NS	-	1	-	-
Echinogorgia sp.3 n.sp.	AZ	NS	-	-	-	1
*Echinogorgi*a sp.4	AZ	*	-	1	2	3
*Echinogorgi*a sp.5	AZ	*	-	-	-	1
*Echinogorgi*a sp.6	AZ	*NT	-	-	-	-
*Echinogorgi*a sp.7	AZ	*NT	-	-	-	-
*Echinogorgi*a sp.8	AZ	*	-	-	-	1
Paracis rigida Thomson & Simpson 1909	AZ	NR	-	-	2	-
Paracis sp.1 n.sp	AZ	NS	-	-	-	1
Paracis sp.2	AZ	*NT	-	-	-	-
Paracis sp.3	AZ	*	-	-	1	1
Echinomuricea cf. *coronalis* Germanos 1896	AZ	NR	-	1	-	-
Echinomuricea indomalaccensis Ridley 1884	AZ	-	-	2	8	7
Echinomuricea ochracea Thomson & Simpson 1909	AZ	NR	-	1	-	-
Echinomuricea pulchra Nutting 1910	AZ	NR	-	-	2	2
Echinomuricea splendens Thomson & Simpson 1909	AZ	-	-	-	5	1
Echinomuricea sp.1 n.sp.	AZ	NS	-	-	-	1
Echinomuricea sp.2 n.sp.	AZ	NS/NT	-	-	-	-
Echinomuricea sp.3	AZ	*	-	-	6	13
Echinomuricea sp.4	AZ	*	-	-	-	1
Euplexaura rhipidalis Studer 1895	AZ	NR	-	1	-	4
Euplexaura sp.1	AZ	*	-	1	3	2
Euplexaura sp.2	AZ	*	-	-	-	1
Euplexaura sp.3 n.sp.	AZ	NS	-	2	1	-
Euplexaura sp.4	AZ	*	-	-	-	1
Euplexaura sp.5	AZ	*	-	-	2	-
Euplexaura sp.6	AZ	*NT	-	-	-	-
Euplexaura sp.7	AZ	*	-	-	1	-
Euplexaura sp.8	AZ	*	-	-	-	1
Euplexaura sp.9	AZ	*	-	-	2	-
Euplexaura sp.10	AZ	*	-	1	1	1

Taxon	Z/AZ	NR/NS/ NT	Sampela	Buoy 3	Pak Kasim's	Ridge 1
Menella indica Ridley 1888	AZ	NR	-	1	-	-
Menella lenzii Studer 1895	AZ	NR	-	1	3	11
Menella praelonga Ridley 1884	AZ	-	-	1	1	
Menella spinifera Kükenthal 1911	AZ	-	-	1	6	13
Menella sp.1	AZ	*	-	1	4	2
Menella sp.2	AZ	*	-	1	-	3
Menella sp.3	AZ	*	-	-	-	3
Trimuricea sp.1 n.sp.	AZ	NS/NT	-	-	-	-
Trimuricea sp.2 n.sp.	AZ	NS	-	-	1	-
Paraplexaura cf. *cimenia* Grasshoff 1999	AZ	NR	-	-	-	1
Paraplexaura sp.1	AZ	*	-	4	4	-
Paraplexaura sp.2	AZ	*	-	1	4	1
Paraplexaura sp.3	AZ	*	-	-	6	19
Paraplexaura sp.4	AZ	*	-	1	-	-
Villogorgia cf. *citrina* Grasshoff 1999	AZ	*NR	-	1	-	7
Villogorgia rubra Nutting 1910	AZ	-	-	-	1	2
Villogorgia sp.1 n.sp.	AZ	NS	-	2	1	9
Villogorgia sp.2	AZ	*	-	-	1	3
Villogorgia sp.3	AZ	*	-	-	-	3
Villogorgia sp.4	AZ	*NT	-	-	-	-
Villogorgia sp.5	AZ	*NT	-	-	-	-
[Suborder: Calcaxonians]		-	-			
Family: Ellisellidae Gray 1859						
Ctenocella sp.1	AZ	*NT	-	-	-	-
Ellisella ceratophyta Linnaeus 1758	AZ	-	-	1	-	16
Ellisella plexauroides Toeplitz 1919	AZ	-	-	-	-	8
Ellisella sp.1	AZ	*	-	2	-	5
Ellisella sp.2	AZ	*	-	1	1	5
Ellisella sp.3	AZ	*	-	-	2	1
Dichotella gemmacea Milne Edwards & Haime 1857	AZ	*	-	-	6	3
Heliania sp.1	AZ	*NR/NT	-	-	-	-
Junceella fragilis Ridley 1884	AZ	-	-	2	-	16
Junceella cf. *juncea* Pallas 1766	AZ	*	-	-	-	1
Nicella sp.1	AZ	*NT	-	-	-	-
Verrucella cf. cerasina Grasshoff 1999	AZ	*NR	-	2	1	5
Verrucella cf. rubra Nutting 1910	AZ	*NR/NT	-	-	-	-
Verrucella sp.1	AZ	*	-	4	3	3
Verrucella sp.2	AZ	*NT	-	-	-	-
Viminella sp.1	AZ	*	-	-	-	1
Family: Ifalukellidae Bayer 1955						
Ifalukella yanii Bayer 1955	Z	NT	-	-	-	-
Plumigorgia hydroides Nutting1910	Z	NT	-	-	-	-
Plumigorgia schuboti Alderslade 1986	Z	NT	-	1	-	-
Family: Isididae Lamouroux 1812						
Isis hippuris[N] Linnaeus 1758	Z	-	41	103	413	278
Isis hippuris[LT] Linnaeus 1758	Z	-	59	7	71	49
Isis hippuris[S] Linnaeus 1758	Z	-	4	20	26	15
Zignisis sp.1	AZ	*NR	-	1	-	-
Unidentified	AZ	*	-	5	10	7
Unidentified, Holaxonian	AZ	*NT	-	-	-	-
Unidentified, Holaxonian, Plexauridae	AZ	*	-	4	4	6
Total # Species:			126	445	1165	1713

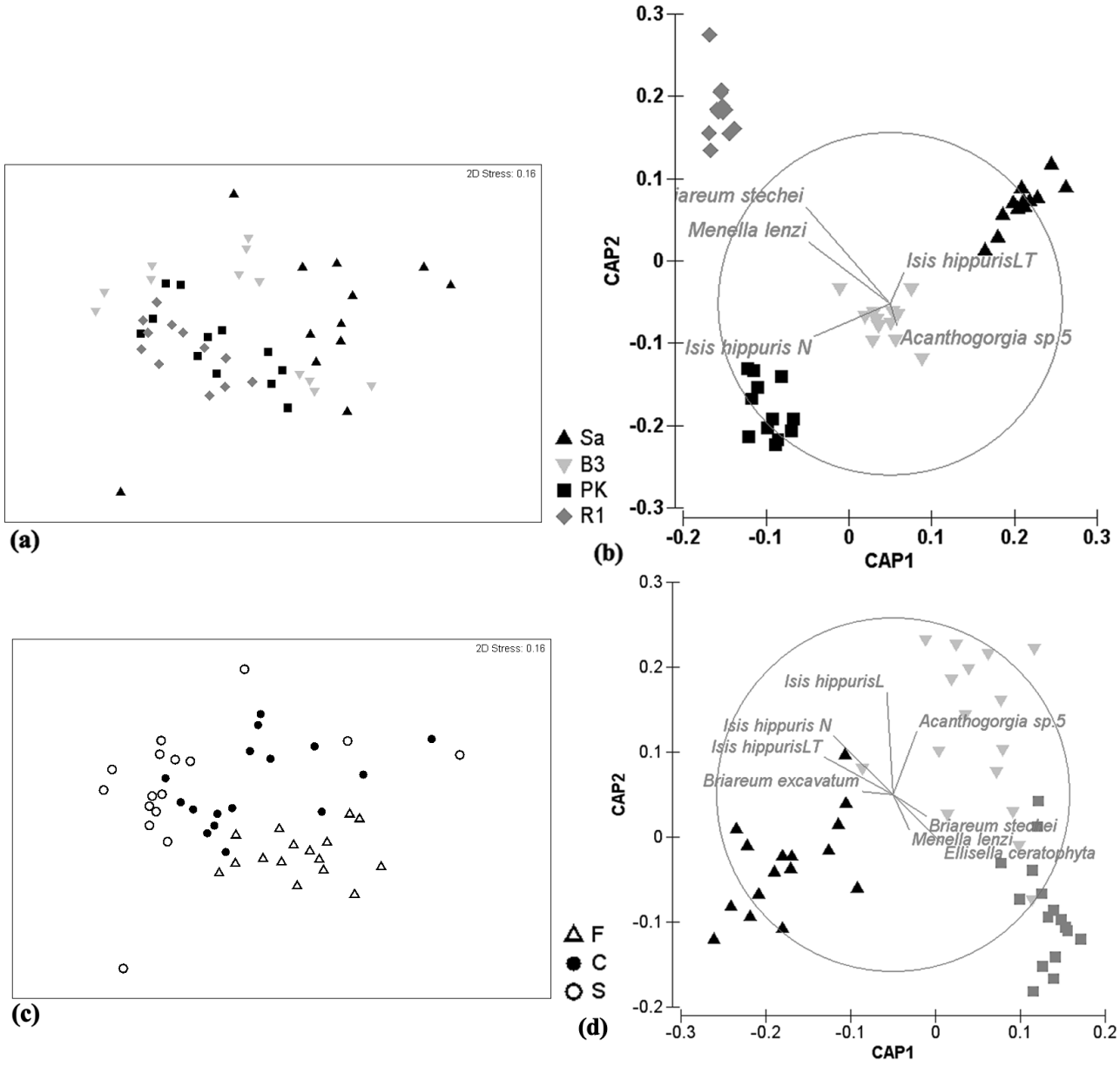


Figure 2.3. (a, c) Constrained and (b, d) unconstrained ordinations of gorgonian assemblages between sites (a, b) and habitats (c, d). Sa, Sampela; B3, Buoy 3; PK, Pak Kasim's; R1, Ridge 1. F, reef flat; C, reef crest; S, reef slope.

species at Ridge 1 (Figure 2.2; Table 2.2). This pattern of increased species richness and diversity was similarly replicated with depth, the inverse evident in Sampela with the majority of colonies and species on the reef crest and flat (Figure 2.2).

PERMANOVA results revealed that differences in gorgonian abundance across all sites and habitats were significant with no interaction effects (pseudo-F = 7.938, P < 0.0001; pseudo-F = 6.714, P < 0.0001). Pair wise comparisons revealed significant differences were between all

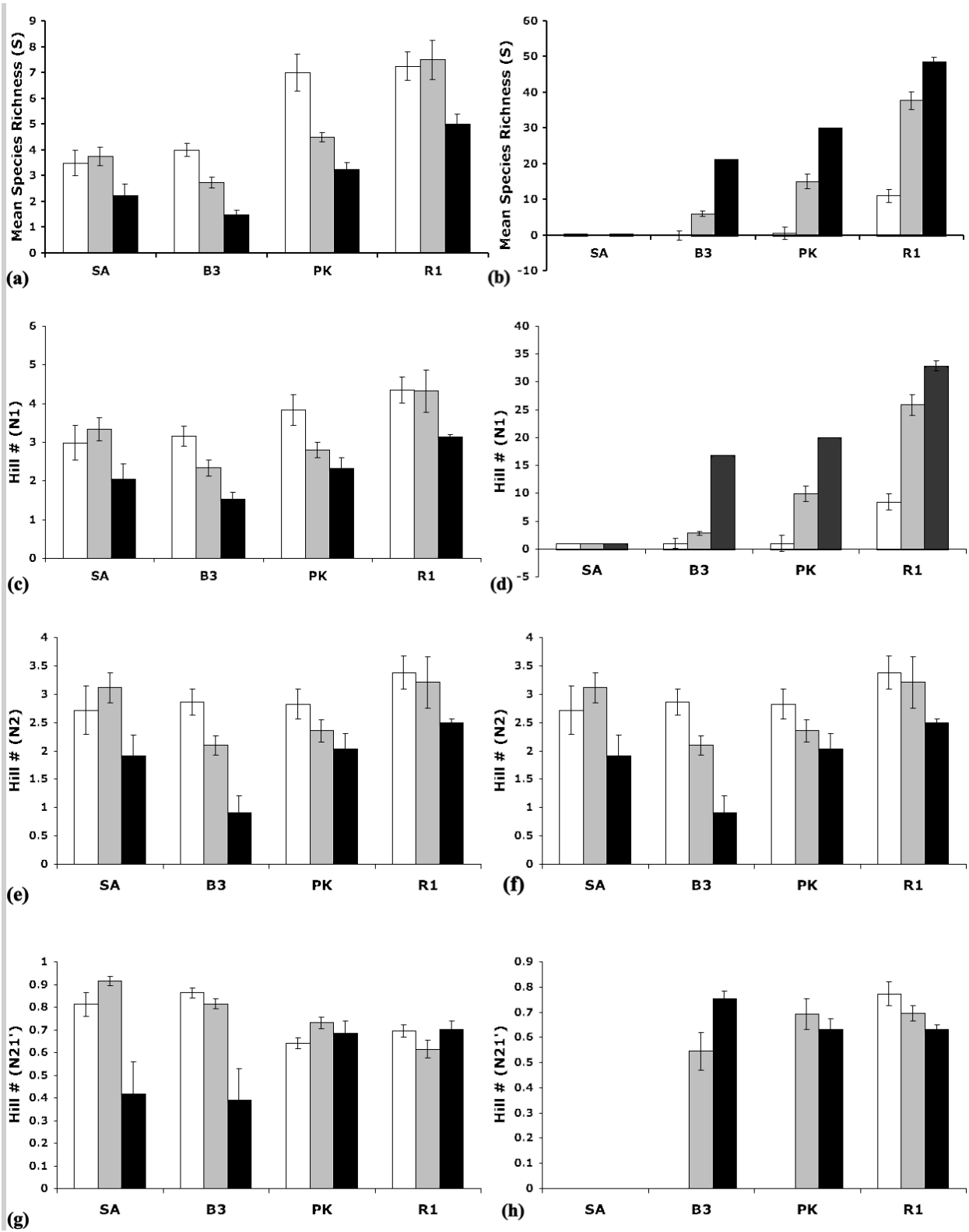


Figure 2.4. Zooxanthellate (a, c, e, g) and azooxanthellate gorgonian species richness (a b), Hill's diversity indices N1 (c, d), N2 (e, f) and modified ratio for evenness N21' (g, h) (mean ± SE) across sites and habitats. Sa, Sampela; B3, Buoy 3; PK, Pak Kasim's; R1, Ridge 1 within the WMNP. White bars, reef flat; grey bars, reef crest; black bars, reef slope.

sites and habitats, most notably Sampela and Ridge 1, and the reef flat and slope respectively (Figure 2.3). CAP analyses were consistent with these results, where strong allocation success clearly defined distinct assemblage variability between sites and habitats (Figure 2.3; Table 2.2). SIMPER further revealed that 'morphotypes' within the zooxanthellate taxa *Isis hippuris* [LT,N] Linnaeus 1758 and *Briareum excavatum* Nutting 1911 accounted most for differences between both site and habitat gorgonian assemblages (Table 2.4). *I. hippuris* colonies with long thick branches were prevalent on the reef flat at Sampela (Figure 2.3b, d, Table 2.4), whereas low-lying branching *Briareum* species more abundant towards the reef slope. In addition, the azooxanthellate *Acanthogorgia* sp.5 contributed considerably towards the difference between the reef crest and flat (Figure 2.3d, Table 2.4). This was due to its exclusive and abundant presence on the ceilings of caves and overhangs, characteristic of Buoy 3.

Zooxanthellate *versus* Azooxanthellate Gorgonians. The dominance of the zooxanthellate gorgonians *I. hippuris* (1094) and *Briareum* spp. (792) obscured distribution patterns of azooxanthellate taxa (Figure 2.3). To highlight indicative distribution patterns and potential interactions between zooxanthellate and azooxanthellate gorgonian assemblages and their environment, tests of 1) diversity and richness; 2) independence; 3) separate assemblage structure, and 4) environmental driver(s) were performed.

A total of 1900 zooxanthellate and 1517 azooxanthellate gorgonian colonies were surveyed across reefs within the WMNP. Calcaxonians, holaxonians, as well as scleraxonians were represented by both zooxanthellate and azooxanthellate taxa with 6 genera belonging to 4 families, and 31 genera belonging to 9 families respectively. Taxonomic richness and diversity for azooxanthellate species largely replicated that of figure 2.2 – all taxa (Figure 2.4b, d, f, h); increasing towards Ridge 1 and with depth. Zooxanthellate taxonomic richness and diversity also increased with site, however showed an inverse relationship with depth, being greatest at the reef crest and flat (Figure 2.4a, c, e, g).

Results from a Wald–Wolfowitz (runs) test revealed the distributions of zooxanthellate and azooxanthellate taxa were non-random, rejecting the null hypothesis of independence ($P < 0.001$). The relative abundance of both zooxanthellate and azooxanthellate taxa differed significantly across sites (PERMANOVA, pseudo-$F = 9.476$, $P < 0.0001$ and pseudo-$F = 3.997$; $P < 0.0001$, respectively) and habitats (PERMANOVA, pseudo-$F = 7.716$, $P < 0.0001$ and pseudo-$F = 4.687$, $P < 0.0001$, respectively). Yet an interaction effect (pseudo-$F = 1.925$; $P = 0.012$) between sites and habitats for azooxanthellate taxa revealed that significance levels were

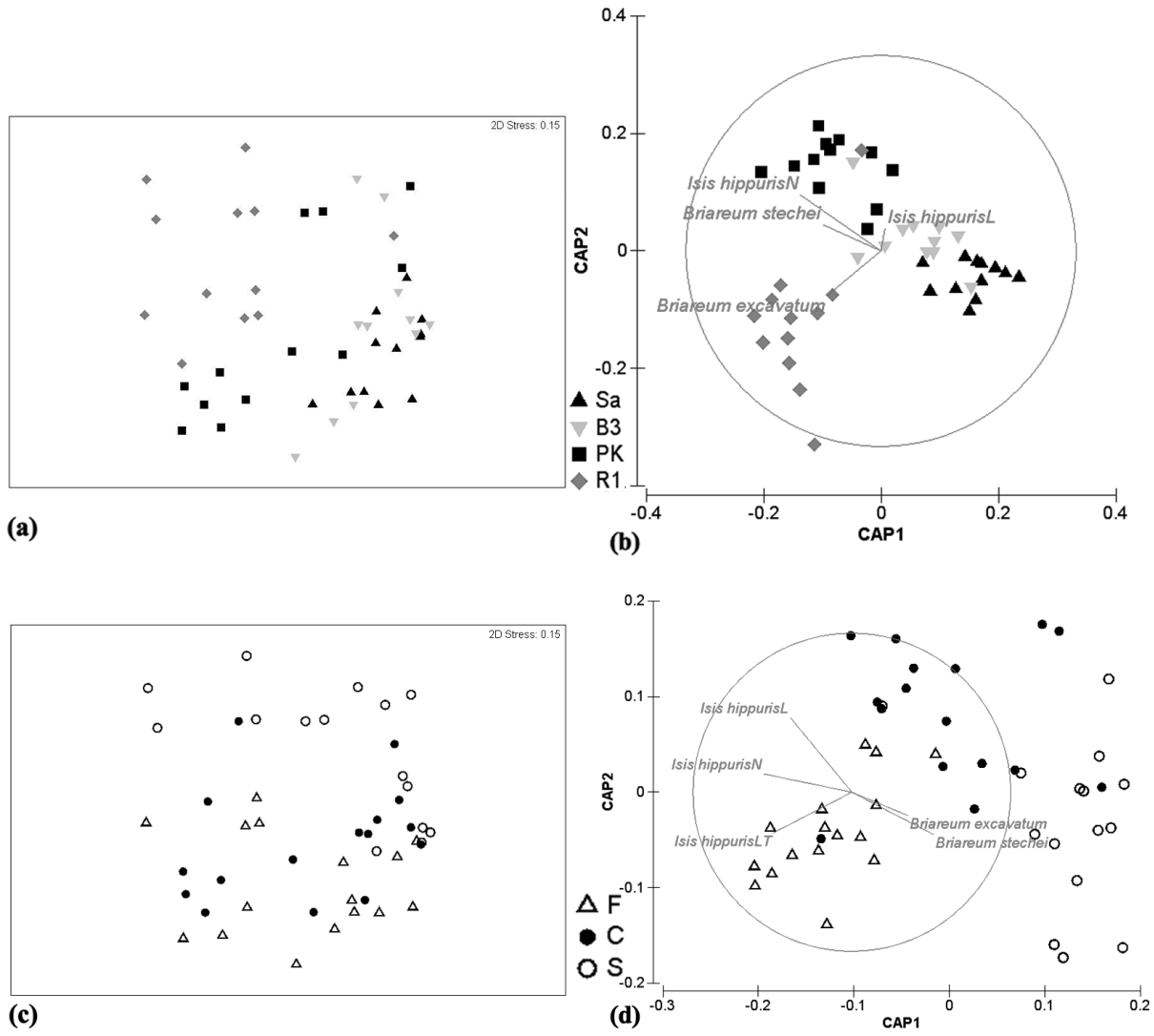


Figure 2.5. (a, c) Constrained and (b, d) unconstrained ordinations of zooxanthellate gorgonian assemblages between sites (a, b) and habitats (c, d). Species vectors are directed where the species were best represented. See Figure 2.3 for factor level codes.

principally driven by zooxanthellate gorgonians. Results were further supported by CAP analyses; allocation success (number of correct allocations to each factor level) was weaker for azooxanthellate taxa at Pak Kasim's, Buoy 3, and the reef crest (Table 2.2). CAP and SIMPER analyses confirmed previous results of *I. hippuris*[LT] on the reef flats at Sampela, *I. hippuris*[N] and *I. hippuris*[L] towards the reef crest (Figure 2.5, Table 2.5 & 6). *Briareum* spp. followed a typical pattern of encrusting on the reef flats at Ridge 1, with low-lying branching colonies characterizing the reef crest and slope, a pattern particularly replicated at Pak Kasim's (Figure 2.5, Table 2.5). It is notable that *Briareum* spp. and *I. hippuris* colonies altered in colouration (magenta to brown or grey and mustard-yellow to beige respectively) at depth and areas of high turbidity.

Table 2.3. CAP analyses results assessing gorgonian species assemblages for all (All spp.), zooxanthellate (Z) and azooxanthellate (AZ) taxa between sites and habitats within the WMNP, Indonesia. *m* is the maximum number of principle coordinate (PCO) axes with minimal misclassification; % var. quantifies total variance explained by the first *m* PCO axes; allocation success denotes the proportion of correct allocations to each group; δ^2 is the first squared canonical correlation size.

Factor	***m***	**% var**	**Allocation Success %**					δ^2	***P***
Site			**Sampela**	**Buoy 3**	**Pak Kasim's**	**Ridge 1**	**Total**		
All spp.	27	97.86	83.33	83.33	83.33	91.67	85.42	0.979	0.0001
Z	10	93.97	83.33	75	83.33	91.67	83.33	0.883	0.0001
AZ	28	99.53	100	66.67	50	100	79.17	0.991	0.0001
Habitat			**Flat**	**Crest**	**Slope**				
All spp.	17	89.55	87.5	68.75	81.25	-	79.17	0.946	0.0001
Z	5	82.24	87.5	56.25	68.75	-	70.83	0.676	0.0001
AZ	8	71.86	100	43.75	62.5	-	68.75	0.516	0.0009

Several azooxanthellate species within 5 families principally defined the reef slope (Figure 2.6). Two azooxanthellate colonies (*Astrogorgia* sp.19, *Melithaea ochracea* Linnaeus 1758) were encountered during the survey at Sampela, 70 species at Buoy 3, 94 species at Pak Kasim's and 161 species at Ridge 1 (Table 2.2). Species richness and diversity, with the exception of Sampela, were similar to the first model (Figure 2.2) for the crest and slope (Figure 2.4b, d, f, h). It is notable that the pattern of *Acanthogorgia* sp.5 on the reef crest at Buoy 3 (Figure 2.6b, d, Table 2.6), was replicated by *Acabaria* sp.23 at Ridge 1, also inhabiting the ceilings of caves, overhangs and crevices. Both species are as yet undescribed. *Melithaea* sp.3 showed distinct assemblages on the ridge top at Ridge 1 (Figure 2.6d). However, the vast majority of azooxanthellate taxa inhabited the reef slope with similar assemblage composition and distribution patterns across Buoy 3 and Pak Kasim's as evident by the reduced allocation success (an indicator of reduced site and habitat distinction; Table 2.3) and site x habitat interaction.

Table 2.4. SIMPER analysis results indicating which gorgonian species (zooxanthellate and azooxanthellate) contributed the greatest dissimilarities between sites and habitats. Results presented as the average abundance (AvAb1 & 2), species average (AvD) and cumulative dissimilarity contribution (AvD Cum%).

All Species SITE	Av. Group Diss %	AvAb1	AvAb2	AvD	AvD Cum %
Sampela & Buoy 3	88.95	**Sampela**	**Buoy 3**		
Isis hippuris[LT]		0.4	0.25	5.79	6.51
Acanthogorgia sp.5		0	0.61	5.55	12.76
Isis hippuris[L]		0.25	0.06	4.49	17.8
Isis hippuris[N]		0.5	0	4.03	22.33
Sampela & Pak Kasim's	93.71	**Sampela**	**Pak Kasim's**		
Isis hippuris[N]		0.12	1.81	9.58	10.23
Briareum excavatum		0	0.67	5.13	15.7
Isis hippuris[LT]		1.16	0.02	4.33	20.32
Isis hippuris[L]		0.79	0.25	3.34	23.88
Sampela & Ridge	97.73	**Sampela**	**Ridge**		
Briareum stechei		0	2.98	7.05	7.21
Isis hippuris[N]		0.12	1.14	3.53	10.82
Menella lenzi		0	0.92	2.4	13.28
Isis hippuris[LT]		0.4	0.38	2.35	15.68
Buoy 3 & Pak Kasim's	84.26	**Buoy 3**	**Pak Kasim's**		
Isis hippuris[N]		0.25	1.81	8.01	9.51
Briareum excavatum		0.08	0.67	4.81	15.22
Isis hippuris[L]		0	1.16	3.71	19.63
Acanthogorgia sp.4		0.06	0.79	2.97	23.15
Ridge & Buoy 3	89.19	**Buoy 3**	**Ridge**		
Briareum stechei		0.03	2.98	6.22	6.98
Isis hippuris[N]		0.25	1.14	2.91	10.24
Menella lenzi		0.08	0.92	2.13	12.63
Astrogorgia sp.4		0.14	0.43	1.96	14.82
Ridge & Pak Kasim's	81.3	**Pak Kasim's**	**Ridge**		
Briareum stechei		0.16	2.98	4.55	5.6
Isis hippuris[N]		1.81	1.14	2.95	9.23
Briareum excavatum		1.16	0.85	1.81	11.45
Menella lenzi		0.25	0.92	1.69	13.53

All Species HABITAT	Av. Group Diss %	AvAb1	AvAb2	AvD	AvD Cum %
Flat & Crest	86.27	**Flat**	**Crest**		
Isis hippuris[LT]		0.53	0.33	6.33	7.34
Acanthogorgia sp.5		0	0.44	4.2	12.21
Isis hippuris[L]		0.19	0.22	3.7	16.5
Isis hippuris[N]		1.29	1.16	3.65	20.73
Flat & Slope	96.14	**Flat**	**Slope**		
Isis hippuris[LT]		0.53	0	6.8	7.07
Isis hippuris[L]		0.22	0.01	3.86	11.09
Isis hippuris[N]		1.29	0.04	3.61	14.84
Briareum excavatum		0.04	0.86	3.18	18.15
Crest & Slope	84.88	**Crest**	**Slope**		
Isis hippuris[L]		0.22	0.12	3.83	4.51
Isis hippuris[N]		0.89	0.01	3.09	8.16
Briareum excavatum		0.06	0.06	2.7	11.33
Briareum stechei		0.28	0.86	2.66	14.47

Table 2.5. SIMPER analysis results indicating which zooxanthellate gorgonian species contributed the greatest dissimilarities between sites and habitats. Results presented as the average abundance (AvAb1 & 2), species average (AvD) and cumulative dissimilarity contribution (AvD Cum%).

Zooxanthellate SITE	Av. Group Diss %	AvAb1	AvAb2	AvD	AvD Cum %
Sampela & Buoy 3	81.37	**Sampela**	**Buoy 3**		
Isis hippuris[L]		0.42	0.06	16.26	19.98
Briareum excavatum		0.06	0.4	11.78	34.46
Isis hippuris[LT]		0.5	0.24	10.2	47
Briareum stechei		0.06	0.34	9.54	58.73
Sampela & Pak Kasim's	84.66	**Sampela**	**Pak Kasim's**		
Isis hippuris[N]		0.16	1.89	18.57	21.93
Briareum excavatum		0.06	0.6	13.97	38.43
Isis hippuris[L]		0.01	0.98	13.43	54.29
Isis hippuris[LT]		0.42	0.91	8.84	64.73
Sampela & Ridge	91.19	**Sampela**	**Ridge**		
Briareum stechei		0	3.09	29.51	32.36
Isis hippuris[N]		0.16	1.15	10.47	43.84
Briareum excavatum		0.01	0.91	8.26	52.9
Isis hippuris[LT]		0.5	0.37	7.17	60.76
Buoy 3 & Pak Kasim's	77.58	**Buoy 3**	**Pak Kasim's**		
Isis hippuris[N]		0.27	1.89	17.56	22.64
Briareum excavatum		0.03	0.98	13.13	39.56
Isis hippuris[L]		0.4	0.6	11.87	54.86
Briareum stechei		0.34	0.91	9.3	66.85
Ridge & Buoy 3	85.38	**Buoy 3**	**Ridge**		
Briareum stechei		0.03	3.09	27.92	32.7
Isis hippuris[N]		0.27	1.15	9.25	43.53
Briareum excavatum		0.03	0.91	7.73	52.58
Isis hippuris[L]		0.4	0.5	6.6	60.31
Ridge & Pak Kasim's	71.56	**Pak Kasim's**	**Ridge**		
Briareum stechei		0.16	3.09	20.11	28.11
Isis hippuris[N]		1.89	1.15	8.98	40.66
Briareum excavatum		0.98	0.91	8.56	52.62
Isis hippuris[L]		0.91	0.4	4.93	59.52

Zooxanthellate HABITAT	Av. Group Diss %	AvAb1	AvAb2	AvD	AvD Cum %
Flat & Crest	71.23	**Flat**	**Crest**		
Isis hippuris[LT]		0.54	0.38	12.77	17.93
Isis hippuris[N]		1.32	1.22	10.71	32.96
Isis hippuris[L]		0.28	1.01	8.46	44.83
Briareum excavatum		0.69	0.1	7.89	55.91
Flat & Slope	87.31	**Flat**	**Slope**		
Isis hippuris[LT]		0.54	0	14.55	16.67
Isis hippuris[N]		1.32	0.05	13.2	31.79
Briareum excavatum		0.04	0.86	10.24	43.51
Briareum stechei		0.69	0.01	9.25	54.1
Crest & Slope	82.8	**Crest**	**Slope**		
Briareum excavatum		0.28	0.86	14.88	17.97
Isis hippuris[N]		1.22	0.05	14.65	35.66
Isis hippuris[L]		1.01	0.05	13.18	51.58
Briareum stechei		0.17	0.47	8.77	62.18

Table 2.6. SIMPER analysis results indicating which azooxanthellate gorgonian species contributed the greatest dissimilarities between sites and habitats. Results presented as the average abundance (AvAb1 & 2), species average (AvD) and cumulative dissimilarity contribution (AvD Cum%).

Azooxanthellate SITE	**Av. Group Diss %**	**AvAb1**	**AvAb2**	**AvD**	**AvD Cum %**	**Azooxanthellate HABITAT**	**Av. Group Diss %**	**AvAb1**	**AvAb2**	**AvD**	**AvD Cum %**
Sampela & Buoy 3	100	**Sampela**	**Buoy 3**			**Flat & Crest**	97.66	**Flat**	**Crest**		
Acanthogorgia sp.5		0	0.61	12.88	12.88	*Acanthogorgia* sp.5		0	0.44	8.8	9.01
Astrogorgia sp.4		0.08	0	11.11	23.99	*Astrogorgia* sp.6		0.06	0	7.69	16.89
Acanthogorgia sp.4		0	0.25	5.75	29.74	*Acanthogorgia* sp.4		0	0.69	6.3	23.33
Acabaria sp.23		0	0.25	4.74	34.48	*Bebryce* cf. *indica*		0.32	0.14	3.1	26.51
Sampela & Pak Kasim's	100	**Sampela**	**Pak Kasim's**			**Flat & Slope**	97.4	**Flat**	**Slope**		
Astrogorgia sp.4		0	0.51	9.75	9.75	*Astrogorgia* sp.6		0.06	0	6.36	6.5
Ellisella ceratophyta		0	0.17	8.77	18.52	*Melithaea* sp.3		0	0.06	6.36	13.07
Acanthogorgia sp.4		0.08	0	7.86	26.38	*Acanthogorgia* sp.4		0	0.81	2.83	15.97
Astrogorgia sp.6		0	1	4.99	31.38	*Annella* sp.1		0	0.56	2.58	18.62
Sampela & Ridge	100	**Sampela**	**Ridge**			**Crest & Slope**	86.38	**Crest**	**Slope**		
Melithaea sp.3		0	0.25	4.32	4.32	*Melithaea* sp.3		0	0.06	7.69	8.9
Menella lenzi		0	0.92	3.74	8.06	*Acanthogorgia* sp.5		0.44	0.08	2.24	11.5
Astrogorgia sp.4		0	0.43	3.3	11.36	*Annella* sp.1		0.25	0.63	2.24	14.09
Astrogorgia sp.6		0	0.38	3.16	14.52	*Acanthogorgia* sp.4		0.69	0.81	2.23	16.67
Buoy 3 & Pak Kasim's	92.46	**Buoy 3**	**Pak Kasim's**								
Astrogorgia sp.4		0.31	0.51	11.75	12.7						
Ellisella ceratophyta		0	0.17	10.25	23.79						
Acanthogorgia sp.5		0.61	0.05	3.67	27.76						
Acanthogorgia sp.4		0.25	1	3.36	31.39						
Ridge & Buoy 3	92.72	**Ridge**	**Buoy 3**								
Melithaea sp.3		0	0.25	4.56	4.92						
Astrogorgia sp.4		0.14	0.43	3.68	8.89						
Menella lenzi		0.08	0.92	3.47	12.63						
Astrogorgia sp.6		0	0.38	3.25	16.13						
Ridge & Pak Kasim's	88.52	**Pak Kasim's**	**Ridge**								
Melithaea sp.3		0	0.25	4.15	4.69						
Astrogorgia sp.4		0.05	0.43	3.36	8.49						
Menella lenzi		0.25	0.92	3.21	12.11						
Astrogorgia sp.6		0.32	0.38	3.19	15.71						

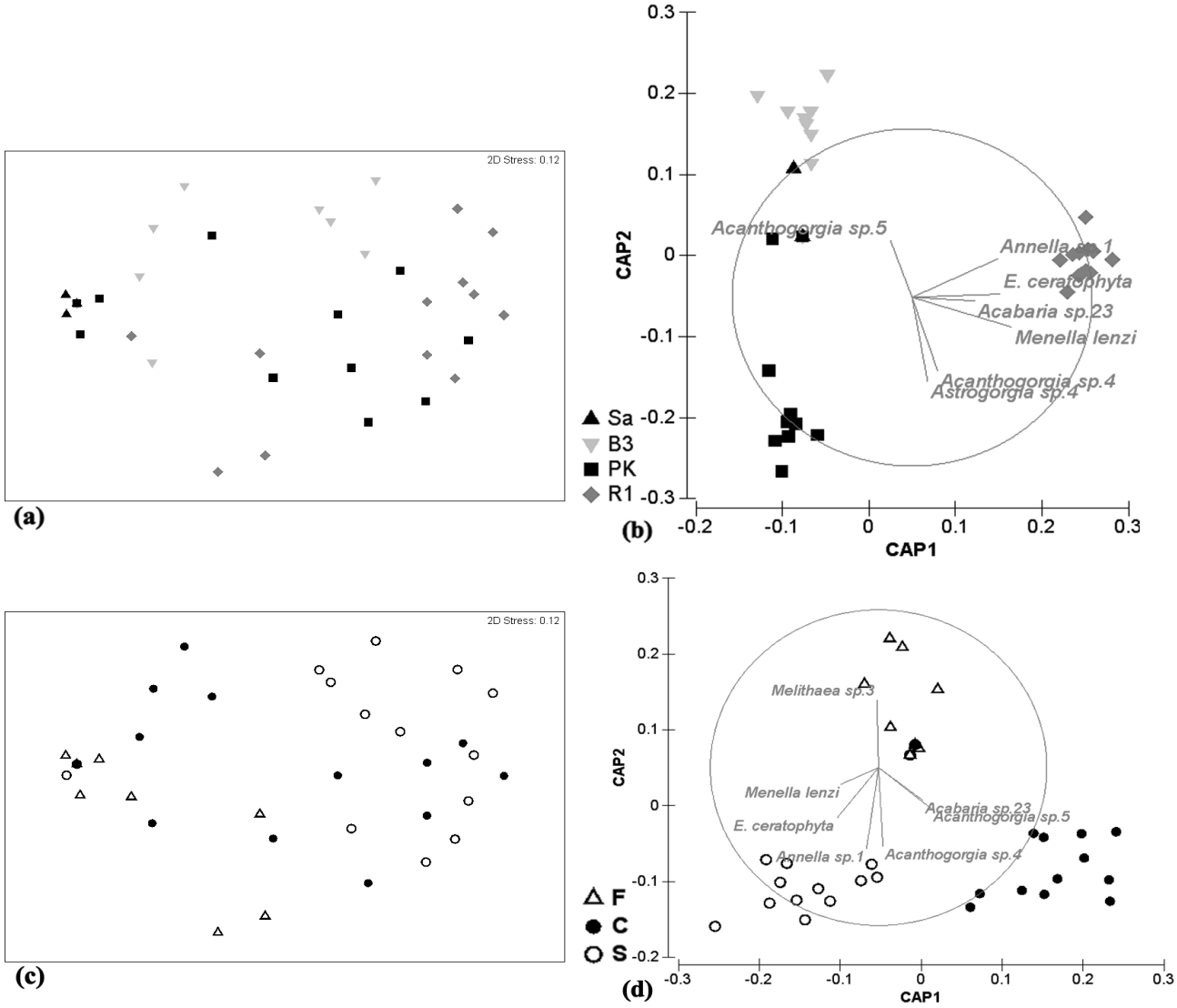


Figure 2.6. (a, c) Constrained and (b, d) unconstrained ordinations of azooxanthellate gorgonian assemblages between sites (a, b) and habitats (c, d). Species vectors are directed where the species were best represented. See Figure 2.3 for factor level codes.

Gorgonian Community Structure. Univariate and multivariate analyses illustrated clear differences between sites and habitats within the WMNP (Figures 2.2 – 2.7). Seven families from the Calcaxonia (Ellisellidae S = 16, Isididae S ~ 2), Holaxonia (Acanthogorgiidae S = 16, Plexauridae S = 87) and Scleraxonia (Briareidae S = 3, Subergorgiidae S = 7, and Melithaeidae S= 44) characterized reef habitats from low diversity and abundance at the impacted site Sampela to high diversity and abundance at Ridge 1 (Figure 2.7).

The Isididae at Sampela were dominant across the flats and crest (11.5 ± 1 & 11.5 ± 1.4 per 20 m^{-2}; mean ± SE), with occasional Briareidae on the slope (2 ± 1). At Buoy 3, Isididae were dominant on

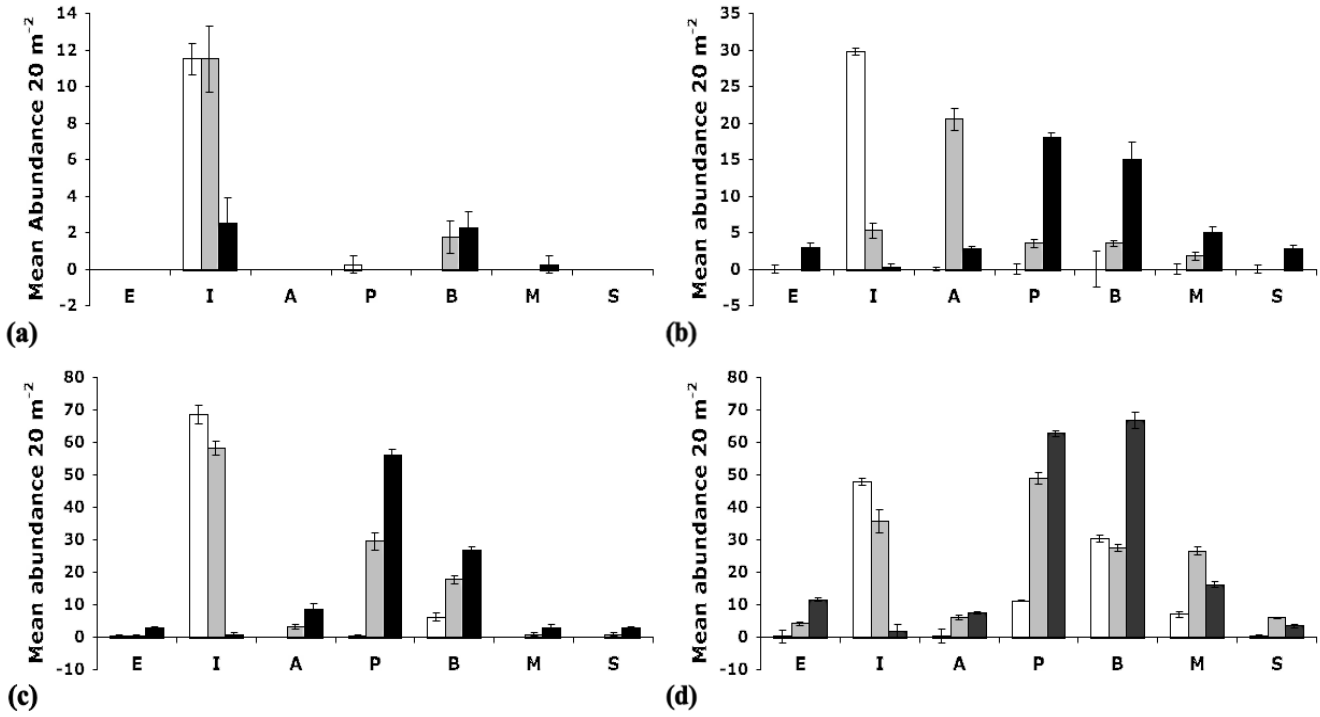


Figure 2.7. Site-specific gorgonian family abundance (mean ± SE) across sites and habitats within the WMNP. (a) Sampela, (b) Buoy 3, (c) Pak Kasim's, and (d) Ridge 1. E, Ellisellidae; I, Isididae; A, Acanthogorgiidae; P, Plexauridae; B, Briareidae; M, Melithaeidae; S, Subergorgiidae. White bars, reef flat; grey bars, reef crest; black bars, reef slope.

the reef flat (30 ± 2), Acanthogorgiidae characterised overhangs on the reef crest (20 ± 1.5), and Plexauridae on the reef slope (18 ± 1). Pak Kasim's was dominated by high numbers of the Isididae on the reef flat and crest (68 ± 3; 58 ± 2), with Plexauridae and Briareidae on the reef slope (56 ± 2; 27 ± 1). Ridge 1 showed Isididae and Briareidae having the greatest relative abundance on the ridge top (48 ± 2; 30 ± 3), Plexauridae, Isididae, Briareidae and Melithaeidae on the reef crest (49 ± 2; 36± 3; 28 ± 1; 27 ± 1), and Plexauridae and Briareidae on the reef slope (63 ± 0.1; 67 ± 1). In sum, phototrophic/zooxanthellate taxa added little diversity but greatest relative abundance to the reef flats and crest for Isididae and reef slope for Briareidae (Figure 2.7). Heterotrophic/azooxanthellate taxa especially within the family Plexauridae, contributed greatest to the increased biodiversity with depth (Figure 2.7).

Colony size was not reported in this study however numerous azooxanthellate species were small and located within sheltered crevices, overhangs, or at the base or under other coral colonies (e.g., the soft coral *Sarcophyton* Lesson 1834 and tabulate scleractinian *Acropora* Oken 1815). Interestingly, small colonies of *Acanthogorgia* sp.4, *Annella* sp.1 and *Bebryce hicksoni* Thomson & Henderson 1905 were frequently encountered at the base of large *Annella reticulata* Ellis &

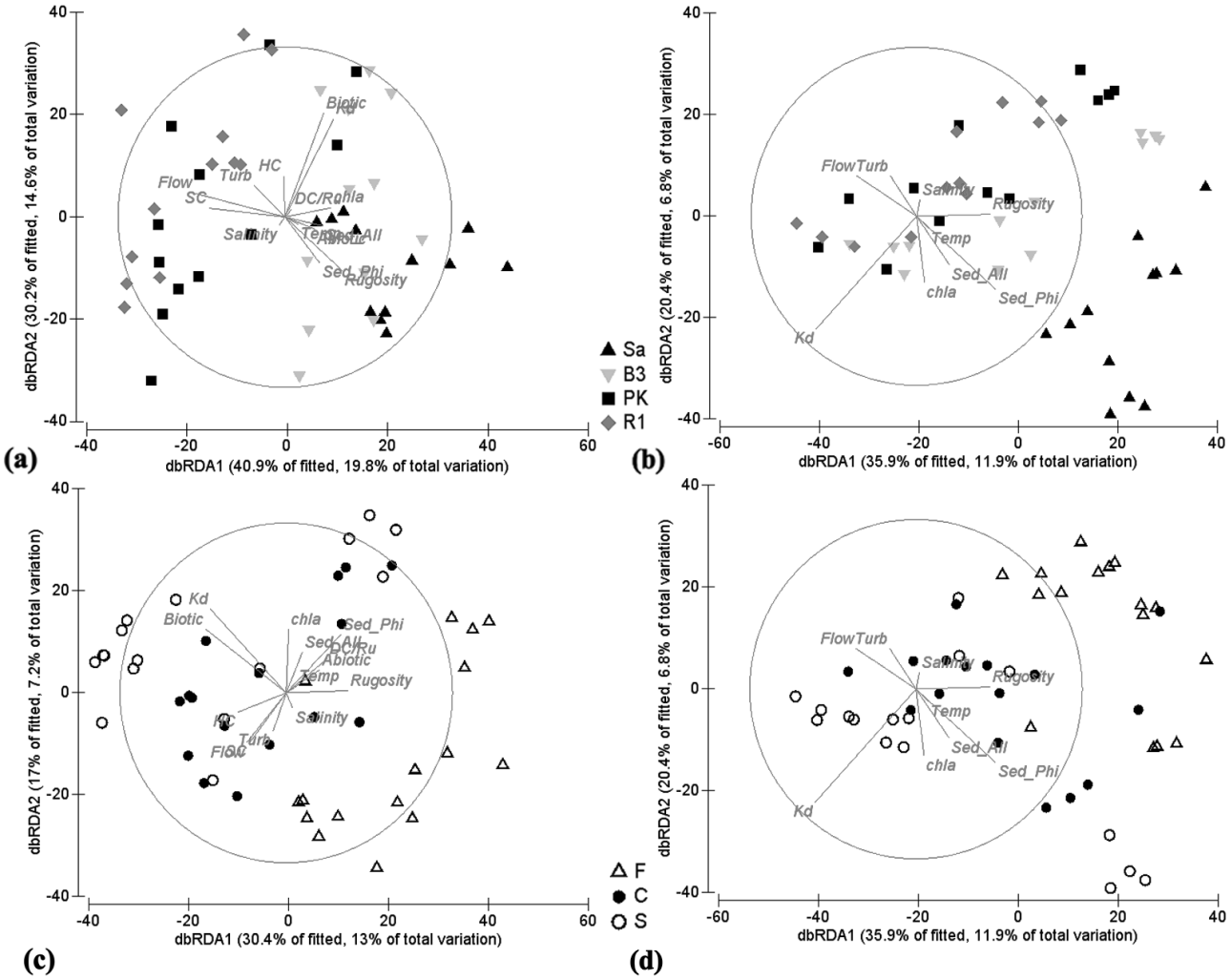


Figure 2.8. DISTLM*forward* models for the best explanatory drivers for variability across site (a, full variables; b, abated variables) and habitats (c, full variables; d, abated variables) of gorgonian variability across sites within the WMNP. See Figure 2.3 for factor level codes.

Solander 1736, *A. mollis* Nutting 1910, and *Melithaea* spp. colonies. Observations at depths greater than those reported here suggest a continual increase in gorgonian diversity, abundance and size, plus a remarkable frequency of new recruits (< 5 cm tall). Additional gorgonian species present within the WMNP not encountered during the surveys are documented in Table 2.2.

2.3.2 Environmental Variables

Results from the nonparametric multivariate regression DISTLM*forward* revealed that taken together biotic (sponges, algae, ascidians, molluscs) variables, grain size, rugosity, and light explained 23.73% (pseudo-F = 2.453, P < 0.001) of the variability in gorgonian assemblage structure (Figure 2.8). Benthic variables, covariates of gorgonian assemblages, were omitted from an additional analysis revealing sediment grain size, light, rugosity and chlorophyll-*a* explaining 25.32% (pseudo-F = 1.999, P < 0.001) of gorgonian assemblage variability (Figure 2.8). The same

model applied separately to zooxanthellate and azooxanthellate species revealed that soft coral, biotic variables and flow explained 29.3% (pseudo-F = 3.601, P = 0.001), and flow, light and chlorophyll-*a* explained 28.13% (pseudo-F = 3.904, P < 0.001) of the variability in both classes respectively. Results from the abated model suggested that water flow, light and chlorophyll-*a* (27.6%; pseudo-F = 3.134, P = 0.003), rugosity, sediment grain size and light (30.04%; pseudo-F = 6.254, P = 0.001) again had a limited, yet significant degree of influence on zooxanthellate and azooxanthellate distributions.

2.4 DISCUSSION

In total, 197 gorgonian species and distinct morphotypes from 42 genera and 12 families were documented across shallow (0-30 m) coral reefs within the WMNP, Indonesia. Comparable with previously described shallow water gorgonians across the Indo-Pacific comprising 51 genera within 14 families (Grasshoff 1999, Fabricius & Alderslade 2001), this study strongly supports the WMNP as an area of high regional gorgonian abundance and diversity. Distinct community types across sites and habitats along an environmental gradient are characterised by non-independence between benthic space competitors, and driven in part by habitat complexity, water flow and natural light.

Gorgonian distribution patterns within the WMNP followed a gradient of low diversity and abundance at the impacted site Sampela to high diversity and abundance at Ridge 1. Species richness and diversity were proportional to depth, a pattern consistent with previous research on benthic invertebrates within the area (e.g., Porifera, Bell & Smith 2004), yet the inverse for Scleractinia (Haapkylä et al. 2007). Similarly, high gorgonian diversity with depth has been shown in other areas (Singapore, Goh & Chou 1994; Caribbean, Sanchez et al. 1997; Marianas, Paulay et al. 2003; Hong Kong, Fabricius & McCory 2006; Japan, Matsumoto et al. 2007; Philippines, Rowley unpublished data) with concomitant zooxanthellate octocoral abundance in the shallows (Great Barrier Reef, Fabricius & Klumpp 1995; Thailand, Chanmethakul et al. 2010).

Differences in gorgonian assemblage structure between sites and habitats were driven by morphotypes of the zooxanthellate isidid *I. hippuris* and species within the genus *Briareum*. *I. hippuris* dominance on the shallow reef flats is likely due, in part, to differential disturbance levels between study sites. Continual disturbance maintains relative species composition, stability and biodiversity within a particular reef community (Connell 1978, Aronson & Precht 1995, Shea et al. 2004, Gouhier & Guichard 2007, Bohn et al. 2014). For example, strong upwellings and water currents at Ridge 1, contrasting with continual resource exploitation and high sedimentation rates at Sampela, are reflected in diverse and impoverished gorgonian communities, respectively. Buoy 3

and Pak Kasim's, both subject to past destructive fishing practices and bleaching on the former reef flats, show an increase in gorgonian abundance and diversity between Sampela and Ridge 1. However, considerable loose rubble and anthropogenic gleaning on the shallow (~1-3 m) reef flats of Buoy 3 likely impede settlement success even at low turbulence (Goh & Chou 1994), thus reflecting minimal recovery and gorgonian presence. Yet high *I. hippuris* abundance on the deeper (~3-5 m) reef flats at Pak Kasim's may indicate that this species is an *r*-selected strategist, with its response to reef recovery unencumbered by inhibitors to settlement and growth, therefore, thriving in elevated current flow, low turbidity and minimal loose substrata.

The ability to colonize disturbed areas reflects reproductive strategy, larval settlement choices and post-settlement mortality. *I. hippuris* is a gonochoristic (Simpson 1906) external brooder (pers. obs.; see also Figure 4.5), yet also displays considerable fragmentation with a proportionally high regeneration capacity on the reef flats across all sites. Asexual propagation through fragmentation is not uncommon in gorgonians yet can show intra- and inter-disturbance sensitivity (Coffroth & Lasker 1998). The proportional success of regenerative fragments, with most fragments being small and apical, across all sites suggests fragmentation in *I. hippuris* is not disturbance sensitive. Interestingly, such fragment success was morphotype-specific and may represent eco-phenotypic specificity or sibling taxa. Nevertheless, asexual propagation through fragmentation facilitates rapid post-disturbance recovery (Dauget 1992, Dahan & Benyahu 1997) resulting in high local population abundance as evident by *I. hippuris* on the reef flats in the WMNP.

The zooxanthellate genus *Briareum* also influenced separations between the factors site and habitat. Low-lying branching species on the reef slope, predominantly at Pak Kasim's and Ridge 1, had a bathymetric distribution counter to that of both *I. hippuris* (this study) and scleractinians (Porter 1976). Furthermore, numerous asexual fragments and juvenile colonies were encountered. This pattern mirrored its Atlantic congener, *Briareum asbestinum* Pallas 1766, which reproduces through asexual fragmentation and external brooding producing low dispersal philopatric larvae (Brazeau & Harvell 1994). It is likely that such a dual reproductive strategy occurs in Indo-Pacific *Briareum* taxa. However, reproductive strategies for Indo-Pacific gorgonian taxa are largely unknown, likely having limited dispersal abilities leading to habitat specialists and high endemism (Grasshoff & Bargibant 2001, Picciano & Ferrier-Pagès 2007).

Acanthogorgia sp.5 was the only azooxanthellate species driving differences between and within factor levels due to its exclusive abundance on the ceilings of caves and overhangs on the reef crest at Buoy 3. This specialized distribution may be due to within-overhang microhabitats, pre-settlement larval preferences such as negative phototaxis (Sánchez et al. 1997), geotaxis, or

differential mortality following settlement in other areas. Species within the *Acanthogorgia* genus do however, possess a degree of habitat selectivity (Goh & Chou 1994, this study), frequently observed at the base of other large chemically well defended gorgonians such as *Annella reticulata*, *A. mollis*, *Melithaea* spp. and the soft coral *Sarcophyton*. Such taxa likely affect recruitment (Yoshioka & Yoshioka 1989, Yoshioka 1996) through waterborne exudates facilitating spatial refugia from predation, competition (Hay 1986) or fouling. Larval settlement preferences have been shown for crustose coralline algae (Harrington et al. 2004), in addition to enhanced photophysiological performance on carbonate substratum (Green et al. 2010*).* Furthermore, azooxanthellate Caribbean gorgonian larvae show settlement preference for consolidated topographically complex reefs in addition to longer pelagic larval duration (PLD; Sánchez et al. 1997) compared to zooxanthellate taxa. Yet both fitness enhancement through substratum selection and PLD are unknown for Indonesian gorgonians, PLD not necessarily predictive of dispersal (Rosen 1988, Weersing & Toonen 2009, but see also Cowen et al. 2006). Nevertheless, diversity and abundance increased markedly with habitat complexity towards Ridge 1 and with depth. This bioenvironmental cline suggests higher larval availability through water currents and self-seeding, as well as selection and post-settlement success for sites with high topographic complexity and consolidated substratum. In contrast, low relief, unconsolidated fine-grain substratum coupled with low water flow, high sediment rate, continuous anthropogenic disturbance and high grazing activity from *Diadema* spp. at Sampela (Hodgson 2008) likely act in concert with reduced larval availability, settlement and survival to result in low biodiversity.

Habitat structural complexity, thus colonizable area, substratum type and light intensity, can determine settlement choices and profoundly influence benthic community structure on coral reefs (Sánchez et al. 1997, Meesters et al 2001, Linares et al. 2008). Yet the combination of predictor variables biotic, sediment grain size, rugosity and light explained only 23% of gorgonian assemblage structure across clear environmental clines. Evidently, two inherently related patterns were occurring. Firstly, benthic variables such as sponges, algae, hard and soft coral all co-vary with gorgonian distribution, themselves members of coral reef benthic communities. Remodeling on such covariate removal revealed sediment grain size, light, rugosity and chlorophyll-*a* still explained only 25% of gorgonian assemblage structure. that, Secondly, this suggests that zooxanthellate and azooxanthellate gorgonian distribution are not independent of each other, essentially reflecting two different trophic groups, heterotrophs and phototrophs. This further confirms that interspecific competitive forces do exist between shallow water gorgonians relative to natural light as a function of bathymetry.

2.4.1. Zooxanthellate versus Azooxanthellate Gorgonians

The dominance of zooxanthellate taxa driving separation between reef areas and location obscured azooxanthellate distribution patterns. Trophic group separation (zooxanthellate = phototrophy; azooxanthellate = heterotrophy) revealed a clear environmental gradient interaction with depth, further confirmed by non-independence between the two groups. Thus both groups displayed contrasting patterns with azooxanthellate species richness and diversity proportional to depth, consistent with other areas (Goldberg 1973, Goh & Chou 1994, Sanchez et al. 1997, Paulay et al. 2003, Fabricius & McCory 2006, Matsumoto et al. 2007, this study), with the inverse being true for zooxanthellate taxa.

Replicate multivariate models for zooxanthellate and azooxanthellate taxa revealed zooxanthellate species drove differences between site and habitat. Distinct *I. hippuris* morphologies showed patterns of variability both within and between sites, most notably bushy colonies with long thick branches on the reef flat at Sampela and planar short tightly packed branched colonies at Ridge 1. Morphological variants in 'sympatry' are not uncommon in Cnidaria (Knowlton 1993, Prada et al. 2008, Forsman et al. 2010) and may be indicative of phenotypic plasticity or incipient ecological divergence in response to natural light and water flow. Morphological variation in *I. hippuris* has previously been noted but not quantified (Simpson 1906, Fabricius & Alderslade 2001). However such variation through increased branching surface area enhancing photosynthetic efficiency in shallow water branching taxa (Hennige et al. 2008), coupled with a previously undocumented dual mode of reproduction, may likely explain the biological success of *I. hippuris* across environmental clines within the WMNP. Colony form can depend on feeding strategy and the same genotype can show different allocation patterns in different environments (Weiner 2004). Yet different morphotypes within the same environment, may represent separate clonal aggregations (Coffroth & Lasker 1998) consequential of fragmentation or the potential for sibling species.

Fitness enhancement through morphological plasticity relative to water flow and light availability may drive *I. hippuris* morphotype differences particularly between Ridge 1 and Sampela. Densely packed, shorter branches in high flow environments - coupled with high irradiance - at Ridge 1 maintains colony integrity and maximizes high particle capture, especially downstream of a colony or aggregation due to turbulence intensity (McFadden 1986, Sebens et al 1997). Conversely, long wide branches with a high surface area likely maximize photosynthetic gain (Hennige et al. 2008) otherwise absorbed by suspended particulate matter in high sedimented reefs (Anthony & Fabricius 2000) such as Sampela, and further reducing sediment accumulation on the colony (Crabbe & Smith 2003). Colony surface area and metabolism are intrinsically linked whereby variations in

branching structure and concomitant within colony canalisation result in resource allocation plasticity, further representing alternate feeding strategies, size and growth patterns (Sebens 1997) to mitigate environmental change. Reciprocal transplant experimentation between Sampela and Ridge 1 assessing a suite of phenotypic traits, in addition to molecular analyses, may be fruitful in ascertaining species delineation or acclimatory capacity between *I. hippuris* morphotypes.

A dual reproductive strategy was also observed in *Briareum* spp. and likely explains, in part, the relative success of this species at depths where few zooxanthellate taxa are encountered and azooxanthellate diversity is high. *Briareum* morphotypes also displayed habitat specificity with branching taxa at depth and encrusting types on the high flow reef flat/Ridge top. Encrusting morphologies reduce drag in such high flow environments (Bell & Barnes 2000, Bell & Smith 2004). However, habitats characterised by low wave action, high turbidity and sedimentation rates, have also been shown to favour encrusting *Briareum* spp. (Fabricius & Alderslade 2001, Fabricius & De'ath 2004), likely due to morphological and behavioural pre-adaptations such as phenotypic and photoacclimatory plasticity, colony and polyp size, reproductive strategy and recruitment survival (Stafford-Smith 1993, Anthony 2000). Yet such patterns are in direct contrast with those in this study. Furthermore, *Briareum* spp. abundance at Sampela compared to *I. hippuris* was considerably less with three out of the seventeen colonies encountered being encrusting. Thus branching and lobe-like, upward projecting *Briareum* morphologies may well be selected for in low light and water flow, high turbidity and sedimented environments; reducing sediment smothering with increased SA:V for photosynthetic efficiency akin to *I. hippuris*.

Predictor variables highlight water flow, light and chlorophyll-*a*, rugosity, sediment grain size and light for zooxanthellate and azooxanthellate species respectively. High water motion and localized upwelling further enhanced by strong water currents at Ridge 1, fertilize the reef with deep benthic nutrients for primary productivity and enhanced food availability (Jokiel 1978, Sebens 1984), maximizing species biodiversity and abundance. Therefore, increased azooxanthellate species richness and diversity on the Ridge top, coupled with slightly reduced zooxanthellate species abundance compared to Pak Kasim's is indicative of a natural reef environment on the Ridge with overall reduced species dominance. Such patterns can again be attributed to intermediate disturbance levels, maintaining relative species diversity within a reef community, and thus acting in concert with nutrient and suitable substrate availability and competition (Connell 1978, Aronson & Precht 1995, Townsend & Scarsbrook 1997, Gouhier & Guichard 2007).

Azooxanthellate gorgonian assemblage structure showed a relatively consistent pattern across sites and habitats with the exception of Sampela. However, an amplitudinal/additive interaction (i.e., not

due to “crossing-over”) revealed that proportionality of abundance between sites and habitats changed markedly for some taxa. Nevertheless, azooxanthellate gorgonians showed assemblage patterns consistent with an environmental decline from the healthy high energy Ridge to the depauperate reef communities at Sampela. Community structure of azooxanthellate taxa varied little within the deeper depths with only Plexauridae and Melithaeidae present across all sites. Species within the most diverse family, Plexauridae, drove diversity with depth, a pattern generally observed in other azooxanthellate families (Goh & Chou 1994, Matsumoto et al. 2007, this study). Increased diversity and a high frequency of recruits with depth suggest a deeper refugia and competitor release from zooxanthellate corals. This pattern is similarly replicated by sponge taxa (Bell & Smith 2004, Powell et al. 2010) inferring no or positive interactions between these two benthic groups (McLean & Yoshioka 2007), both typically possessing powerful secondary metabolites. Moreover, increased azooxanthellate diversity with depth may likely represent a consistent biological source pool. Such taxa being invaluable in the event of past sea level variance in addition to current and future natural and anthropogenic disturbance particularly with regards the insidious effects of destructive fishing practices and global climate change.

Taken together, sedimentation, rugosity, light, and water flow have been shown to be major factors controlling local gorgonian populations (Sánchez et al. 1997, Meesters et al 2001, Linares et al. 2008). This pattern is true, in part, across environmental gradients within the WMNP. However, non-independence between zooxanthellate and azooxanthellate gorgonians and coral reef benthic variables likely explain the large amount of gorgonian assemblage variation unexplained by the predictor variable model. Niche partitioning through trophic differentiation (phototrophy and heterotrophy) is thus epitomized by gorgonian corals, greatly contributing towards coral reef biodiversity in the WMNP and undoubtedly the Coral Triangle as a whole.

2.5 CONCLUSION

In sum, gorgonian distribution patterns within the WMNP follow a gradient from low diversity and abundance at the impacted site at Sampela to high diversity and abundance at Ridge. Moreover, this environmental gradient response interacts with habitat primarily as a function of depth (thus light) structuring zooxanthellate and azooxanthellate taxa on shallow and slope reef habitats respectively. Light availability and benthic competitors define distribution and abundance for most gorgonian taxa. Most notable are morphological variants of the zooxanthellate species *I. hippuris* and species within the genus *Briareum*, such biological success likely being a consequence of dual reproductive strategies and morphological responses to different environments. However, whether such morphological variability, particularly in *I. hippuris*, is due to physiological capacity or fixed

adaptations inferring incipient divergence due to differing resource allocation structure from prolonged exposure to sub-optimal environments, is unknown. Tests of physiological resilience of respective morphotypes would indeed be informative to management plans and coral reef biodiversity assessments. By determining species delineation and/or potential ‘eco-morphotype’ environmental specificity, monitoring of gorgonian taxa, in particular *I. hippuris*, can therefore greatly assist environmental impact assessments and identify areas of habitat degredation.

CHAPTER 3: ENVIRONMENTAL INFLUENCES ON THE INDO-PACIFIC GORGONIAN *ISIS HIPPURIS* LINNAEUS 1758: PLASTICITY CAPACITY OR GENETICALLY FIXED?

ABSTRACT

As conspicuous modular components of benthic marine habitats, gorgonian (sea fan) corals have perplexed taxonomists for centuries through their shear biodiversity, particularly throughout the Indo-Pacific. Phenotypic incongruence within and between seeming unitary lineages across contrasting environments can provide the raw material to investigate processes of disruptive selection. Two distinct phenotypes of the Isidid *Isis hippuris* Linnaeus 1758 partition across environmental clines: long-branched bushy colonies on degraded reefs, and short-branched multi/planar colonies on healthy reefs within the Wakatobi Marine National Park (WMNP), Indonesia. Multivariate analyses reveal phenotypic traits between morphotypes were likely integrated primarily at the colony level with increased polyp density and consistently smaller sclerite dimensions at the degraded site. Sediment load and turbidity, hence light availability, primarily influenced phenotypic division between the two sites. Thus the distinct morphological variability between the two sites is a reliable indicator of reef health; selection primarily acting on colony morphology, porosity through branching structure, as well as sclerite diversity and size. ITS2 sequence and predicted RNA secondary structure further revealed intraspecific variation between *I. hippuris* morphotypes relative to such environments (Φ_{ST} = 0.7683, P <0.001). This evidence suggests – but does not confirm - that *I. hippuris* morphotypes within the WMNP are two separate species, however to what extent and taxonomic assignment requires further investigation across its full geographic distribution. Incongruence between colonies present in the WMNP and tenuously described *Isis* alternatives (*Isis reticulata* Nutting 1910, *Isis minorbrachyblasta* Zou, Huang & Wang 1991), questions the validity of such assignments. Furthermore, phylogenetic analyses corroborate early taxonomic suggestion that the characteristic jointed axis of the Isididae is in fact a convergent trait. Thus the polyphyletic nature of the Isididae lies in its type species *I. hippuris,* being unrelated to the rest of its family members.

Key words: *Isis hippuris* • Gorgonian coral • Isididae • Morphology • Indonesia • ITS2

3.1 INTRODUCTION

Reef biodiversity reflects that of its environment, those within the Indo-Pacific Coral Triangle the most diverse of all. Intense competition in such environments leads to niche partitioning through resource acquisition, typically leading to coevolutionary divergence. Such diversification, the pre-requisite for speciation, can occur with or without extrinsic barriers to gene flow and is particularly marked in sessile modular organisms such as cnidarians, far from passive to their environment (Cossins et al. 2006) in terms of growth form and chemical complement. However, delimitation between closely related species across steep environmental clines on coral reefs may be confounded by phenotypic plasticity, homoplasy, cryptic and sibling taxa (Knowlton 1993). It is, therefore, necessary to define environmentally driven divergent mechanisms on select phenotypic traits to accurately assess species biodiversity and endorse effective conservation management strategies (Taylor et al. 2006, Ladner & Palumbi 2012).

Gorgonian corals (Cnidaria: Anthozoa: Octocorallia) are conspicuous modular organisms, the greatest diversity occurring within the Indo-Pacific coral triangle yet remarkably 'poorly known' (van Ofwegen 2004). Intra- and inter-specific morphological variability in gorgonians is influenced by environmental factors such as light, temperature, sedimentation and flow rates. However, little is known about the responses of gorgonian taxa to environmental parameters within the Coral Triangle. Distinct morphotypes of the isidid gorgonian *Isis hippuris* Linnaeus 1758 exist between healthy (Ridge 1) and degraded (Sampela) reefs within the Wakatobi Marine National Park (WMNP), SE Sulawesi, Indonesia; short-branched multi/planar colonies, and long-branched bushy colonies, respectively. Whether such morphological differentiation is a consequence of plasticity capacity, plasticity as an adaptation (Gotthard & Nylin 1995, Hoogenboom et al. 2008), or genetically fixed leading to two species, is unclear.

Isis hippuris within the WMNP may be indicative of 'robust' canalisation where morphotypes are in fact two previously diverged species through disruptive (in sympatry) selection on traits between environments (Schluter, 1998, 2001, Campbell, 2003). Alternatively, physiological developmental constraints may have become decanalised through an acute perturbation or more likely accumulative cryptic genetic variation leading to evolutionary capacitance breakdown with subsequent fixation through genetic assimilation, itself a consequence of epigenetic heritability. In the first scenario, existence in low water velocity, high turbid environments typical of lagoon, semi-lagoon or sea-grass beds, as seen in Sampela, gave rise to an accumulation of pre- or postzygotic isolation between populations leading to separate adaptive fitness peaks representing ecological niches of long standing. Divergent morphotypes would therefore be robust to environmental

change, maintaining native phenotypic traits. In the second scenario, cryptic variation more likely to be adaptive than random mutations, facilitate rapid mutation, as can acute perturbations (Flatt 2005). Either case can be accelerated by pleiotropy, linkage disequilibrium or concerted evolution (Sánchez & Lasker 2003) leading to population level genetic assimilation of a particular phenotype and thus provides a testable level of emergent trait integration. Moreover, phenotypic variation can be largely attributed to rapid evolutionary events (Eldredge & Gould 1972, Mattila & Bokma 2008, Simpson 2013).

The phenomenon of species delimitation in closely related modular organisms can be investigated through models of integration (Magwene 2001). Growth persists through the iterative addition of modules (e.g., polyps, branching properties), which may develop independently or in concert (by trait integration, see Magwene 2001, Sánchez & Lasker 2003, de Kroon et al. 2005, Sánchez et al. 2007) leading to differential integration in response to environmental perturbations. The co- and multi-variance of certain phenotypic traits may differ due to inextricably linked developmental (e.g., heterochrony; Sánchez 2004) or functional integration leading to patterns of diversity through plasticity or divergent selection directly (extrinsic) or indirectly (intrinsic) on traits between populations or subpopulations (Schluter, 2001, Turelli et al. 2001). By measuring five morphological traits in twenty-one Caribbean gorgonian species, Sánchez & Lasker (2003) revealed integration within both branching and polyp dynamics yet were independent of each other. Furthermore, colony form and growth via branching were interconnected through the ratio of 'mother' branches to 'daughter' branches (Sánchez et al. 2004). Whether this is replicated across all gorgonians i.e. from different regions and habitats, is unclear, however species-specific trait integration particularly in response to environmental change has been shown in other taxonomic groups (e.g., plants; Xu et al. 2012).

Isis hippuris may simply possess high plasticity capacity, itself an adaptation facilitating considerable physiological tolerance to environmental heterogeneity, not uncommon in gorgonians (West et al. 1993, West 1997, Brazeau & Harvell 1994, Kim et al. 1997, 2004, Skoufas 2006). Long-branched bushy, porous colonies reduce sediment settlement and maximise light capture through increased surface area and decreased self-shading in reduced light and water flow environments as seen in the scleractinian *Stylophora pistilata* Esper 1797 (Shaish et al. 2006). Whereas the densely packed short branches of planar colonies in high light and water flow, coupled with greater densities of small micro-skeletal elements (sclerites) provide mechanical strength (Grigg 1972, West et al. 1993, Kim et al. 2004, but see Skoufas 2006). Sclerites are key characters for species delineation within Octocorallia, those of the coenenchyme surface and polyps most

susceptible to environmental variation (Bayer & Stefani 1987). Reduced polyp density with depth as a function of light in zooxanthellate taxa (West et al. 1993, Kim et al. 2004, Prada et al. 2008, Prada & Hellberg 2013) increases photosynthetic gain through surface area, yet polyp dimensions decouple integration by remaining independent of branching dynamics in Caribbean taxa (Sanchez & Lasker 2003, Sanchez et al. 2004, 2007). A broad trait assessment including genetic analyses would therefore provide further insight into the relationship between *I. hippuris* and its environment, if such trait patterns are commensurate with those described in other gorgonians, and thus fixed within the phylogenetic group (e.g., Lasker & Sánchez 2003, Sánchez 2004, Sánchez et al. 2004, 2007).

A single representative of its genus, *I. hippuris* has a recognised plasticity (Wright & Studer 1889, Simpson 1906, Thomson & Simpson 1909, Bayer & Stefani 1987, Fabricius & Alderslade 2001) and taxonomic uncertainty (Watling et al. 2012), which obscures any possible species boundaries. In order to fully elucidate the nature of *I. hippuris* phenotypic variation between reef sites, a brief taxonomic and historical account of the genus is presented with subsequent investigation into potential adherence to previously documented lower taxonomic assignments.

The family Isididae Lamouroux 1812 [nom. correct. Kükenthal 1915 (pro Isidae Lamouroux 1812)], itself currently within the sub-Order Calcaxonia, is characterised by a unique axis of alternating calcareous internodes and proteinaceous (gorgonin) nodes giving a bamboo appearance. Calcareous internodes can be hollow or solid and are not sclerobastic (i.e., consisting of fused sclerites *sensu* the sub-group Scleraxonia). The Isididae was further subdivided into four currently accepted subfamilies (see Bayer & Stefani 1987, Alderslade 1998) primarily based on polyp retractability and sclerite composition and arrangement. Pertinent to this study, the sub-family Isidinae Lamouroux 1812 (*sensu* Studer 1887) is distinguished by small, warty and usually irregular sclerite forms and contains the genera *Isis* Linnaeus 1758 and *Chelidonisis* Studer 1890. Within the *Isis* genus 20 species have been assigned with currently only *Isis hippuris* Linnaeus 1758, the type species for the Isididae, Isidinae and genus *Isis* being widely accepted (Bayer & Stefani 1987, Fabricius & Alderslade 2001). *I. reticulata* Nutting 1910 and *I. minorbrachyblasta* Zou, Huang & Wang 1991 have occasional reference yet with some taxonomic misgiving (see Bayer & Stefani 1987, but also Mai-Bao-Thu & Domantay 1971), therefore are briefly discussed to investigate divergent character traits similar to those found within the WMNP.

Originally (pre-Linnaen) *Hippuris saxea* Clusius 1605 (“the stony horse-tail”), *Isis hippuris* Linnaeus 1758 has for centuries been admired for its distinct articulated axis (Figure 3.1a) and thus named after the most important ancient Egyptian deity, the Goddess Isis. To this day the axis can

be found in jewellery, art, souvenirs and even Royal collections; however, not until Ellis & Solander (1786) was this taxon documented with its somewhat uninspiring mustard-brown outer soft tissue (coenenchyme: Figure 3.2). Nevertheless, conspicuous on coral reefs and in sea grass beds throughout the Central Indo-Pacific, *I. hippuris* contains potent secondary metabolites with anti-viral (Bordeleau et al. 2006, Chen et al. 2011) and anti-cancer (Susilaningsih et al. 2009) properties, likely contributing to its ecological success (Chapter 2). That said, correct taxonomic assignment using multiple lines of evidence across morphotypes within different geographical locations, are necessary given the clear ecological, pharmaceutical and inherent conservation importance of *I. hippuris*.

Sub-Class OCTOCORALLIA

Order ALCYONACEA Lamouroux 1812

Sub-Order CALCAXONIA Grasshoff 1999

Family ISIDIDAE Lamouroux 1812

Sub-Family ISIDINAE Lamouroux 1812

Isis hippuris Linnaeus 1758

(Figure 3.1a)

See Bayer & Stefani 1987 for list of references [p. 55]

Type Material – Unfound, however 'authentic' specimens were collected and fully defined from Amboina, Indonesia (Milne-Edwards & Haime 1857).

Diagnosis – These arborescent colonies can be up to 1 m tall, planar or bushy with lateral or partially dichotomous branching but rarely anastomosing (net-like). Branch formations may also give a candelabrum appearance. The axis consists of alternating calcareous internodes that reduces to a fine rod through the non-scleritic and convex, dark proteinaceous (gorgonin) nodes, the former typically longer than the latter. Branching is internodal in single or multiple planes, the latter giving rise to the bushy appearance. Branch lengths and diameters are variable, and up to three short branches can arise per internode with some so close they appear nodal in highly

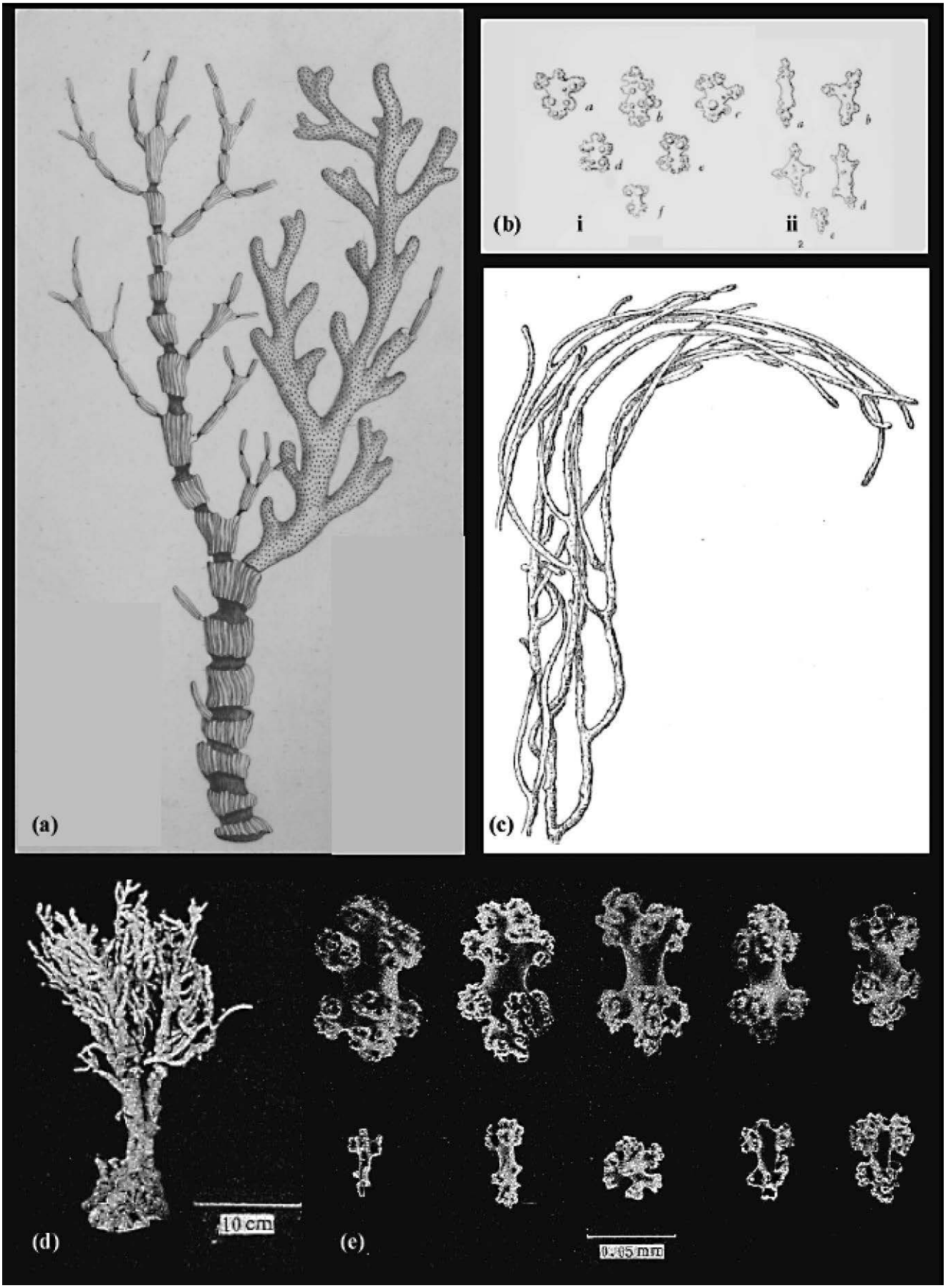

(a)
(b)
i
ii
(c)
(d)
10 cm
(e)
0.05 mm

Figure 3.1. (Previous page) *Isis* Linnaeus 1758 comparisons of (a) *Isis hippuris* Linnaeus 1758 colony in Ellis & Solander 1786; (b) sclerites of: i. *I. hippuris* and ii. *Isis reticulata* in Nutting 1910; (c) *I. reticulata* in Nutting 1910; (d) *Isis minorbrachyblasta* Zou et al. 1991 colony and (e) sclerites. Note, images sourced from each citation respectively.

branched colonies. The expanded calcareous cup-shaped base obliterates any trace of nodal composition particularly in older colonies. Calcareous internodes are sclerobastic (consisting of fused sclerites; but also see Nutting 1910c) with fibres arranged radially from a central core, akin to that of the sclerites bearing close resemblance to the Scleraxonia (Bayer 1955). Internodes possess longitudinal grooves corresponding with 8 – 12 sinuous water vascular canals of ~1 mm diameter. Polyps distributed all around the branches are 0.5 – 1.25 mm apart and fully retractile to ~1.25 mm deep (and wide) into the thick coenenchyme. Such polyps bear eight lanceolate pinnate tentacles, and can possess up to three large (max. 1 mm diameter) round eggs, likely explaining the often swollen appearance of the branch tips as opposed to being a diagnostic feature (but see Nutting 1910c, Mai-Bao-Thu & Domante 1971).

A diverse range of sclerites (Figure 3.1b, e) exists within the thick coenenchyme. The surface layer consists of small warty clubs 0.08 x 0.001 mm [note: typographical error pg. 55, 2nd paragraph, Bayer & Stefani 1987] typically bearing three large warts below the head wart (Figure 3.1b.i). Throughout the sub-surface layer some or all of the following are present in varying dimensions, asymmetry and commonly girdled: 6-, 7-, or 8-radiate capstans up to 0.19 mm in length, dumbbells/double heads (considered derivatives of 6-radiates; Bayer & Stefani 1987) up to 0.32 mm long, warty or tuberculate spindles up to 0.25 mm long, and crosses. Very small e.g. rods of 0.07 x 0.01 mm (Bayer & Stefani 1987), or no sclerites may be present within the polyp structures (Kölliker 1865). However smaller forms from those found within the coenenchyme (Simpson 1906, Thomson & Simpson 1909, Kükenthal 1919, 1924) as well as small warty clubs with short handles ~0.055 x 0.045 mm located within the tentacles have been reported (Simpson 1906, Thomson & Simpson 1909).

Sclerites colourless and colonies typically light brown to mustard yellow, with slightly darker polyps.

Distribution – Central Indo-Pacific including Great Barrier Reef, Vanuatu, Papua New Guinea, Indonesia, Malaysia, Andaman, Philippines, Taiwan, Palau, South China Sea, Japan including Okinawa and the Ryukyu Islands.

Remarks – As evident from the description above, substantial phenotypic plasticity, from colony and branching structure to sclerite composition, exists in described specimens of *I. hippuris* (Wright & Studer 1889, Simpson 1906, Thomson & Simpson 1909, Bayer & Stefani 1987, Fabricius & Alderslade 2001). Whether such plasticity is a consequence of environmental influence within and between its distributions, or significantly structured to be more than one species is unclear. Thus, for such a ubiquitous and well-known species it "has been very imperfectly described" (Thomson & Simpson 1909) leading to "a slender basis on which to raise a superstructure of classification" (Wright & Studer 1889). Clearly a revision of the *Isis* genus is required including thorough analyses of specimens throughout its geographic range, as such character trait variability may be ecologically dependent (Bayer & Stefani 1987). Attempts, however, have been made to differentiate phenotypic patterns within the *Isis* genus that, even though somewhat tenuous, may equate to the morphotypes found within the WMNP (Chapter 2). *I. reticulata* Nutting 1910 and *I. minorbrachyblasta* Zou, Huang & Wang 1991 are therefore summarized below highlighting differences between the selected taxa.

Isis reticulata Nutting 1910

(Figure 3.1b)

See Mai-Bao-Thu & Domantay (1971) for list of references [p. 28]

Type Material – Syntypes: several fragments of varying sizes, ZMA COEL. no. 2721, Siboga Expedition, station 149 or 273 at Pulu Jedan, Aru Islands, Maluku, Indonesia, 13 meters on sand and shells. Fragment donated to State University of Iowa (van Soest 1979). Specimens not located on request.

Diagnosis – Slender colonies, typically arborescent with long slim terminal branches that are not swollen at the ends. Few very small polyps irregularly distributed around the branches, the latter occasionally anastomosing. Sclerites of the coenenchyme bear sharp rough warts symmetrically distributed around delicate spindles and clubs the latter 0.04 – 0.06 mm in length. Some spindles curved possessing large tubercules. Irregular radiates 0.06 x 0.03 mm to 0.2 x 0.1 mm in length and

width respectively, smooth warty rods 0.1 - 0.15 mm long and occasional crosses present. No polyp sclerites reported.

Sclerites colourless, colony reddish brown with slightly darker polyps in alcohol. Also noted as "brownish white" by Mai-Bao-Thu and Domantay (1971).

Distribution – I. reticulata has been documented in Indonesia (fragments from a single location 13 m depth), the Philippines (2 specimens and some fragments from a single location 12 - 15 m depth; Mai-Bao-Thu & Domantay 1971), and Xisha Islands of China (single specimen and location; Zou, et al. 1991).

Remarks – I. reticulata is thus differentiated from *I. hippuris* on the basis of planar *versus* bushy colonies, long thin sinuous branches without swollen ends *versus* short thick antler-like branches with swollen ends, and all sclerites of a smaller size with sharp rough warts in *I. reticulata*. Sclerite differences between *I. hippuris* and *I. reticulata* have been considered questionable owing to the huge diversity in form (Stiasny 1940, Bayer & Stefani 1987). However, Nutting (1910c) observed smaller and more sharply warted sclerites further corroborated by Kükenthal (1924) and Mai-Bao-Thu & Domantay (1971), with illustrations showing marked asymmetry (Figure 3.1b.ii) contrary to that described. Curiously, Nutting (1910c) noted *I. reticulata* having flaccid polyps if preserved when extended due to their lack of sclerites. However, in *I. hippuris*, polyp sclerites were "not being evident on account of their small size" (Nutting 1910c) with no further discussion, lending question to their presence at all (Simpson 1906, Simpson & Thomson 1909, Fabricius & Alderslade 2001, but see Bayer & Stefani 1987, Kölliker 1865). Conflicting sclerite images between Nutting (1910c) and Mai-Bao-Thu & Domantay (1971), in addition to regional differences between specimens of *I. hippuris* (see Bayer & Stefani 1987) having some adherence to *I. reticulata*, lends further question to its validity as a taxon. Finally, colony and polyp colouration may be an artifact of preservation; Nutting's 'pink' likely from buffered formalin used at that time (note: Rowley, S.J., examined a 'pink' specimen from the Siboga expedition at the British Natural History Museum [BNHM. 1889.6.28.18], which adhered closely to the *I. hippuris* description above and not the proposed *I. reticulata*), and Mai-Bao-Thu & Domantay's 'white' from endosymbiotic bleaching not uncommon with, in particular, damaged *Isis* specimens (e.g., Thomson & Simpson 1909). In summary, the distinction between *I. hippuris* and *I. reticulata* is conflicting and unclear, requiring further investigation.

Isis minorbrachyblasta Zou, Huang & Wang 1991

(Figure 3.1c)

Type Material – Holotype (G85-001) and paratype (G87-031) from two locations of the Nansha Islands, China.

Diagnosis – Colonies bushy with distal branches densely aggregated, themselves bearing tufts of branchlets no longer than 5 cm (ave. 3.5 cm). The short, fine branches arise from the scleritic internodes. Tiny polyps are equally distributed around the branches. Coenenchyme sclerites up to 0.140 x 0.091 mm being predominantly dumbbells and double heads with tubercules generally symmetrically arranged. Assortment of small clubs also present 0.06 x 0.025 mm with occasional crosses.

Sclerites colourless, colonies light brown in alcohol.

Distribution – Nansha Islands, China.

Remarks – Zou et al. (1991) state that the bushy non-planar colonies of *I. minorbrachyblasta* differ from the planar ones of *I. hippuris* and *I. reticulata*, in direct contrast to previous reports (e.g., Nutting 1910, Mai-Bao-Thu & Domantay 1971). Furthermore, the branches for *I. minorbrachyblasta* are fine, short and densely packed, whereby *I. hippuris* and *I. reticulata* are thick, short, dense, and fine, long, anastomosing and loosely packed respectively; thus *I. minorbrachyblasta* an intermediate between the two. Statistical significance between select morphological traits (branchlet and sclerite length and width) revealed differences among taxa were between *I. minorbrachyblasta* and *I. reticulata*. However, it is unclear what sclerites were used for comparative analyses, and n = 1 in all cases. Based on the information presented here, any appreciable difference in colony, branch and sclerite composition especially between *I. minorbrachyblasta* and *I. hippuris* (e.g. Nutting 1910, Bayer & Stefani 1987) is nebulous. Finally, Zou et al. (1991) propose *I. minorbrachyblasta* based on one or two specimens per taxon, somewhat unsatisfactory given both the nature of *Isis* phenotypic variability and analyses taken from a single region.

Isis *within the Wakatobi* - *Isis* morphotypes found within the WMNP bear only partial adherence to those described above at the colony level. The long-branched bushy colonies on degraded reefs, and short-branched multi/planar colonies on healthy reefs may reflect *I. reticulata* and *I. hippuris* respectively, or simply the widely accepted plasticity of the latter (Wright & Studer 1889, Simpson

1906, Thomson & Simpson 1909, Bayer & Stefani 1987, Fabricius & Alderslade 2001), likely through an integration effect (Magwene 2001, Sanchez & Lasker 2003, de Kroon et al. 2005).

Clearly *Isis* taxonomy is in a state of flux, compromising conservation efforts due to difficulties in species assignment. Here an assessment of morphotypes found within the WMNP relative to reef health is presented, with brief comparisons to those previously described. A thorough and necessary examination of *Isis* specimens throughout its distribution is however, underway and outside of the scope of this study. Given the tenuous nature of previously described *Isis* species bar *I. hippuris*, from here on in all specimens will remain assigned to *I. hippuris*, unless otherwise specified.

Research Question - Are populations of *I. hippuris* morphotypes phenotypically and genetically subdivided relative to environmental gradients within the WMNP, Indonesia? Do the *I. hippuris* morphotypes represent previously described species, or a single species with highly variant, integrated phenotypic traits?

This study therefore, aims to: (1) investigate morphological variability in the zooxanthellate gorgonian *I. hippuris* across environmental gradients on coral reefs within the WMNP, SE Sulawesi, Indonesia; (2) identify patterns of genetic variability relative to such morphotypes using population genetics and predicted RNA secondary structure of the nuclear ribosomal ITS2 region, (3) to subsequently infer mechanisms of speciation or phenotypic plasticity as a consequence of environmental change, and (4) investigate the currently assigned phylogenetic position of *I. hippuris* within the Octocorallia using the ITS2 region.

3.2 METHODS

3.2.1 Study Area

The Wakatobi Marine National Park (WMNP) is a remote archipelago of ca. 13,900 km^2 in S.E. Sulawesi, Indonesia (Figure 2.1a). The epicentre of the Coral Triangle and Indonesia's second largest marine park, the WMNP comprises ca. 600 km^2 of the most biodiverse coral reefs on earth (Scaps & Denis 2007). Such marine biodiversity sustains >100,000 people with alarming human population expansion and consequential marine resource dependence and destructive commercial fisheries (Clifton et al. 2010, Clifton 2013). Coral reefs within the Wakatobi range from low current, high turbidity lagoons to highly exposed sites with strong water currents and high nutrient deep-water upwellings. Therefore, strong environmental gradients of natural and anthropogenic

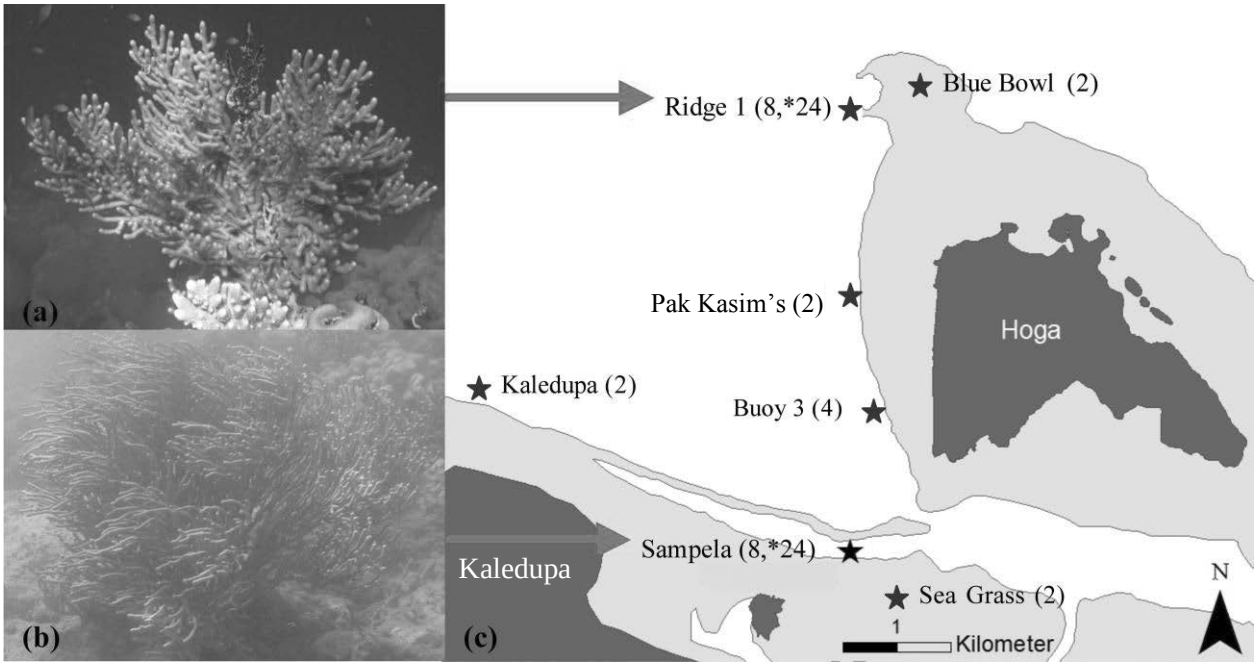


Figure 3.2. *Isis hippuris* morphotypes: (a) short branched predominantly planar or multiplanar colonies at the healthy site Ridge 1, and (b) long branched bushy colonies at the impacted site Sampela, with additional (c) collection localities within the WMNP. Sample number in brackets for molecular and asterisk for morphological analyses.

disturbance exist across reefs within the Wakatobi, providing a novel natural laboratory for studies of environmentally induced change on reef components.

Research was conducted between two sites spanning 5 km of anthropogenic and natural environmental gradients during July and August 2010. Ridge 1 (healthy) is an exposed reef ridge with high nutrient upwellings and water currents; Sampela (impacted) is a semi-lagoonal reef with low water flow and high turbidity. Sampela is situated ca. 400 m from a Bajo (sea gypsy) village of ca. 1600 people and subject to continuous marine resource exploitation and community waste disposal. Previously informative environmental variables driving morphotype distribution for the two study sites (summarized in Table 3.1 adapted from Table 2.1) were selected for further analyses. Light ($K_{d(PAR)}$) was measured using HOBO® data loggers, turbidity (NTU) expressed as an inverse values, and chlorophyll-*a* (µg L^{-1}) were measured using an RBR® XR-420 CTD data logger, a General Oceanics® flow meter was used to measure water flow velocity, and sediment grain size was estimated using Retsch Technology® test sieves, with logarithmically converted diameters expressed as *phi* (Φ) and classified using the Wentworth scale (Wentworth 1922). Environmental variables, with the exception of latitude and longitude,

Table 3.1. Environmental characteristics of the two study sites in the WMNP, Indonesia. All values expressed as mean (± SE). Data summarised of significant variables from Table 2.1.

Parameter Recorded	**Mean value ± SE (where appropriate)**	
Site	**Sampela**	**Ridge 1**
Latitude (S)	005˚ 29'01"	005˚ 26'57"
Longitude (E)	123˚45'08"	123˚45'38"
Temperature (˚C min-max)	25.61 – 29.36	24.06 – 28.07
Light ($K_{d(PAR)}$ min-max)	0.31 – 3.14	0.1 – 1.56
Flow (cm/s)	5.02 ± 2.18	30.54 ± 2.61
Chlorophyll-*a* (µg 1^{-1})	0.3 ± 0.01	0.35 ± 0.03
Turbidity (NTU)	4.38 ± 1.80	0.17 ± 0.33
Sedimentation (g d^{-1}, n = 12)	3.28 ± 0.26	1.16 ± 0.07
Sediment grain size (Φ, n = 12)	5 [31.25–62.5 µm]	1 [0.5–1 mm]

were edited visually with significant outliers removed, and entered into statistical models as raw values.

3.2.2 Sample Collection

Isis hippuris colonies were sampled from the healthy site Ridge 1 (n = 24) and the anthropogenically impacted site Sampela (n = 24; total n = 48), where the two distinct morphotypes at densities of 18 and 6 colonies per 10 m^{-2} respectively were previously documented (Chapter 2; Figure 3.3). A further twelve clippings were taken from five additional sites (Blue Bowl n = 2; Pak Kasim's n = 2; Buoy 3 n = 4; Kaledupa n = 2; Sea Grass beds n = 2; Figure 3.2c) to investigate and compare genetic differences between colonies within the study area. Sample numbers for molecular analyses were low due to financial constraints, yet provide valuable insights for further study. All colonies were randomly selected within 2 - 5 m depth and a minimum of 10 m distance apart to avoid sampling asexual clone mates. Each colony was subject to *in situ* scaled digital photography using a Canon IXUS 900Ti, WP-DC7 u/w housing and INON UWL-105 AD x 0.51 lens, with duplicate samples preserved in 96% EtOH and Guanidinium solution for morphological and molecular analyses respectively. Colonies were photographed both parallel and overhead for planar and bushy colonies as appropriate with a ruler for scale.

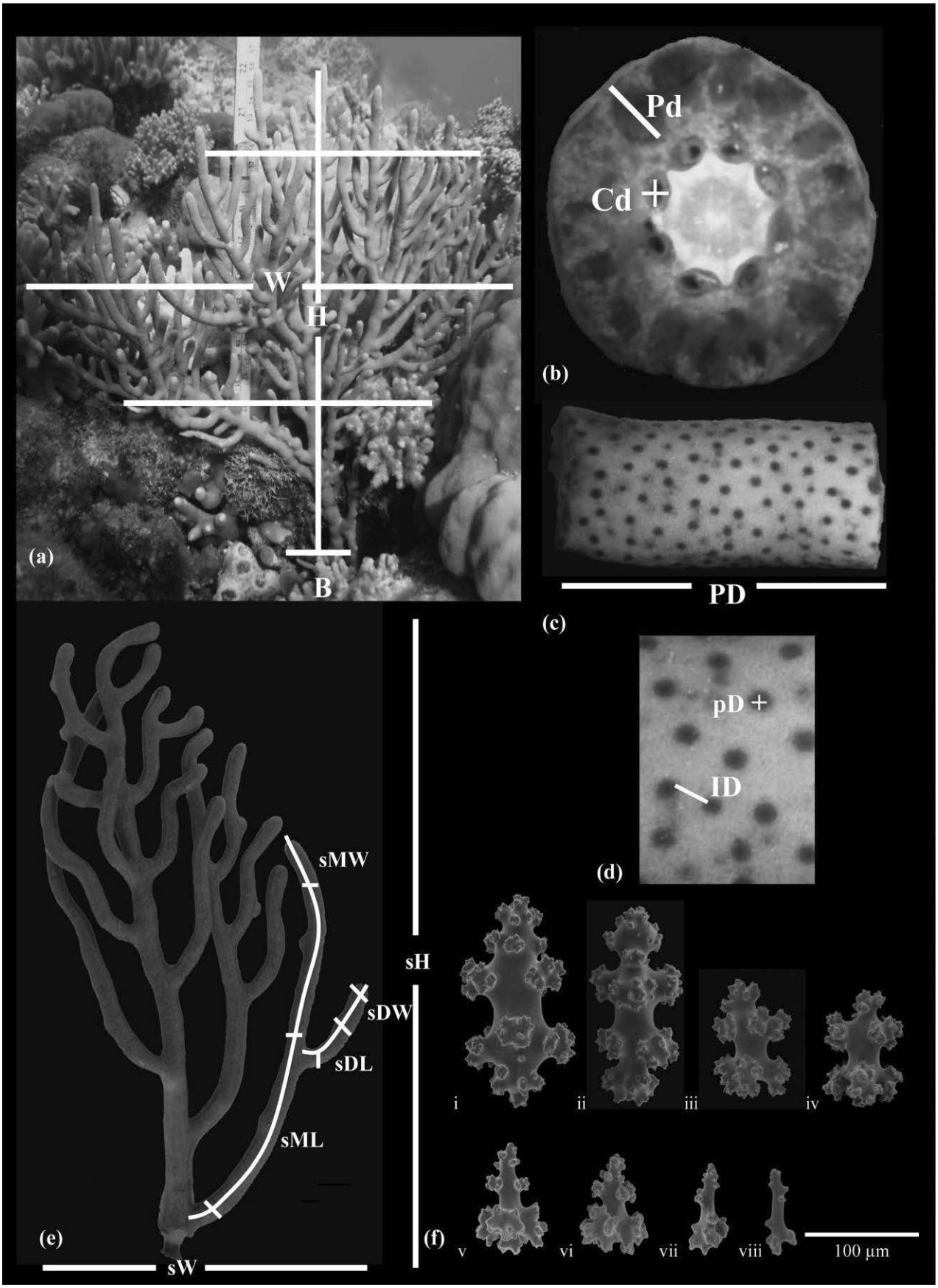

W
H
B
(a)
Pd
Cd
(b)
PD
(c)
pD
ID
(d)
sMW
sDW
sDL
sML
sW
(e)
sH
(f)
i
ii
iii
iv
v
vi
vii
viii
100 µm

Figure 3.3 (Previous page). *Isis hippuris* morphological trait measurements of the (a) colony; (b, c, d) canal and polyp dynamics; (e) sub-colony (branching) dynamics; (f) sclerites site/morphotype comparisons of i and ii spindles, iii and iv capstan 7-radiates, v - viii clubs from Ridge 1 and Sampela respectively. All abbreviations are described in Table 3.2.

3.2.3 Data Analyses

Morphological Measurements

Population comparisons of morphological traits were conducted on 24 *I. hippuris* colonies from both Ridge 1 and Sampela (total 48 colonies). A total of 57 morphological traits were quantified and divided into 32 macro- and 25 micro-morphological traits (Figure 3.3; Table 3.2). Due to the variable nature of *I. hippuris* colonies (planar, multiplanar or bushy), particularly between sites, whole colony height [H], mean width [W] taken equidistant apart, and colony spread [CS] were measured with CS as the mean of two measurements taken above the colony. Branch tips [T], mid main branch [M] and base [B] width were also recorded but limited access to the latter meant data were omitted from further analyses (n = 13 & 5 for Ridge 1 & Sampela respectively). Colony sub-sections of ~20 cm in height were selected for further comparable macro-morphometric analyses: sub height [sH], mean width [sW], and projected sub-colony area [PA] estimated by sH x sW which was then used to calculate sub-colony porosity [Po] as a ratio of PA and the projected branch area [PBA] itself the total branch length multiplied by the mean branch thickness (see below). Branch articulation was assessed using a hierarchical generation ordering system (Lasker et al. 2003, Sánchez & Lasker 2003, Sánchez et al. 2003a) where each branch was ascribed as either a 'mother' branch or 'daughter' branch, the latter emerging from the former. As the colony develops, daughter branches may also become mother branches (e.g. second generation mother branch; see Figure 3.3) quantified as follows: mother branch length [sML], mean mother branch width [sMW], daughter branch length [sDL], mean daughter branch width [sDW], total branch number [sTB#], total branch length [sTBL], and mean branch width [MBW]. Branch surface area was calculated on the geometric approximation of a cylinder from branch length and mean width as the radius, with subsequent polyp density [PD] per cm^2. Twenty random measurements were made of both polyp diameter ([pD] mean of 2 measurements see Figure 3.3) and inter-polyp distance [ID]. All polyp, branch cross-section and canal [C#/Cd; Cd see Figure 3.3] quantification were visualised under an Olympus SZX16® stereomicroscope at 10x magnification with 0.5x objective.

Isis hippuris has considerable diversity in sclerite form (Simpson 1906, Bayer & Stefani 1987, Fabricius & Alderslade 2001). For consistency, only those represented in all test colonies were

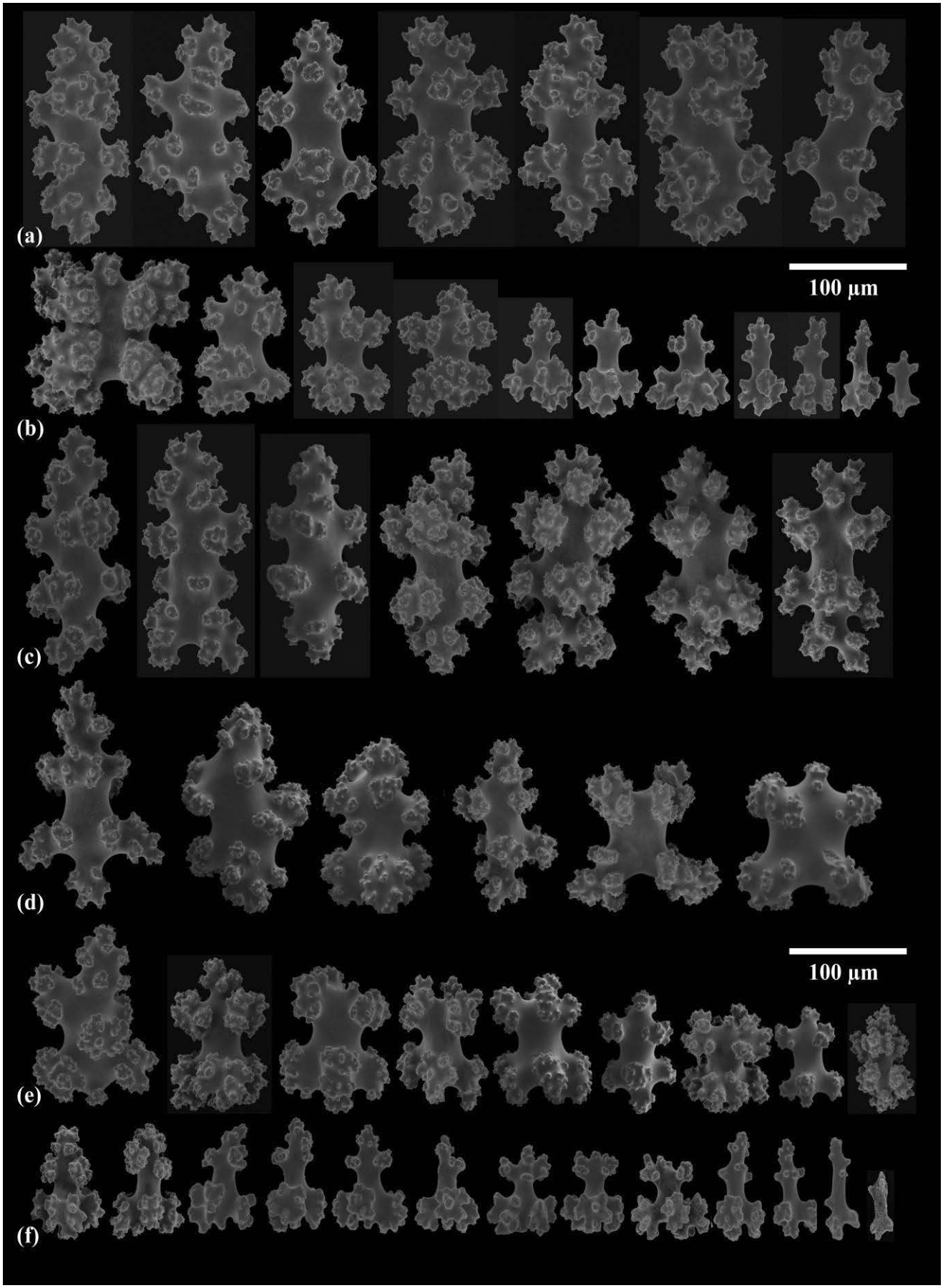

(a)
100 μm
(b)
(c)
(d)
100 μm
(e)
(f)

Figure 3.4 (Previous page) Scanning electron micrographs showing sclerite diversity of *Isis hippuris* from (a - b) Ridge 1 and (c - f) Sampela within the WMNP. Inner coenenchyme spindles (a, c, d), surface capstans and clubs (b, e, f). Small rods at the end of both (a & f).

selected for quantitative analyses. Length and mean width of three measurements were made on 20 randomly selected sclerites per sclerite type; surface clubs [CL1/2, CW1/2]; and sub-surface capstans [7-radiates: CaL/W] and spindles [SL/W](Figure 3.3). Additional sclerite diversity is shown in Figure 3.4. Sclerites were removed by dissolving the surrounding tissue in 5% sodium hypochlorite solution and visualized using optical microscopy (Olympus BX51®) and scanning electron microscopy (SEM) which was performed on a Hitachi S-800 SEM at the University of Hawai‘i at Mānoa, USA. All micro-morphological measurements and sclerite preparation were taken 2 cm below the branch tip to avoid underdeveloped traits due to sub-apical branch growth (Lasker et al. 2003) and photographed using an Olympus 3.3MPX™ camera and Rincon software (ImagingPlanet®). All macro- and micro-morphological characteristics were measured using ImageJ64 (Abràmoff et al. 2004).

Phenotypic traits were analysed using routines within the PRIMER-E v6.1.12 statistical package (Clarke and Gorley 2006), with PERMANOVA+ v1.02 extension (Anderson 2001). Specifically, character traits (untransformed) were simultaneously correlated in a Draftsman plot to eliminate uninformative traits ($P > 0.95$) and to establish appropriate transformation in downstream analyses (Clarke and Ainsworth 1993). Informative phenotypic trait data were subsequently standardized and a ‘zero-adjusted’ Bray-Curtis similarity matrix (Clarke et al. 2006b) constructed for tests of morphological divergence between the two sites; Ridge 1 and Sampela. A single-factor model with 9999 permutations (PERMANOVA; Anderson 2001) was performed and further visualised utilizing non-metric multidimensional scaling (nMDS) and constrained canonical analysis of principal coordinates (CAP; Anderson & Willis 2003). Informative traits contributing most to the dissimilarities between sites, thus specific morphotypes were investigated using similarity percentages (SIMPER; Clarke 1993) and displayed as a vector overlay on the CAP ordination.

The relationship between *I. hippuris* morphotypes and their environment was investigated using nonparametric multivariate regression (McArdle & Anderson 2001) with the DISTLM*forward* routine (Anderson 2003). Based on a Euclidean distance matrix, all raw environmental variable data were normalised (Table 3.1) and significance tested using 9999 permutations (Anderson 2001).

Table 3.2. *Isis hippuris* morphological traits summary table. All values expressed as metric or counts (± SE). Asterisk (*) indicate significantly (< *P* 0.05) informative traits selected for multivariate analyses. † Depicts low sample size.

Morphological Trait		Measures/ Counts	Dimensions (± SE) Ridge 1	Dimensions (± SE) Sampela
Macromorphology (cm)				
Colony				
*H	Colony Height	48	49.74 ± 3.99	58.78 ± 3.10
*W	Colony Mean Width	48	39.65 ± 2.24	58.03 ± 2.94
*w1	width 1	144	40.53 ± 3.24	65.31 ± 5.35
*w2	width 2	144	57.02 ± 3.06	76.74 ± 3.6
*w3	width 3	144	22.0 ± 1.35	31.86 ± 1.93
*CS	Colony overhead Spread	48	45.5 ± 5.10	78.86 ± 3.10
*cs1	colony spread 1	48	52.06 ± 5.34	78.86 ± 3.92
*cs2	colony spread 2	48	38.94 ± 4.85	62.12 ± 3.99
†B	Colony Base Width	18	3.47 ± 0.40	5.43 ± 0.64
*M	Colony Mid Branch Width	48	1.10 ± 0.08	1.53 ± 0.48
*T	Colony Tip Branch Width	960	0.63 ± 0.02	0.95 ± 0.05
sub Colony				
*sH	sHeight	48	10.8 ± 0.33	11.55 ± 0.24
*sW	sMean Width	48	3.15 ± 0.21	2.66 ± 0.17
sw1	swidth 1	144	2.50 ± 0.14	2.46 ± 0.15
sw2	swidth 2	144	4.18 ± 0.28	3.37 ± 0.22
sw3	swidth 3	144	2.76 ± 0.32	1.94 ± 0.23
sML	sMean Mother Length	48	8.41 ± 0.33	9.77 ± 0.35
*sMW	sMean Mother Width	48	0.44 ± 0.02	0.55 ± 0.02
mw1	mwidth 1	144	0.51 ± 0.01	0.62 ± 0.03
mw2	mwidth 2	144	0.48 ± 0.01	0.57 ± 0.02
mw3	mwidth 3	144	0.42 ± 0.01	0.54 ± 0.02
*sDL	sMean Daughter Branch Length	48	3.08 ± 0.21	4.44 ± 0.18
*sDW	sMean Daughter Branch Width	48	0.42 ± 0.01	0.48 ± 0.01
dw1	dwidth 1	480	0.44 ± 0.01	0.51 ± 0.01
dw2	dwidth 2	480	0.41 ± 0.01	0.49 ± 0.01
dw3	dwidth 3	480	0.38 ± 0.01	0.45 ± 0.01
*MBW	Mean Branch Width	48	0.43 ± 0.01	0.52 ± 0.01
*sTB#	sTotal Branch#	48	13.5 ± 1.10	10.83 ± 1.42
sTBL	sTotal Branch Length	48	51.86 ± 3.49	52.57 ± 3.76
*PA	Projected sub-colony Area	48	34.28 ± 2.65	30.79 ± 2.03
*PBA	Projected Branch Area	48	22.41 ± 1.69	26.96 ± 2.06
*Po	Porosity	48	1.61 ± 0.10	1.19 ± 0.06

Morphological Trait		Measures/ Counts	Dimensions (± SE) Ridge 1	Dimensions (± SE) Sampela
Micromorphology (mm)				
sub Colony				
*PD	Polyp Density	31,761	88.23 ± 2.6	100.21 ± 5.32
Pd	Polyp Depth	398	0.08 ± 0.001	0.08 ± 0.002
pD	Polyp Diameter	1920	0.04 ± 0.002	0.03 ± 0.001
ID	Inter-polyp Distance	1920	0.05 ± 0.002	0.05 ± 0.001
Cd	Canal diameter	1,920	0.02 ± 0.001	0.02 ± 0.002
C#	Canal#	1,677	8.17 ± 0.14	8.33 ± 0.21
Sclerites				
*CL1	Club Length 1	960	0.073 ± 0.001	0.068 ± 0.001
*CW1	Club Mean Width 1	48	0.021 ± 0.000	0.020 ± 0.000
c1w1	c1width 1	960	0.022 ± 0.001	0.021 ± 0.001
c1w2	c1width 2	960	0.012 ± 0.000	0.011 ± 0.001
c1w3	c1width 3	960	0.030 ± 0.002	0.028 ± 0.001
*CL2	Club Length 2	960	0.072 ± 0.000	0.068 ± 0.002
*CW2	Club Mean Width 2	48	0.033 ± 0.000	0.031 ± 0.001
c2w1	c2width 1	960	0.032 ± 0.001	0.030 ± 0.001
c2w2	c2width 2	960	0.016 ± 0.000	0.016 ± 0.001
c2w3	c2width 3	960	0.051 ± 0.001	0.047 ± 0.002
*CaL	Capstan Length	960	0.114 ± 0.004	0.101 ± 0.002
*CaW	Capstan Mean Width	48	0.075 ± 0.001	0.066 ± 0.001
*caw1	cawidth 1	960	0.086 ± 0.004	0.076 ± 0.003
*caw2	cawidth 2	960	0.041 ± 0.003	0.036 ± 0.020
*caw3	cawidth 3	960	0.090 ± 0.003	0.080 ± 0.002
SL	Spindle Length	960	0.164 ± 0.003	0.162 ± 0.003
*SW	Spindle Mean Width	48	0.069 ± 0.001	0.065 ± 0.001
sw1	swidth 1	960	0.038 ± 0.002	0.037 ± 0.002
sw2	swidth 2	960	0.080 ± 0.003	0.076 ± 0.002
*sw3	swidth 3	960	0.081 ± 0.003	0.077 ± 0.002
Total		**59,328**		

Genetics Analyses

Genomic DNA of *I. hippuris* and endosymbiotic dinoflagellates (for the latter see Chapter 4) were extracted from 28 colonies, 8 from each of the two test sites (n = 16) and 12 from additional site populations for area and morphotype comparison as described above. Approximately 2 - 3 mm of fresh soft tissue was immediately cut and stored in 400 µl Guanidinium lysis buffer (4 M guanidinium isothiocyanate, 0.05 M Tris pH 7.6, 0.01 M EDTA, 0.07 M Sarkosyl, β-mercaptoethanol 1% v/v) (Pochon et al. 2001) for 14 days at room temperature during transit from the field, with subsequent storage at 4ºC. Preserved samples were incubated at 72ºC for 20 min, vortexed prior, during and after incubation, then centrifuged at 16,000 *g* for 5 min. The resulting DNA-containing supernatant was precipitated with an equal volume of 100% isopropanol, vortexed and stored over night at -20ºC. DNA was pelleted via centrifugation at 16,000 *g* for 15 min, washed with 70% EtOH, centrifuged for 10 min, dried and resuspended in 0.1 M Tris Buffer pH 8. The DNA solution was placed on ice for 1 hour with frequent vortexing and stored at -20ºC. DNA was visualized on 1% agarose gel. PCR amplifications of the ITS2 rDNA marker were conducted using the primers itsD (forward; 5'-GTGAATTGCAGAACTCCGTG-3') and ITS2Rev2 (reverse; 5'-CCTCCGCTTACTTATATGCTT-3') (Pochon et al. 2005, 2007, 2010). Total PCR volume was 50 µl constituting: 5.0 µL of 10x PCR Buffer (Bioline), 2.0 µL of $MgCl_2$ (2 mM), 1.0 µL of each primer (10 mM), 1 µL (2.5 mM of each dATP, dCTP, dGTP, and dTTP), 0.2 µL of Hotstart Immolase *Taq* polymerase (Bioline Incl., London, UK), 1.0 µL of DNA, and 39 µL of sterile water. Touchdown amplification was conducted as follows: denaturation at 95ºC for 10 min, 25 cycles at 94ºC then 35 s at 65ºC (reduction in annealing temperature of 0.5ºC per cycle), and 2 min at 72ºC. A further 14 cycles of 30 s at 94ºC, 35 s at 52ºC, 2 min at 72ºC, and a final 10 min extension at 72ºC. All amplicons were purified using the QIAquick™ PCR Purification Kit (Qiagen), and separated by cloning for haplotype verification. Purified products were ligated into the pGEM®-T Easy vector™ (Promega), transformed into α-Select Gold Efficiency™ competent cells (Bioline), with subsequent positive inserts verified by PCR using plasmid specific primers (M13). Positive inserts (8-12 per library) were purified with an ExoSAP-IT kit, sequenced in both directions using the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit and run on an ABI 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) at the University of Hawai'i at Mānoa, USA.

ITS2 clone libraries from 28 individuals were aligned using ClustalW2 (Thompson et al. 2002) and manually edited in Geneious Pro v.5.6.2 (Biomatters Ltd., NZ). A selection criterion of

Table 3.3. ITS2 Accessions of octocoral outgroups used in the analyses.

Taxon	GenBank	Reference
[Group: Alcyoniinans]		
Family: Alcyoniidae Lamouroux 1812		
Alcyonium digitatum Linnaeus 1758	AF262347	McFadden et al. 2001
[Group: Scleraxonians]		
Family: Coralliidae Lamouroux 1812		
Corallium rubrum Linnaeus 1758	AF413059	Constantini et al. 2003
Corallium sp. 1	GQ358526	Herrera et al. 2010
Family: Paragorgiidae Kükenthal 1916		
Paragorgia kaupeka Sánchez 2005	GQ293292	Herrera et al. 2010
Sibogagorgia cauliflora Herrera, Baco & Sánchez 2010	GQ293288	Herrera et al. 2010
[Suborder: Holaxonians]		
Family: Gorgoniidae Lamouroux 1812		
Africagorgia schoutedeni Stiasny 1939	AY587533	Aguilar & Sánchez 2007a
Gorgonia flabellum Linnaeus 1758	AY587521	Aguilar & Sánchez 2007a
Leptogorgia violacea Pallas 1766	AY587527	Aguilar & Sánchez 2007a
Lophogorgia [Synonym of *Leptogorgia*] *euryale* Bayer 1952	AY587530	Aguilar & Sánchez 2007a
Pacifigorgia stenobrochis Valenciennes 1846	AY587531	Aguilar & Sánchez 2007a
Pinnigorgia platysoma Nutting 1910	AY587536	Aguilar & Sánchez 2007a
Pseudopterogorgia [Synonym of *Antillogorgia*] *bipinnata* Verrill 1864	AY587524	Aguilar & Sánchez 2007a
Family: Plexauridae Gray 1859		
Eunicea tourneforti Milne Edwards & Haime 1857	EF490982	Grajales et al. 2007
Muriceopsis bayeri Sánchez 2001	AY587538	Aguilar & Sánchez 2007a
[Suborder: Calcaxonians]		
Family: Isididae Lamouroux 1812		
Acanella weberi Nutting 1910	FJ790943	Dueñas & Sánchez 2009
Acanella sp.	FJ790921	Dueñas & Sánchez 2009
Isidella tentaculum Etnoyer 2008	FJ790944	Dueñas & Sánchez 2009
Keratoisis zelandica Grant 1976	FJ790939	Dueñas & Sánchez 2009
Lepidisis olapa Muzik 1978	FJ790908	Dueñas & Sánchez 2009
Family: Primnoidae Milne Edwards 1857		
Calyptrophora japonica Gray 1866	EF090735	Aguilar & Sánchez 2007a

identical sequences from two or more clone libraries was established to minimize the effect of intragenomic variation and/or PCR artefacts on further analyses. On average 4 - 6 host clones were recovered per library due to simultaneous recovery of both host and endosymbionts (see Chapter 4).

Estimates of genetic differentiation relative to morphotype were investigated via an analysis of molecular variance (AMOVA) with pairwise population comparisons (Φ_{ST}) between sites using ARLEQUIN v.3.5 (Excoffier & Lischer 2010). Haplotype (h_d), nucleotide diversity (π) and substitution rate (JC) were calculated with DNAsp v.5.0 (Librado & Rozas 2009). A parsimony haplotype network with a 95% confidence level and gaps treated as a fifth state was constructed using Network v.4.6.1.1 on sample sequences only.

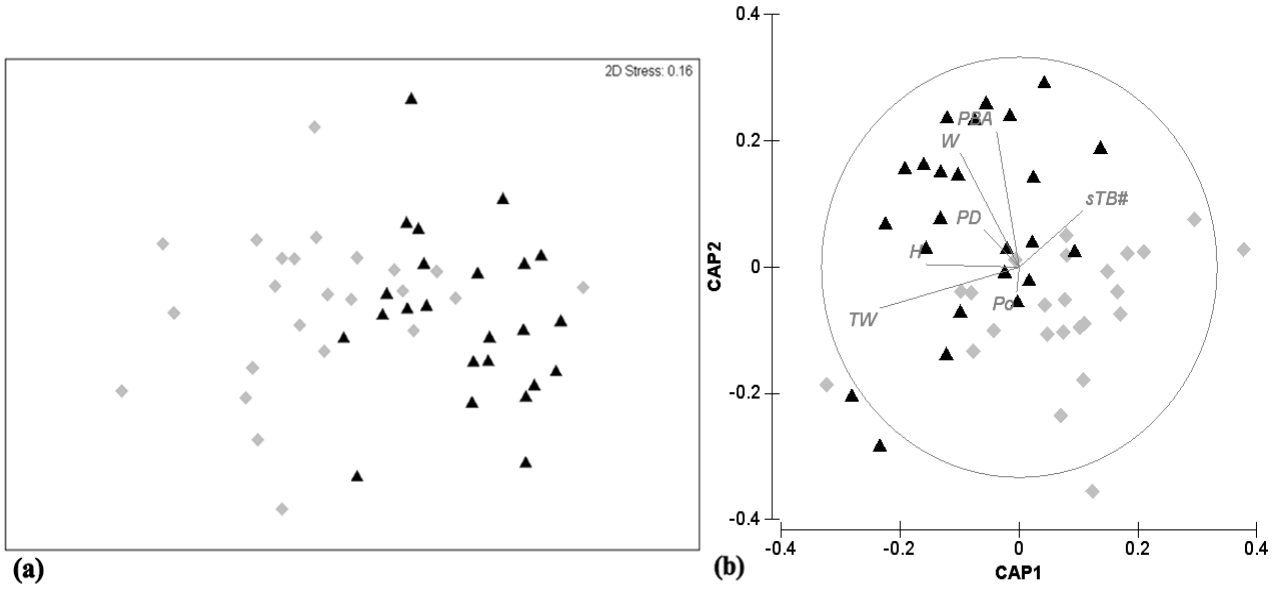


Figure 3.5. (a) Unconstrained and (b) constrained ordinations of *Isis hippuris* character traits between Ridge 1 [◆] and Sampela [▲].

ITS2 Predicted RNA Secondary Structure

ITS2 RNA secondary structures were predicted to further investigate haplotype differences specifically between Ridge 1 and Sampela at a more conserved level. *Alcyonium digitatum* Linnaeus 1758 (Genbank Acc. # AF262347; McFadden et al. 2001) was used as a template for conserved motif identification with subsequent constraints implemented into MFOLD (Zuker 2003) using default parameters. RNA was folded at 37°C and structures with the highest negative free energy values, thus stability, were selected, manually edited in 4SALE (Seibel et al. 2006, 2008) and visually annotated in VARNA (Darty et al. 2009).

Phylogenetic reconstructions between *Isis* haplotypes and twenty octocoral ITS2 outgroups obtained from GenBank (see Table 3.3) were conducted using the PHYML 2.1.0 (Guindon & Gascuel 2003) and MrBayes 2.0.5 (Huelsenbeck & Ronquist 2001) plugins within Geneious. Indels (insertion and deletion mutations) were considered phylogenetically informative and treated as separate characters using the 'simple indel coding' gap method (Simmons & Ochoterena 2000) in GapCoder v.1.0 (Young & Healy 2003). Maximum likelihood (ML) phylogeny was conducted using the best-fit model (JC) of nucleotide substitution as selected in jModelTest 2 (Darriba et al. 2012) through Akaike Information Criterion (AIC). Bayesian inference (BI) phylogeny was made with a JC69 substitution model and burn-in of 100,000. Phylogenetic trees were rooted with *Paragorgia kaupeka* Sánchez 2005 and node support values set at ≥ 70% for both ML and BI.

Table 3.4. *Isis hippuris* ITS2 haplotype sequence view from the seven test sites within the WMNP. Each sequence represents haplotypes present in each sample per site. Colour codes depict gaps (lilac), transitions (red), and transversions (yellow).

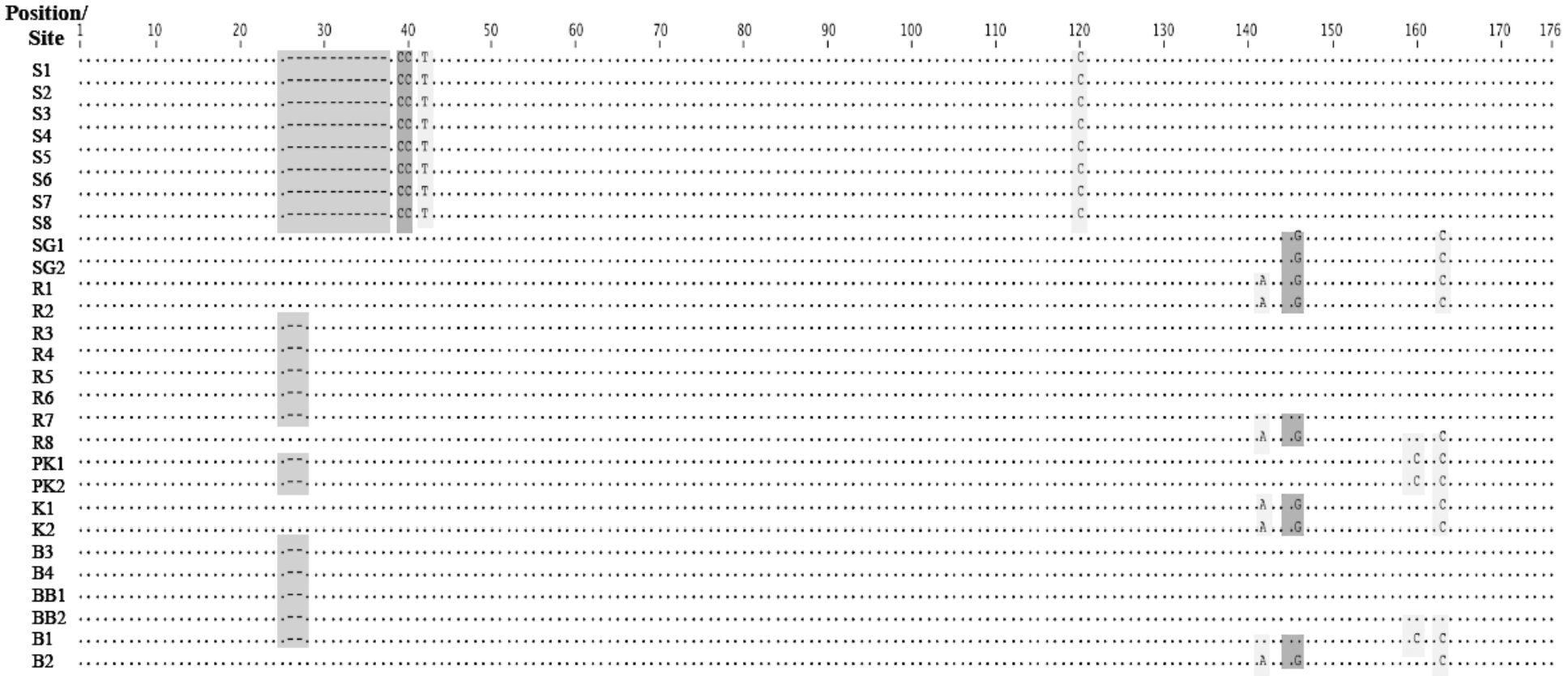


Table 3.5. AMOVA of genetic structure between sites within the WMNP from both cloned and sample sequences. R1 denotes Ridge 1; S denotes Sampela. *$P < 0.001$ significant.

Source of Variation	*df*	SS	Variance Component	Variance %	Φ_{ST}
7 Populations: Clones					
Among populations	6	122.248	$V_a = 1.237$	80.97	0.80974*
Within populations	113	32.844	$V_b = 0.291$	19.03	
Total	119	155.092	1.528		
7 Populations: Samples					
Among populations	6	33.768	$V_a = 1.401$	76.83	0.76831*
Within populations	21	8.875	$V_b = 0.423$	23.17	
Total	27	42.643	1.824		
2 Populations (R1 & S): Samples					
Among populations	1	17.688	$V_a = 2.161$	84.32	0.84321*
Within populations	14	5.625	$V_b = 0.402$	15.68	
Total	15	23.312	2.563		

3.3 RESULTS

3.3.1 Morphometrics

Of the 57 morphological traits measured, 32 were selected for further analyses (Table 3.2). Based on these traits, PERMANOVA revealed significant differences between morphotypes across the two study sites Sampela and Ridge 1 (pseudo-$F = 14.489$; $P < 0.0001$), further corroborated by the CAP analysis ($\delta^2 = 0.995$, $P = <0.0001$; Figure 3.5b) with 89% variance (% var.) as the total variance explained by the first *m* PCO axes. Prominent morphological traits contributing most to dissimilarities between sites were primarily at the colony level (TW, W, H, PBA, Po, & sTB#) with the exception of a higher polyp density (PD) at Sampela (Figure 3.5). From both Figure 3.5b and Table 3.2 it is clear that larger colonies present at Sampela have a reduction in branch density yet larger colony size and spread (PBA, sTB#, Po, and H, W, TW respectively). Branches were also consistently longer and thicker including the branch tips at Sampela, however polyp parameters were relatively invariable despite significantly high polyp density. It is noteworthy that all sclerite trait measurements were consistently smaller at Sampela (Table 3.2), particularly capstans (Figure 3.3f.iii, iv & 3.4 for variability) were variable throughout *I. hippuris* distribution (Simpson 1906, Bayer & Stefani 1987, Fabricius & Alderslade 2001). Yet irrespective of pre-treatment the magnitude of differences between sclerite measurements were not that of the colony level. Nevertheless, separation and re-analyses under the same models for macro (e.g., colony and sub-colony: pseudo-$F = 15.255$; $P < 0.0001$;

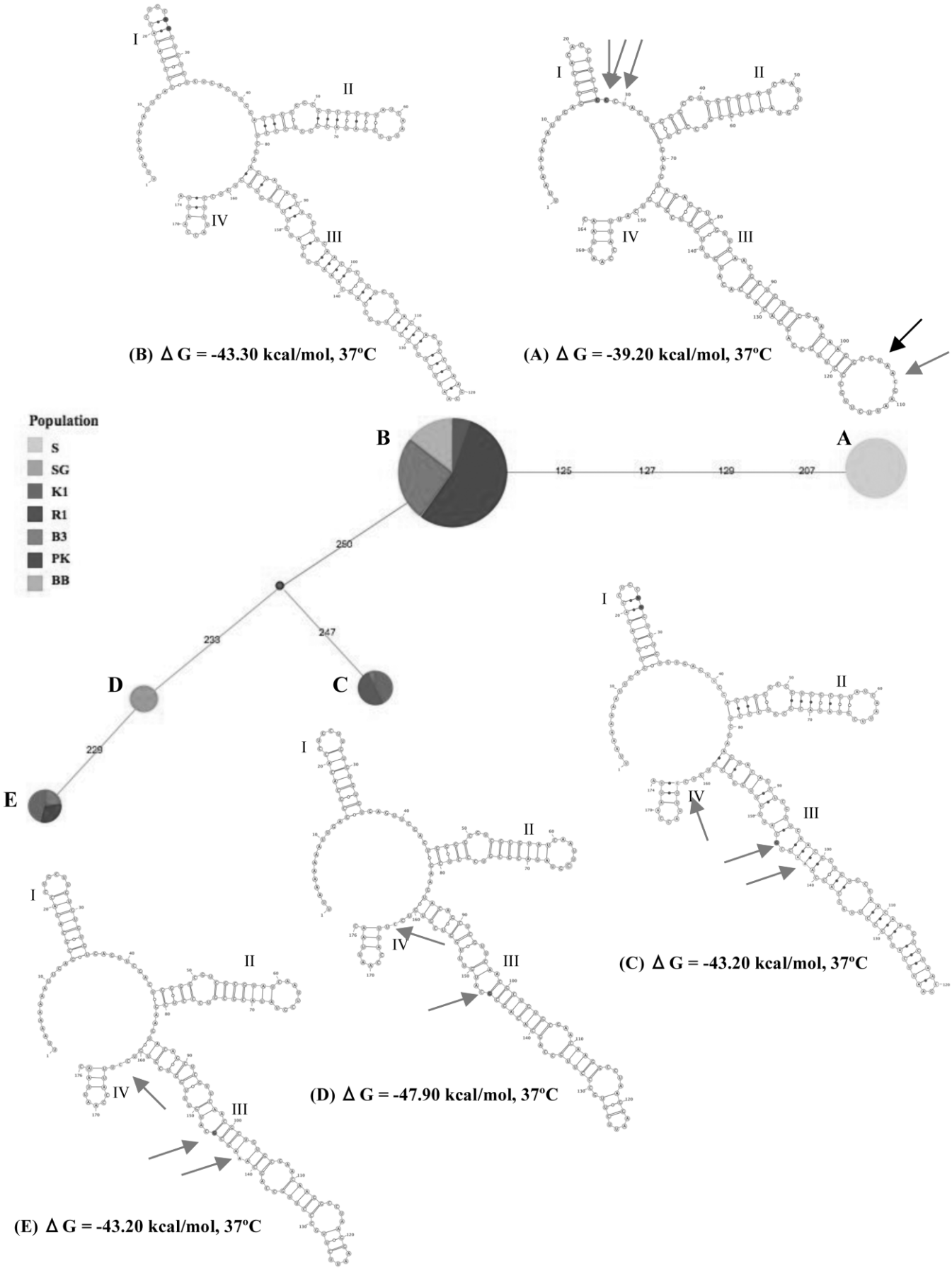

I
II
IV
III
(B) Δ G = -43.30 kcal/mol, 37ºC
(A) Δ G = -39.20 kcal/mol, 37ºC
Population
S
SG
K1
R1
B3
PK
BB
B
A
125
127
129
207
250
233
247
D
C
229
E
(C) Δ G = -43.20 kcal/mol, 37ºC
(D) Δ G = -47.90 kcal/mol, 37ºC
(E) Δ G = -43.20 kcal/mol, 37ºC

Figure 3.6. (Previous page) *Isis* haplotype network with corresponding ITS2 RNA predicted secondary structure relative to haplotype (A – E, see also Figure 3.7) and enthalpy values according to MFOLD. Roman numerals (I – IV) represent helices; red and black arrows indicate point mutations and loop differences respectively. Coloured bases according to transitions (red), transversions (yellow) and gaps (lilac), Table 3.5. Haplotype circle diameters are proportional to identical clone sequences.

CAP $\delta 2 = 0.976$, $P = <0.0001$, 91% var.) and micro (e.g. sclerite: pseudo-$F = 11.727$; $P < 0.001$; CAP $\delta^2 = 0.996$, $P = <0.0001$, 85% var.) measurements did not significantly alter results from the full model, demonstrating a lack of redundancy in selected character traits.

Results from the distance-based nonparametric regression (DISTLM*forward*) revealed that turbidity and sediment load explained 27.31% (pseudo-$F = 5.100$; $P < 0.001$) of morphotype differences between the two sites.

3.3.2 ITS2 Sequence Diversity

Of the 28 *Isis* samples 120 clones were recovered; Ridge (34), Sampela (29), Sea Grass (9), Kaledupa (12), Buoy 3 (18), Pak Kasim's (10), and Blue Bowl (8). ITS2 sequences revealed five haplotypes: 1-3 per sample population with up to 8 substitutions (Table 3.4, Figure 3.6). In keeping with morphological traits, colonies found at Sampela were significantly different from all other sample sites (Table 3.5, Figure 3.6). Haplotype diversity was greatest across Hoga Island with overall haplotype (h_d) and nucleotide diversity (π) measured as 0.780 and 0.0197 respectively with just (JC) 0.0313 substitutions per site. Population division was strongly inferred by all AMOVA models (Table 3.5) and haplotype network analysis, the latter showing no evidence of reticulation through homoplasy (Figure 3.6). Curiously, the single haplotype present in the sea-grass beds (D) shared no nucleotide differences with Sampela (A) despite its relatively close proximity, however little can be determined without greater sampling effort. Pairwise Φ_{ST} estimates of ITS2 sequences from Sampela ranged from 1.000 (Sampela vs. Sea Grass, Kaledupa, Pak Kasim's, Blue Bowl, Buoy 3) to 0.843 (Sampela vs. Ridge 1; $P < 0.0001$ in all cases), and from 0.467 (Ridge 1 vs. Pak Kasim's; $P < 0.05$) to no structure (Ridge 1 vs. Blue Bowl and Buoy 3) at Ridge 1. Note, such values, in particular the P – values, should be treated with caution. Low sample sizes reduce fine-scale structure detection, thus more data would likely yield greater insight into the level of haplotype and nucleotide diversity observed across sites with an increase in taxonomic certainty.

ITS2 predicted RNA secondary structure analyses revealed minimal variation between haplotypes with the exception of Sampela (Figure 3.6) providing greater confidence in phenotypic trait differences. Clones were collapsed into haplotypes per sample for phylogenetic analyses. Phylogenetic topologies using Maximum Likelihood and Bayesian Inference were very similar including all outgroups and unambiguously identical with regard to WMNP haplotypes (Figure 3.7). Branch support was typically stronger with BI particularly regarding outgroup species where recognised taxonomic suborders and groups were distinct. Phylogenetic uncertainty leading to the addition of multiple outgroups, confirmed *Isis* sequences from the WMNP were not grouped with morphologically described sister taxa within the Isididae (highlighted red, Figure 3.7). Reducing the outgroup number did not alter the integrity of the phylogenetic signal, in fact irrespective of model or selected root *Alcyonium digitatum* Linnaeus 1758 consistently positioned directly above *Isis* haplotypes.

3.4 DISCUSSION

Isis hippuris morphotypes were clearly defined both morphologically and genetically between the two sites (Φ_{ST} = 0.7683, P <0.001). Even with a reduced ITS2 sample size, corroboration of both morphometric and molecular results reveal a powerful indication that divergence has or is taking place, the nature of which is unclear. Multivariate trait integration at the colony level (including branching parameters), polyp density and sclerite size define significant differences between morphotypes indicative of trait dependency, yet polyp dimension and canal width appear canalised (genetically fixed). Nevertheless, inherent phenotypic plasticity and/or disruptive selection may enhance the success of two phenotypes particularly across contrasting environments. Trophic level interaction through differential light and nutrient exposure may drive such phenotypic differences, further reinforced by population structure by asexual fragmentation and external brooding (Chapter 2 & 4). Taxonomic assignment maybe tenuous, however, considering the partial adherence of morphotypes to previously described species within the *Isis* genus in addition to polyphyly within the Isididae.

3.4.1 Isis: *1 species or 2?*

Of the 48 colonies (28 used for genetic analyses) studied here and 1094 recorded in Chapter 2, it cannot be said with confidence that *I. hippuris* morphotypes at either site within the WMNP adhere to the descriptions as outlined for *I. reticulata* (Nutting 1910, Kükenthal 1919, 1924, Stiasny 1940, Mai-Bao-Thu 1971) or *I. minorbrachyblasta* (Zou et al. 1991), and may just be an artifact of depth in those previously described. *I. hippuris* contrasts with *I. reticulata* on the basis

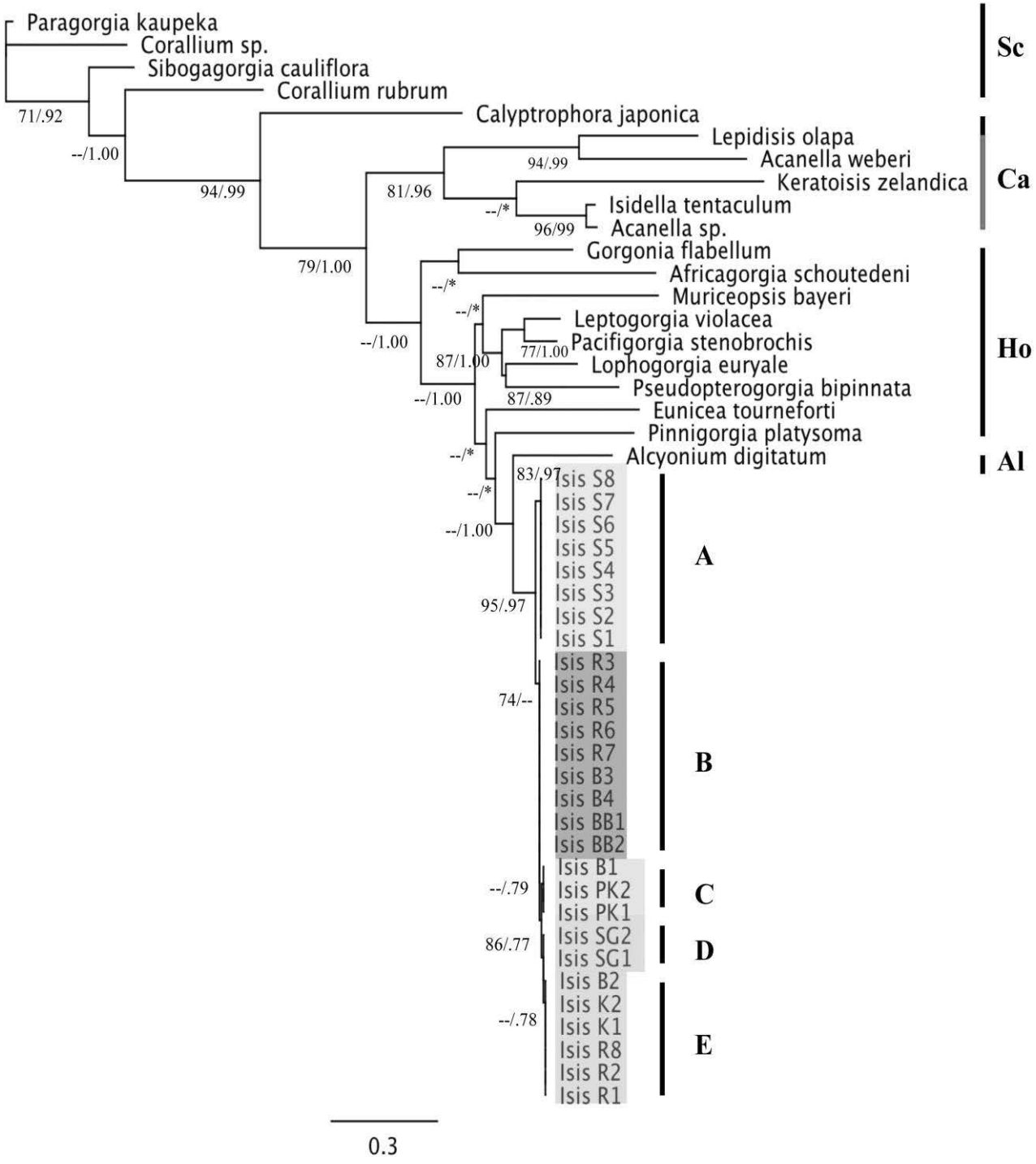


Figure 3.7. Phylogram based on maximum likelihood (ML) analyses of the ITS2 region from twenty Octocoral taxa in GenBank and *Isis* haplotypes within the WMNP. Branch numbers represent ML/BI support with low values expressed as a hyphen (--) ≤ 70% and asterisk (*) indicative of differences from MrBayes phylogenetic inference. Letters Sc = Scleraxonia, Ca = Calcaxonia, Ho = Holaxonia, Al = Alcyoniina, and A – E represent *Isis* haplotypes as depicted in Figure 3.6.

of short thick branches in the former, and long thin branches with smaller, coarsely articulated sclerites in the latter. *I. minorbrachyblasta* has bushy colonies with short densely packed branches, but considering both the lack of sampling for this taxon, documented panmixia and phenotypic plasticity of *I. hippuris* (e.g., Simpson 1906, Thomson & Simpson 1909, Bayer & Stefani 1987) in addition to the potential for parallel evolution throughout the *I. hippuris* distributional range relative to environmental clines, this latter taxonomic assignment is treated with extreme caution and may simply be an intermediate form. The two morphotypes of *I. hippuris* presented here have but partial adherence to those previously described. The short-branched predominantly planar colonies at Ridge 1 are more akin to *I. hippuris* whereas the more open, long-branched colonies at Sampela resemble *I. reticulata* but with thick branches as opposed to thin. Swollen branch tips characteristic of *I. hippuris* were observed in both morphotypes is not a reliable trait. Such swollen branch tips were more prevalent at Sampela, which was the only site where external brooding was observed (Chapter 4; Figure 4.5), and therefore, swollen branch tips may pertain to the presence of eggs within the polyps.

3.4.2 Isis hippuris *phenotypic variability*

Measuring a broad range of phenotypic traits between *I. hippuris* morphotypes highlights trait integration, canalisation and thus those acted on by selection which may differ from those previously described for other gorgonian taxa (e.g., Sánchez & Lasker 2003, Sánchez et al. 2003, 2004, 2007, Sánchez 2004, Dueñas & Sánchez 2009). Here clear patterns of colony, therefore branching, integration coupled with sclerite-level traits and polyp density are consistent between the two morphotypes. Specifically, branching dynamics and colony size (colony porosity as a function of total branch number and size [projected branch area]) appear to have a negative association with sclerite size. Whether these traits are negatively associated as emergent properties or of longstanding, would necessitate further investigation using reciprocal transplant experimentation and population coalescence (Prada et al. 2008, Prada & Hellberg 2013). In either case, differential light attenuation and nutrient components between the two sites are not unnatural phenomenon, which may or may not be exacerbated by the reef resource dependent anthropogenic influence from Sampela.

Colony surface area and metabolism are intrinsically linked whereby a cascade effect of concomitant variations in branching, polyp, canal and sclerite dynamics would be expected. However disintegration or canalisation was evident in both polyp and canal dimensions consistent with previous work (Sánchez 2004). Responses to variations in water quality, thus heterotrophic feeding capacity, are incurred through polyp density as opposed to size, yet both

canal number and dimensions remained unchanged in both morphotypes. The exact function of stem canals is unclear (Cadena et al. 2011), although suggested to circulate and exchange water and nutrients throughout the coral colony (Ellis & Solander 1786, Grillo et al. 1993, Gateño et al. 1998). Canalisation at this level further suggests that photosynthetic gain with nutrient translocation at the cellular level between endosymbionts and host, are the primary trophic resource. Optimal allocation theory posits an increase in the uptake of resource(s) that are most limiting growth (Bloom et al. 1985, Weiner 2004). Moreover, the same genotype can show resource allocation plasticity (Sebens 1997) in alternate environments consistent with the 'partitioning' hypothesis (Poorter & Nagel 2000, Weiner 2004). Plasticity as a response is an emergent property of divergence (Schlichting & Pigliucci 1998, Pigliucci 2005). Therefore to further elucidate energy allocation patterns between morphotypes, physiological tests coupled with morphological and genetic analyses on reciprocal transplants between reefs would establish phenotypic trait plasticity, thus plasticity capacity, or ecological divergence through disruptive selection (Schluter 1998, 2001) in *I. hippuris*.

Sclerite composition can vary relative to light and/or water motion (Muzik & Wainwright 1977, West et al. 1993, Kim et al. 2004, Skoufas 2006, Clavico et al. 2007, Prada et al. 2008, 2013). Here the presence of numerous small, articulate interlocking sclerites could provide additional structural support for larger colonies found at Sampela, which lack the close branching structure present at Ridge 1. Smaller sclerites may mitigate mechanical constraints on the axis of increased colony size and bushy morphology through long thick branches, and provide greater soft tissue support as surface area increases (Clavico et al. 2007). Small clubs increase both flexion and torsion capacity in less exposed areas of *Eunicella singularis* Esper 1791 whereas larger spindles were prevalent in the exposed peripheral branches, yet *Eunicella cavolinii* Koch 1887 showed no selective difference (Skoufas 2006). A decrease in sclerite size with increased density in shallow conspecifics has been shown (e.g., Prada et al. 2008), typically due to increased water flow (West et al. 1993, West 1997, Brazeau & Harvell 1994, Kim et al. 1997, 2004). Here, regardless of both morphotypes containing high densities of small sclerites, the consistency in small size at Sampela coupled with thicker longer branches and higher polyp density likely increases photosynthetic gain through greater surface area, as well as heterotrophic feeding. Canalisation due to a lack of variability in canal size or number, as well as polyp dimensions, suggests that photosynthetic gain from dinoflagellate endosymbionts is the primary resource for *I. hippuris*.

Sclerites are key characters for species identification yet susceptible to environmental

perturbation and selection. All sclerites were consistently smaller in colonies from Sampela. The overall dimensions between the two morphotypes from both sites were within those described for *I. hippuris* with regards clubs and radiates from both *I. reticulata* and *I. minorbrachyblasta*. Sclerite differences between morphotypes compared to those published appeared inconclusive with notable crossover. For example, bent spindles characteristic of *I. reticulata* were present in colonies from Ridge 1, themselves bearing closer resemblance to *I. hippuris*. Interestingly, sclerite diversity was greater at Sampela with closer resemblance to *I. reticulata,* particularly considering sclerite asymmetry and crosses. No sclerites were found within the polyps or tentacles in either morphotype in this study, unlike in *I. reticulata*. However the small rods (0.07 x 0.01 mm) of Bayer & Stefani (1987) were present, but their precise location within *I. hippuris* soft tissue could not be determined, questioning their presence at all.

Enhanced fitness through an individual's (genotype) capacity to respond to environmental heterogeneity - specific morphotypes predominating in certain habitats - maximises survival through resource acquisition and minimises metabolic costs. Most corals are polymorphic under varying environmental conditions (West et al. 1993), with differential phenotypic expression of a genotype as a consequence of astogeny (colony development), itself genetically and/or environmentally mediated (De Rosa et al. 1999, Sánchez & Lasker 2003, Garland & Kelly 2006). Environmental influences on larval settlement, such as high sedimentation at Sampela or competition and high water flow at Ridge 1, may lead to developmental adaptational responses. Moreover, *I. hippuris* colonies survive and replicate through external brooding and asexual fragmentation with a propensity for philopatry and upward growth (Dauget 1992), increasing population structure and expansion on such degraded reefs over time.

Phenotypic divergence and biological success in *I. hippuris* within the WMNP may be a consequence of intraspecific polymorphism due to plasticity capacity with no barriers to gene flow between morphotypes. This, in part, can be a consequence of epigenetic effects, which may be heritable and become fixed through genetic assimilation if conditions persist. This, particularly in the presence of evolutionary capacitance whereby suppressed variation becomes functionally overwhelmed or initiated by the environment, can exert pleiotropic effects on poignant developmental processes (Rice 2008). Such non-additive genetic covariance wields a stronger influence on mutation than random drift, itself much stronger in small populations typical of brooding and asexual taxa. Thus, phenotypic divergence as seen in *I. hippuris* across sites within the WMNP, may be a consequence of hidden genetic variation leading to emergent environmentally mediated fixation accelerated by anthropogenic impact. Peripheral haplotypes

reveal emergent lineages (Forsman 2003), those at Sampela up to seven base pairs differences between primary ITS2 sequence comparisons, none of which are shared with other haplotypes within the region. Furthermore, shared haplotypes and thus gene flow at the remaining test sites, with the exception of haplotype D (sea grass), suggests assortative mating with the onset of reproductive isolation at Sampela, the remaining haplotypes more frequent and broadly adapted, likely being ancestral. Greater sampling with genetic and coalescent analyses is required to confirm such supposition, particularly considering a minority presence of opposing morphotypes at each site (pers. obs.).

The consistent mutational differences both within (clones) and between sequenced samples, renders PCR or base calling errors unlikely. Given the renowned caveats associated with the ITS2 region such as intragenomic variation, secondary structure of each haplotype confirmed molecular morphometric differences most notably between Sampela (haplotype A) and the remaining sites, yet with strong sequence similarities between the remaining haplotypes. Furthermore, lack of network reticulation suggests no indication of hybridization, validating confidence in two species taxonomic assignment, emergent or previously diverged. However, hybridization at this juncture cannot be overlooked, *I. hippuris* morphotypes across its distributional range may also represent an Indo-Pacific syngameon as seen in the diverse and polymorphic scleractinian *Acropora* Oken 1815 (Ladner & Palumbi 2012), with widespread gene flow through introgression (Vollmer & Palumbi 2002, Palumbi et al. 2011, Ladner & Palumbi 2012). However, any species delimitation within the *Isis* genus in addition to *I. hippuris* is necessary before further inference can be made.

It is clear that pertinent crossover exists between previously described *Isis* taxa and those present within the WMNP. It is tempting to conclude that *I. hippuris* is a single species with an extensive phenotypic and geographical range, or that only *I. hippuris* are present in the Wakatobi with other taxa within the genus elsewhere. Tolerant taxa tend to possess wide geographic distributions compared to those that are not (Calosi et al. 2010). However, the historically perceived panmixia of *I. hippuris* is likely more than a single species and not that of a complex when considering similar repetitive phenotypic trait differences across its distributional range. Previous alternative taxonomic assignments are therefore questionable, as the standard error of phenotypic variance would be greatly improved by assessing differences between *I. hippuris* morphotypes with increased specimen analyses from throughout its distributional range; a beneficial strategy when dealing with highly polymorphic taxa. Again, tests of coalescence on numerous independent highly polymorphic markers (SNPs; Ladner &

Palumbi 2012) would be required in order to fully elucidate convergent environment-by-genotypic effects in *I. hippuris* across its distributional range.

3.4.3 Isididae polyphyly

Phylogenetic analyses confirm haplotype differences as well as polyphyly within the Isididae; a phenomenon recently reported using the putative octocoral mitochondrial marker *msh1* (Watling et al. 2012). Even as far back as the earliest part of the last century, Kükenthal (1919) considered the Isididae to be polyphyletic; the subfamilies within as independent groups and the axis a "convergent phenomenon." Furthermore, *I. hippuris*, itself the type species of this family and the subfamily Isidinae, appears to have minimal phenotypic similarities to virtually all other isidid taxa with the exception of the axis, yet even this has been shown to be scleritic (consist of fused sclerites; Milne-Edwards & Haime 1857, Kükenthal 1919, 1924, Bayer 1955, Watling et al. 2012, in prep., but see Nutting 1910c). Such evidence naturally brings into question the validity of *I. hippuris* in its current classification. Polyphyly within gorgonian groups across bathymetry is not unknown (McFadden et al. 2006). *I. hippuris* is the only shallow and zooxanthellate representative of the Isidinae and Isididae respectively, the remainder being characteristic of the deep ocean.

The scleritic composition of the *I. hippuris* axis further sets it apart from both the Isididae and the suborder Calcaxonians, these being more closely affiliated with the Alcyoniinan-Holaxonian clade as phylogenetically determined by Bernston et al. (2001) and McFadden et al. (2006). However this convergent trait holds significant evolutionary intrigue. The fused scleritic internodes with gorgonin nodes of the *I. hippuris* axis, ensures flexibility and durability in high water energy conditions. Yet what is the selective advantage of a jointed axis in deep-sea isidids? Empirically, this is undetermined but it is not unreasonable to propose that the jointed axis is a relictual anachronism consequential of geological (e.g., opening of the Drake Passage, Watling pers. comm.) as well as later glacio-eustatic sea-level changes resulting in bathymetric refugia from turbulent shallow coastal waters (Helm & Schülke 2003). Thus, the functional significance of an articulated axis at depth is still a mystery; however longer internodes in the colonies at Sampela – like those seen in the benign deep ocean Isidids - compared to Ridge 1 were observed but not quantified (pers. obs.). Interestingly, deep-sea low flow specialists *Isidella* Gray 1857, have long elegant calcareous internodes compared to the larger much more robust internodes of *Keratoisis* Wright 1869, characteristic of moderate flow environments in the deep-sea (Watling pers. comm.), yet with no appreciable flexibility. A deep divergence with stabilizing selection regards a non-sclerite axis in deep-sea isidids may have occurred. Whether

the *I. hippuris* axis is a consequence of convergent evolution based on ecological necessity in heterogeneous environments typical of shallow reefs or deep inheritance is unclear and under investigation (Watling et al. in prep.).

3.5 CONCLUSION

The two distinct *I. hippuris* morphotypes within the WMNP are phenotypically segregated through trait integration between healthy and degraded reefs, likely reinforced through reproductive strategy. The co-variability of light, sediment and water flow between sites fortify directional trait selection (Feder 1998); colony, branching dynamics, polyp density, sclerite size and diversity all vary significantly between sites. Moreover, polyp and nutrient canals appear canalized due to the additive effect of modules to the colony as opposed to an increase in size, raising inference to maximizing photosynthetic yield and heterotrophy, both mitigating and capitalizing on environmental conditions particularly at Sampela. Diverse phenotypic trait assessment through character trait integration using reciprocal transplant experiments across the two sites would undoubtedly be insightful, particularly as shifts in metabolic function are subject to selection at opposite ends of environmental gradients (Feder 1998). Selection acts on phenotypic variation (reflecting variation in gene expression), which may have become fixed over time leading to ecological divergence. *I. hippuris* morphotypes, tentatively confirmed by ITS2 sequences and secondary structure analyses, have only partial adherence to previously described taxa. It lacks prudence to assign species at this juncture necessitating integrative classical taxonomic, genomic, axis composition, biogeographical and ecological analyses across its distributional range. Furthermore, compelling phylogenetic evidence not only confirms *I. hippuris* morphotype differences, but also reveals its disassociation within the Isididae. Phylogenetic discernment investigating congruence between skeletal structure, multi-locus next-generation sequencing and coalescence modelling (Puritz & Toonen 2011, Puritz et al. 2012), will assist unresolved hypotheses within this group. Thus is the continuum of evolution, compartmentalized for the necessity of biodiversity assessment and conservation management, itself a human construct against its own influence.

CHAPTER 4: ACCLIMATORY CAPACITY OF THE GORGONIAN *ISIS HIPPURIS* LINNAEUS 1758 TO ENVIRONMENTAL CHANGE IN SE SULAWESI, INDONESIA.

ABSTRACT

Coral reefs within the Indonesian archipelago are some of the most exquisite yet anthropogenically compromised marine ecosystems. Within the Wakatobi Marine National Park (WMNP), SE Sulawesi, Indonesia, pronounced environmental clines are either caused or exacerbated by marine resource subsistence and destruction. The zooxanthellate gorgonian (sea fan coral) *Isis hippuris* Linnaeus 1758 however, thrives on degraded reefs, with distinct morphotypes across contrasting reef environments within the region. To further investigate whether the biological success of *I. hippuris* morphotypes are a consequence of selective mechanisms acting on phenotypic plasticity capacity or ecological divergence, reciprocal transplant experiments (RTEs) measuring multiple traits (morphological, physiological and environmental) were conducted across environmental gradients of light attenuation and anthropogenic disturbance. After one-year survivorship was lowest in clones transplanted from healthy to the degraded reef, suggesting the onset of immigrant inviability. Phenotypic traits were grouped into modules (colony, polyps, sclerites, optical parameters) and subject to duplicate multivariate models between the two sites. Significance values were consistently driven by differences between resident morphotype colonies from the healthy and impacted sites. The phenotypic traits in healthy source colonies consistently showed significant trait plasticity, whereas impacted residents were relatively insensitive to environmental change. Of the 38 phenotypic traits assessed, 17 were identified as driving test dissimilarities most notably in branching dynamics, polyp density, capstan and spindle sclerite dimensions, and *Symbiodinium* chlorophyll *a* light energy absorbance efficiency (for photosynthesis). Specifically, photoacclimatory responses were integrated at the morphological and bio-optical levels, with chlorophyll *a* light harvesting efficiency maintained during reduced pigment density through an increase in host sclerite articulation actually maximizing the internal light field in healthy clones on degraded reefs. Variable optical responses were not however, attributed to endosymbiont type as all test colonies possessed a novel *Symbiodinium* Clade D1a. In summary, patterns of phenotypic variability within the *I. hippuris* holobiont likely represent incipient ecological divergence, with high plasticity capacity becoming fixed through ongoing anthropogenic disturbance on degraded reefs.

Key words: Gorgonian coral · Plasticity capacity · Holobiont · *Symbiodinium* · Reciprocal transplants · Indonesia

4.1 INTRODUCTION

Environmental heterogeneity and perturbation may enhance or diminish biodiversity through differential species responses. High-energy biodiversity hotspots such as coral reefs within the Coral Triangle provide ideal environments for the investigation and interpretation of phenotypic variation within and between populations, particularly in response to anthropogenic disturbance. The biological success of such reef inhabitants may be a consequence of divergent selection or acclimatory capacity at the phenotypic level (Weiner 2004). Mechanisms of phenotypic variation can be extrinsic (e.g., substrate, light, temperature, sedimentation, competition, predation, and hydrodynamics), intrinsic (e.g., developmental, life history, physiological, or genetic), a combination, or interaction of the two. Fitness enhancement producing phenotypic novelty through such interactions may lead to ecological divergence either as a by-product or direct selection if conditions persist (Schluter 2001, Hatfield & Schluter 1999). Thus, phenotypic variability, once considered an inconvenience (West-Eberhard 1989, DeWitt & Scheiner 2004), is in fact, the raw material of evolutionary processes that maximizes survival at the individual and population level particularly in the face of environmental change.

Colonial sessile marine taxa are arguably the most pliable to changes in environmental regime through hierarchical modularity necessitated by their physical inability to relocate. Gorgonian corals (Cnidaria: Octocorallia) exhibit a diverse complexity, which is much greater and older than that of Scleractinia (Waggoner & Collins 2004), yet they are poorly understood particularly throughout the Indonesian archipelago (van Ofwegen 2004). Within the WMNP, two distinct morphotypes of the zooxanthellate gorgonian *Isis hippuris* Linnaeus 1758 were found to be segregated between healthy and exploited reefs; short-branched multi/planar colonies and long-branched bushy colonies respectively (Chapters 2 & 3). Inferred integrated phenotypic traits at the colony, polyp and sclerite (skeletal element) levels were corroborated by haplotype differences, which suggest an emergent lineage on exploited reefs with remaining and likely ancestral haplotypes broadly distributed throughout the surrounding area. No indication of hybridization further reinforced the notion of two separate lineages whether emergent or previously diverged. Satisfactory taxonomic assignment to either morphotype however, was compromised by the historically recognised plasticity of *I. hippuris* (Wright & Studer 1889, Simpson 1906, Thomson & Simpson 1909, Bayer & Stefani 1987, Fabricius & Alderslade 2001) and tenuously described alternatives (*Isis reticulata* Nutting 1910, *Isis minorbrachyblasta* Zou,

Huang & Wang 1991). Furthermore, such phenotypic patterns have been repetitively documented in different regions (e.g., Philippines, Mai-Bao-Thu & Domantay 1971, Bayer & Stefani 1987; China, Zou et al. 1991; Okinawa, Muzik & Rowley pers. obs.), which may suggest selective convergence indicative of parallel evolution (Schluter & Nagel 1995). Thus, investigations into *I. hippuris* phenotypic segregation within the WMNP may act as a surrogate for determining the selective mechanisms that underlie the phenotypic differentiation recapitulated across its geographic range.

Concomitant phenotypic and genetic differentiation between *I. hippuris* morphotypes across two contrasting reef environments clearly indicates ecological divergence is at play. Yet both taxonomic ambiguity and likely divergence by gene flow obscures definitive species and ecological boundaries at the mechanistic level. Akin to terrestrial seed dispersal, allopatric barriers to gene flow in the aquatic realm are seldom applicable, with arguably greater reinforcement of disruptive selective mechanisms in sympatry. Ecological boundaries have been shown to be as powerful as they are numerous, specifically with the added dimension of human encroachment (Palumbi 2001, Puritz & Toonen 2011). Therefore, *I. hippuris* on degraded reefs within the WMNP likely represent morphological stability that has come from phenotypic plasticity which has become fixed on degraded reefs, in other words, an environment-by-genotype effect that has become (or in the process of becoming) fixed over time in a degraded reef environment. Phenotypic divergence may have become a necessity to maintain survivorship on such reefs, with the cost of plasticity capacity greater than phenotypic stability over time. Therefore, time to divergence would be reflected in immigrant inviability (Prada & Hellberg 2013) and further reinforced through assortative mating, particularly considering that *I. hippuris* has a propensity for philopatry coupled with replication through external brooding and asexual fragmentation leading to population structure and expansion on degraded reefs. Thus the eventual effect of directional and stabilizing components such as prolonged ecological disturbance, on disruptive selection between *I. hippuris* morphotypes could evolve through gene flow (Johnson 1976) within the Wakatobi.

Determining mechanisms of phenotypic variation provides valuable insight into the processes of divergence between morphotypes. Comparative measurements of *I. hippuris* phenotypic traits between contrasting environments over time are indicative of phenotypic expression patterns and can be visualised through the slope of a reaction norm (summarised in Chapter 1; Figure 1.5). In sum, trait fixation depicts environmental insensitivity and therefore no slope/change; trait plasticity depicts environmental sensitivity through a slope in the reaction norm. Phenotypic

comparisons between morphotypes denote differential responses to environmental change, providing hypotheses for potential evolutionary mechanisms of selection (Pigliucci et al. 2006). However, whether trait plasticity is adaptive (genetic) or epigenetic (developmental) is undetermined (DeWitt & Scheiner 2004, Schluter 2000). Environmentally induced change within and between *I. hippuris* morphotypes demonstrates plasticity capacity; but phenotypic changes in healthy reef morphotypes only, would demonstrate plastic and canalised phenotypes for healthy and degraded reefs respectively, therefore two different species as would be the case with no change visualised in either morphotype. Multivariate trait analyses engaging a "pluralistic approach" (Gould & Lewontin 1979) would help discern mechanisms of evolutionary change within the holobiont and its environment. Yet it is surprising that studies on zooxanthellate gorgonians predominantly from the Caribbean, overlook the functional significance of their photosynthetic endosymbionts (West et al. 1993, Sánchez & Lasker 2003, Kim et al. 2004, Sánchez 2004, Prada et al. 2008, Prada & Hellberg 2013). Reciprocity between endosymbiont and host must surely bring about changes in the holobiont phenotype (Johnson 1976, Gilbert et al. 2010): the integrated whole being greater than the sum of its parts.

Zooxanthellate gorgonian corals typically show endosymbiont specificity (Goulet et al. 2008) with *I. hippuris* from the Great Barrier Reef known to harbour the putatively stress-tolerant clade D *Symbiodinium* (van Oppen et al. 2005). Technological advances reveal specificity gradients within endogenous *Symbiodinium* communities, irrespective of host – algal symbiosis assignment ('specificity' or 'flexibility'; Silverstein et al. 2012). Endogenous symbiont 'shuffling' between cryptic and dominant clades (A - I; Pochon & Gates 2010), or types within clades in response to environmental perturbation, is quite logical in terms of community ecology. It is not unlikely that endosymbiont community shifts function as a mechanism of enhanced physiological performance, enabling holobiont persistence particularly on degraded reefs. Alternatively, endosymbiont communities can remain constant with greater acclimatory capacity (Bellantuono et al. 2012) as a consequence of heritable (Molinier et al. 2006) epigenetic effects (Chinnusamy et al. 2009). However, both the methods and molecular markers of *Symbiodinium* detection are controversial (e.g., Apprill & Gates 2007, LaJeunesse & Thornhill 2011, Stat et al. 2011, LaJeunesse et al. 2012, Pochon et al. 2012) with the highly variable internal transcribed spacer (ITS2) region detecting over 400 within clade rDNA 'types' (LaJeunesse 2002, 2005, LaJeunesse et al. 2003, 2004a, b), lending skepticism to its taxonomic efficacy (Stat et al. 2011). Nonetheless, the reported presence of *Symbiodinium* clade D within *I. hippuris* colonies may well contribute to its biological success on degraded reefs within the WMNP. Moreover, holobiont physiological plasticity is suggested to be of a greater fitness

advantage regards mechanisms of resilience to environmental change, than the shifting or alteration of photosynthetic endosymbiont cladal type alone (Bellantuono et al. 2012).

The interplay between *I. hippuris* morphotypes at the morphological and physiological level through phenotypic trait integration would reveal mechanisms of physiological tolerance to environmental change. Primarily, photoacclimatory responses of the *I. hippuris* holobiont through the adjustment of optical and biophysical properties could maximize light harvesting and photosynthetic efficiency, key to its survival and biological success. Translocation of photosynthetically fixed carbon from *Symbiodinium,* as well as the conservation and recycling of essential nutrients such as nitrogen from the host, enables the holobiont to persist in nutrient-poor tropical waters, with concomitant calcium carbonate deposition by the symbionts (Muscatine & Weis 1992). Variations in physiological photoacclimatory properties are indicative of holobiont responses but are not necessarily limited by *Symbiodinium* (Falkowski & Dubinsky 1981), with photophysiological optima controlled at both the colony (Kim & Lasker 1998, Enríquez et al. 2005, Shaish et al. 2006) and cellular levels (Kirk 1994). High light intensities can provoke increased branching and/or pigment concentrations within endosymbiont cells minimizing photoinhibition (irradiance damage to photosystem II [PSII]) through self-shading (Hoegh-Guldberg & Jones 1999, Enríquez et al. 2005). Self-shading at the cellular level - a more rapid and possibly *only* self-shading response if colony morphology is fixed – can maintain relative zooxanthellar cell densities in fluctuating irradiances (Falkowski & Dubinsky 1981, Porter et al. 1984, Dubinsky et al. 1990). However, acute environmental perturbation(s) such as increased nutrient levels, light and/or temperature can lead to marked endosymbiont proliferation (Muscatine et al. 1989, Dubinsky et al. 1984, 1990) or reduction (Wilkerson et al. 1988), particularly in non-acclimated colonies (e.g., Bellantuono et al. 2012). Still, trait mitigation of environmental susceptibility through prolonged exposure (Brown et al. 2000, 2002, Middlebrook et al. 2008, Bellantuono et al. 2012) may eventually become fixed (Molinier et al. 2006). Furthermore, utilization of the internal light field through skeletal light scattering reduces pigment investment and self-shading, whilst maintaining light-harvesting efficiency (Enríquez et al. 2005, Stambler & Dubinsky 2005). Therefore, particular sclerite morphology and size may affect internal light reflection thereby facilitating and maximizing the harvesting of solar energy within *I. hippuris* morphotypes. Subsequent photosynthetic yield and hence productivity will therefore be dependent on integrative phenotypic mechanisms within the holobiont and should be considered in its entirety when assessing differential phenotypic responses of *I. hippuris* morphotypes to environmental change.

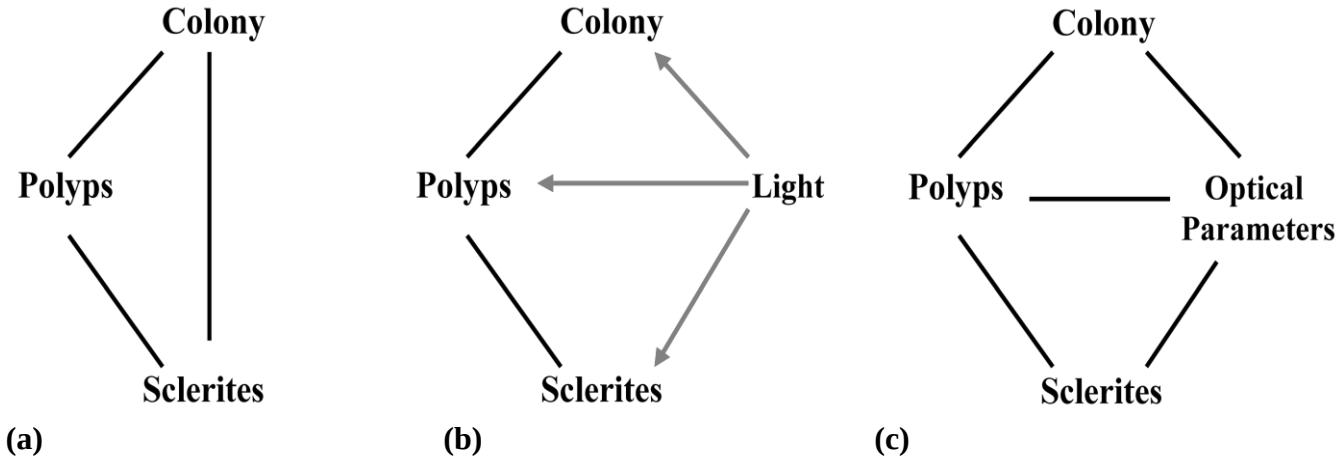


Figure 4.1 *Isis hippuris* (a) descriptive, (b) hypothesized causal and (c) proposed phenotypic module integration models.

Previous descriptive results segregate three phenotypic modules for *I. hippuris* morphotypes at opposing reef environments within the WMNP: colony, polyp, and sclerite level traits (Figure 4.1a; Chapter 3). The probability that any number of possible trait combinations can give rise to a novel phenotype, maximizing individual and/or population fitness in any one environment (Santelices 1999, Magwene 2001b) appears vast. However, multivariate selection recognises and delimits integrated traits, leading to tests of plasticity capacity or divergence through reaction norms as a product of reciprocal transplant experiments (RTEs). Here, it is hypothesized that light availability is a primary vector (causal; Figure 4.1b) of *I. hippuris* morphotypes between opposing sites (with distinct light regimes) within the WMNP, further driving integration among phenotypic traits. The quantum efficiency of the *I. hippuris* holobiont through the functional integration of optical traits (as a phenotypic module *sensu lato*; Figure 4.1c) was therefore assessed for evidence for the onset of light-induced directional selection or plasticity capacity. This was achieved through a one-year RTE measuring multiple traits (morphological, physiological, and environmental) between sites of contrasting reef health at comparable optical depths. Research objectives were as follows: (1) determine if *I. hippuris* morphotypes across environmental gradients are environmentally induced (plastic) or genetically derived (canalised/fixed); (2) assess differential physiological responses of the *I. hippuris* holobiont to environmental change; (3) investigate host algal endosymbiont specificity between morphotypes across and as a consequence of environmental change; (4) determine integrated phenotypic traits which interact to delimit *I. hippuris* morphotypes suggesting mechanisms of divergence through phenotypic trait integration in response to environmental perturbation.

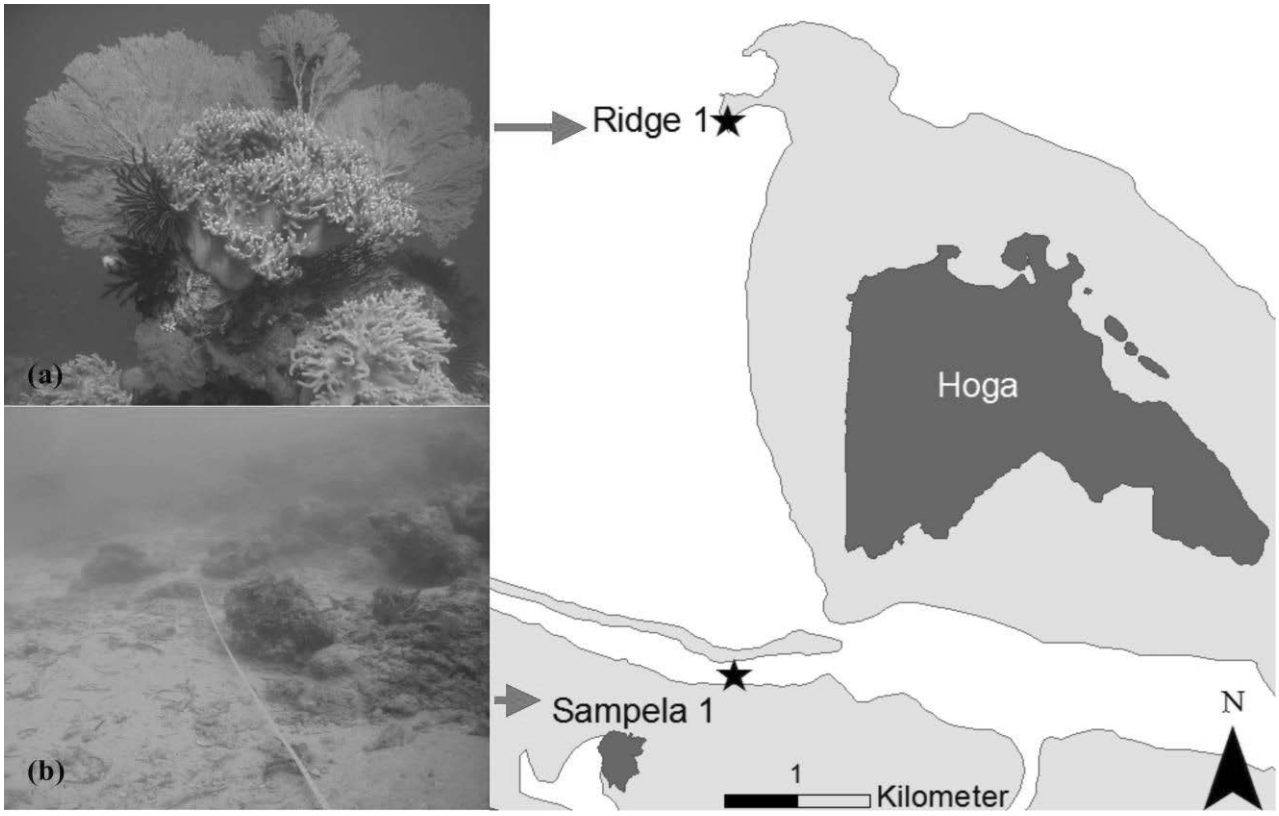


Figure 4.2. Comparative test site location map of (a) Ridge 1 and (b) Sampela off the islands of Hoga and Kaledupa respectively, within the WMNP, Indonesia.

4.2 METHODS

4.2.1 Study Area

The Wakatobi Marine National Park (WMNP) is the second largest national marine park in Indonesia (ca. 13,900 km^2). Comprising ca. 600 km^2 of the most biodiverse coral reefs centered within the Coral Triangle, the WMNP sustains a burgeoning human population of >100,000 people within S.E. Sulawesi supporting extensive marine resource reliance and subsequent destructive marine fisheries (Clifton et al. 2010, Clifton 2013). The dynamic coalescence between natural coral reef environments including the interplay between sea-grass beds, mangroves and human settlements renders the WMNP an ideal natural laboratory in which to study the effects of environmentally induced plasticity or divergence, particularly in the case of *I. hippuris.*

This research was conducted between two contrasting reef sites at opposite ends of a marked environmental gradient ca. 5 km apart (Figure 4.2) characterised by differential light attenuation, sedimentation and hydrodynamic regime (Table 4.1). Ridge 1 (healthy) is a high hydrodynamic, biodiverse offshore reef ridge with shear walls, strong deep-water nutrient rich upwellings and

Table 4.1. Characteristic environmental variables at optical equivalent depths **(ζ)** between the two study sites in the WMNP, Indonesia.

Parameter Recorded	**Mean value ± SE (where appropriate)**			
Site	**Sampela**		**Ridge 1**	
Optical Depth (ζ)	**2-3 m**	**10 m**	**5 m**	**18 m**
Latitude, Longitude	005˚ 29'01" S, 005˚ 26'57" E		005˚ 26'57" S, 123˚45'38" E	
Temperature (˚C min-max)	27.88 - 29.26	25.61 - 28.09	26.96 - 28.10	24.06 - 28.07
Light ($K_{d(PAR)}$ min-max)	0.44 - 0.66	2.21 - 2.65	0.5 - 0.625	2.25 - 2.5
Flow (cm/s)	0.86 ± 0.19	1.54 ± 0.62	34.38 ± 1.33	29.28 ± 2.85
Chlorophyll-*a* (μg 1^{-1})	0.38 ± 0.001	0.39 ± 0.001	0.30 ± 0.001	0.30 ± 0.001
Turbidity (NTU)	4.73 ± 0.72	3.76 ± 0.24	0.15 ± 0.43	0.11 ± 0.21
Sedimentation (g d^{-1}, n = 12)	3.85 ± 0.11	3.22 ± 0.10	1.2 ± 0.05	1.14 ± 0.05
Sediment grain size (Φ, n = 12)	2.5 [125–250 μm]	5 [31.25–62.5 μm]	1 [0.5–1 mm]	1 [0.5–1 mm]

low turbidity. Sampela (impacted) is a low water flow, high sedimented, semi-lagoonal reef ca. 400 m distance from a sea gypsy (Bajo) village of ca. 1600, resulting in continual reef resource exploitation, destructive fisheries and wastewater exposure for at least 90 years (Webber H., pers. comm.). Additional sites within the area were not considered due to logistical constraints.

Characteristic environmental variables suggested to drive morphotype distribution at the two study sites (Chapter 2 & 3) were quantified (Table 4.1). Optical equivalent depths ($\zeta = K_{d(PAR)}$) were calculated from the average $K_{d(400\text{-}700\ nm)}$ for source colony collection and transplant block deployment at the two study sites. Light $K_{d(PAR)}$ was taken every 1 m depth at 12:00 pm over consecutive days using the external photosynthetically active radiation (PAR) sensor on a Diving-Pulse Amplitude Modulation (Diving-PAM) fluorometer (Walz GmbH), calibrated against a Li-Cor quantum sensor. The average site K_d per optical equivalent test depth was calculated as described by Hennige et al. (2010) and is expressed in a reverse scale. Surface layer (i.e., recently settled) sedimentation rate was assessed using four replicate 1 litre sediment traps (English et al. 1997; Chapter 2) deployed for ca. 10 days each year at the calculated optical equivalent site depths. Grain size was estimated using Retsch Technology® test sieves, with logarithmically converted diameters expressed as phi (Φ) and classified using the Wentworth scale (Wentworth 1922). Additional suspended material was determined through turbidity (NTU as an inverse measure) and chlorophyll-*a* (as μg L^{-1}) measurements, using RBR® XR-420 data loggers placed adjacent to each block repetitively throughout the duration of the experiment. Temperature (°C) was determined using HOBO® data loggers placed on a single block at each depth per site, recording every 15 min throughout

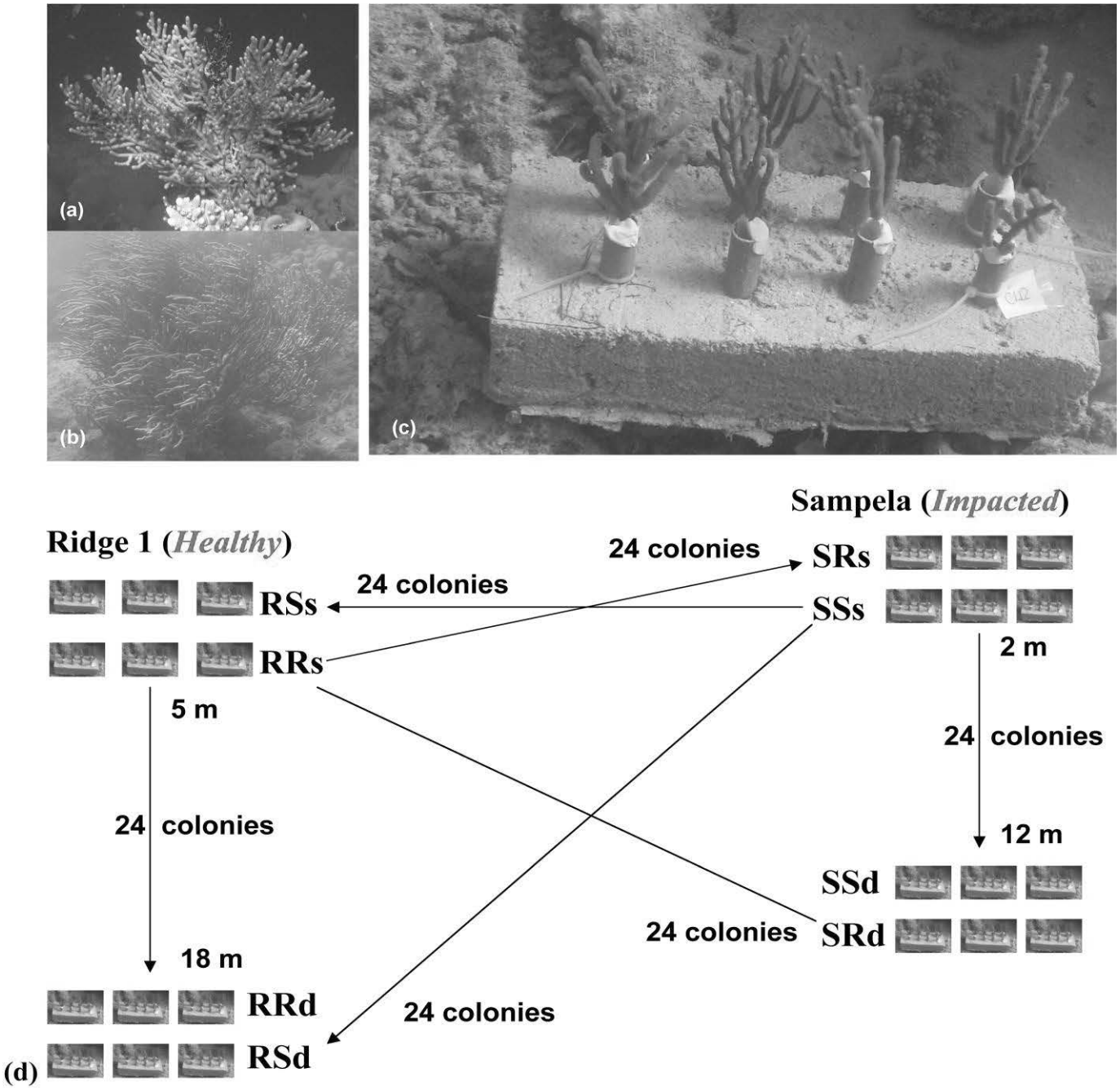


Figure. 4.3. *Isis hippuris* reciprocal transplant experimental design of source morphotypes from (a) Ridge 1, and (b) Sampela. (c) Transplant blocks baring eight clippings were placed (d) reciprocally with controls between optical equivalent (ζ) depths 5 & 18 m at the Ridge, and 2 & 12 m at Sampela. Arrows indicate direction of reciprocity and the number of transplanted test clippings. Transplant codes: RRs, Ridge – Ridge shallow; RSs, Sampela – Ridge shallow; RRd – Ridge – Ridge deep; RSd, Sampela – Ridge deep; SSs, Sampela – Sampela shallow; SRs, Ridge – Sampela shallow; SSd, Sampela – Sampela deep; SRd, Ridge – Sampela deep.

the test period. Water flow velocity was measured using a General Oceanics® flow meter with a low velocity rotor and custom made aluminum pipes for reef placement and expressed as cm s^{-1}. A hand-held GPS meter (GARMIN eTrex®) determined latitude and longitude site coordinates which were not used in the statistical analyses.

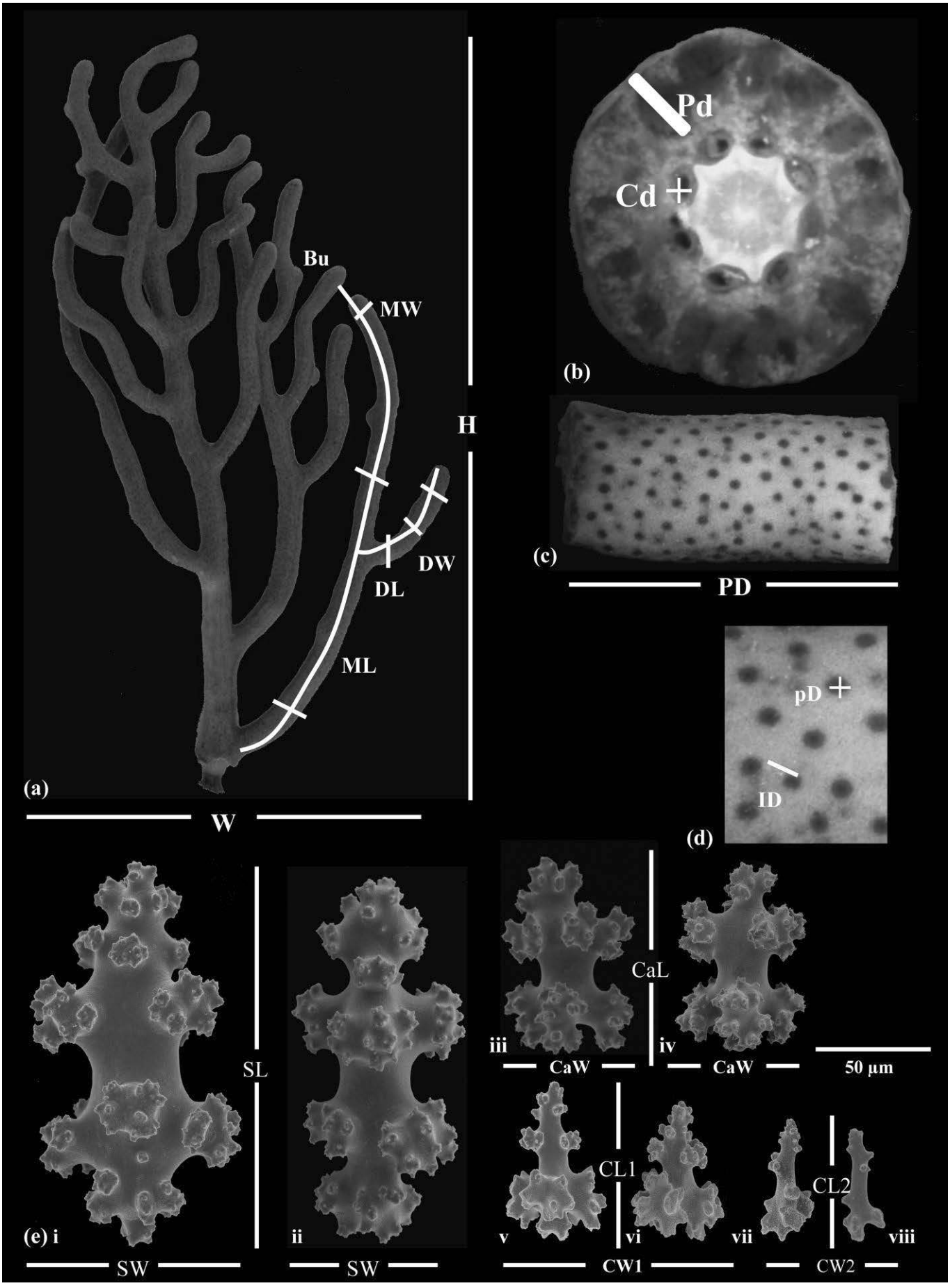
4.2.2 Field Experimentation

I. hippuris test colony cuttings were reciprocally transplanted between the healthy reef Ridge 1 and impacted reef Sampela from June 2010 – July 2011 (Figure 4.3). Source colonies from each site (n = 24; total n = 48) were selected at optical equivalent ($\zeta = K_{d(PAR)}$) depths from the Ridge top (5 m) and Sampela reef crest (3 m) ≥ 10 m apart and of similar size to counter colony-level surplus resource variance. Scaled photographs were taken directly opposite and above each source colony using a Canon IXUS 900Ti, WP-DC7 u/w housing and INON UWL-105 AD x 0.51 lens. For each source colony, five ~10cm cuttings were sub-sampled, four for transplantation and one for comparative down stream analyses (see below). Scaled digital photographs were further taken of each cutting before and after transplantation for comparative annual growth and morphological measurements. A total of 24 cement blocks bearing eight colony cuttings (192 in total), secured using standard marine epoxy, were reciprocally transplanted between the two sites with additional blocks at optical equivalent depths as described above (Figure 4.3), to test for the effect of light on colony morphology, symbiont type, photobiology and stable isotope ratio (see below). Each block was placed such that transplant cuttings were perpendicular to the prevailing water flow.

All test colonies were sampled (n = 48 in 2010, n = 192 in 2011; total n = 240) 2 cm below the apex and preserved in 95% EtOH, 70% EtOH with prior overnight 4% formalin wash, and guanidinium solution for morphometric, taxonomic and zooxanthellar density, and genetic analyses respectively. Chlorophyll was immediately methanol extracted upon live sample recovery on site (see "Optical Assessments" section below and Enriquez et al. 2005).

4.2.3 Comparative Phenotypic Traits

To investigate potential mechanisms underlying distinct *I. hippuris* morphotypes, test (including controls) colony clippings between 2010 and 2011, were subject to a suite of phenotypic trait measurements with subsequent modelling for integrative traits.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CL2
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viii
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SW
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CW2

Figure 4.4. (Previous page) *Isis hippuris* morphological trait measurements of (a) test colony cuttings and branch dynamics; (b, c, d) canal and polyp dynamics; (e) sclerite site/morphotype comparisons of i and ii spindles, iii and iv capstan 7-radiates, v-viii clubs from Ridge 1 and Sampela respectively. Abbreviations as described in the above text and Tables 4.2 - 5.

Morphological Measurements

Morphological trait comparisons were conducted on all *I. hippuris* test and control colony clippings, and divided into 14 macro-morphological (colony level) and 13 micro-morphological (polyp dynamics and sclerite level) traits (Figure 4.3, Table 4.3 - 5). Colony level measurements included colony height [H], and mean width [W] taken equidistant apart, colony base [B] and the projected colony area [PA] estimated by H x W. The PA was subsequently used to calculate colony porosity [Po] as a ratio of PA; the projected branch area [PBA] itself as the total branch length multiplied by the mean branch thickness (see below). Branch growth and development were further assessed using the hierarchical 'mother'/'daughter' branch ordering system (Lasker et al. 2003, Sánchez & Lasker 2003, Sánchez et al. 2003a). To correct for both temporal growth and positive association, thus size variation not related simply to growth, PA [incl. Po], PBA and TBL (see below) were also adjusted in equation (1) from Bayer et al. (1994):

$$\text{e.g., } PA(\log) = (\log[\text{final PA}] - \log[\text{initial PA}])/t \quad (1)$$

where t represents experimental duration in days. As growth persists nascent branches emerge (Bud# [Bu]), daughter branches typically also become mother branches (e.g., second generation mother branch; see Figure 4.4) with the assumption of increased branch density over time. However, resource allocation change with growth would alter essential branching parameters such as [*c*; Sánchez 2004] defined as the ratio between the total branch number [TB#] and total mother branch number [MB#]; and [*r*] (Weinbauer & Velimirov 1995) as the ramification rate defined as the annual growth difference between total branch length [TBL] and [TB#]. Additional branching parameters were assessed via the quantification of daughter branch number [DB#], mother branch length [ML], mean mother branch width [MW], daughter branch length [DL], and mean daughter branch width [DW]. Branch surface area was calculated on the geometric approximation of a cylinder from branch length and mean width as the radius, with subsequent polyp density [PD] per cm^2. Twenty random measurements were taken per sample for inter-polyp distance [ID], and polyp diameter [pD] as the mean of two measurements (see Figure 4.4c). All polyp, branch cross-section and canal ([Cd] see Figure 4.4b) quantification were visualised under an Olympus SZX16® stereomicroscope at 10x magnification with 0.5x

objective.

Sclerites, typically the initial objects of selection (Bayer & Stefani 1987), were quantified through the length and mean width of three measurements on 20 randomly selected sclerites per sclerite type; surface clubs [CL1/2, CW1/2]; and sub-surface capstans [7-radiates: CaL/W] and spindles [SL/W](Figure 4.4e,i - viii). Sclerites were removed by soft tissue dissolution in 5% sodium hypochlorite solution and observed using optical microscopy (Olympus BX51®) and scanning electron microscopy (SEM) performed on a Hitachi S-800 SEM at the University of Hawai'i at Mānoa, USA. All micro-morphological measurements and sclerite preparation were taken 2 cm below the branch tip to avoid underdeveloped traits due to sub-apical branch growth (Lasker et al. 2003) and photographed using an Olympus 3.3MPX™ camera and Rincon software (ImagingPlanet®). All macro- and micro-morphological characteristics were measured using ImageJ64 (Abràmoff et al. 2004).

Optical Assessments

Population dynamics of zooxanthellae within all test *I. hippuris* morphotypes were characterized from ~1 cm fixed (4% O/N formalin wash and storage at -40ºC in 70% EtOH) host branches. Cells were isolated via repetitive (3 x at 4,000 g) centrifugation-wash cycles (Muscatine et al. 1989) in filtered sea-water, and eight replicate 15 µL aliquots of suspended cells per sample were enumerated using a haemocytometer. Zooxanthellar cell density [ZD] was normalized to coral surface area through the approximation of a cylinder as described above and expressed as cells cm^{-2}. Cell division (cytokinesis) was qualified by a doublet appearance with observable plates and expressed as the percentage of total cells (mitotic index [MI], Wilkerson et al. 1988). Zooxanthellae mean diameter [Zd] and surface area to volume ratio [SA:V] were measured using light microscopy at 200x magnification with an Olympus 3.3MPX™ camera and ImageJ64.

Optical density was determined using a U-3000 spectrophotometer with φ−60 integrating sphere (Hitachi) on live samples to assess chlorophyll-*a* specific absorption [a^*] (Enriquez et al. 2005), concentration [*A*], and subsequent concentration per cell [CZ], testing the "packaging effect" or "self-shading" (Dubinsky et al. 1986, Kirk 1994). Live colonies were measured in triplicate with absorbance *D* values taken from a single peak at 675 nm to minimize accessory host and algal pigment interference, whereby:

$$a^*_{chla} = (D/p)\ln 10 \qquad (2)$$

with *D* as the spectrophotometry absorbance via reflectance measurements for absorptance [*A*], *p* is the pigment content per unit branch surface area expressed as mg m^{-2} (Enríquez & Sand-Jensen 2003, Enríquez et al. 2005, Hennige et al. 2010). Bleached (24 h chloride emersion) skeletal elements (axis and sclerites) were further measured as described by Enríquez et al. (2005) and the corresponding live soft tissue (ca. 1 cm^2) was weighed, measured and dissolved in 5 mL of 100% methanol (Porra et al. 1989, Ritchie 2006) for 36 h at 4ºC in darkness. Chlorophyll-*a* concentration was then calculated from the dichroic equation:

$$[A] = 13.6849\ E_{665} - 3.4551\ E_{632}\ \mu g\ mL^{-1} \quad (3)$$

where E_λ is the extinction coefficient of light (photon) absorption at the given wavelength. All optical values were normalised to branch surface area, and chlorophyll-*a* content per cell expressed as pg $cell^{-1}$.

Prior to sub-sampling, light adapted (open photosystem II reaction centres) maximal quantum yield (ΔF/Fm') of PSII (photosystem II) was measured using a Diving-PAM fluorometer set at measuring light intensity = 8; actinic light factor = 0.5; saturation pulse width = 0.8; saturation intensity = 3; gain = 3; and signal damping = 2. Distance between the coral branch surface tissue and the fiber optic probe was standardized (10 mm) using a DIVING-SH Walz surface holder. Light adapted yield (ΔF/Fm') was assessed averaging measurements taken in triplicate from the top (2 cm from the branch tip), middle, and base of each colony parallel to the coral-water interface. This was replicated on transplant retrieval in 2011, where measurements were taken 2 cm (top), 6 cm (middle), and 10 - 12 cm (base) from the apex of the colony. In both years quantum yield was consistently taken at 07:20 h ± 10 min over eight consecutive days.

Symbiodinium Genetics Analyses

Genomic DNA of endosymbiotic dinoflagellates was extracted from all test colony clippings (n = 48 in 2010, n = 192 in 2011; total n = 240) using a Guanidinium procedure as previously described (Pochon et al. 2001, Chapter 3). Three molecular markers were used to test for *Symbiodinium* diversity and marker utility. The relatively conservative mitochondrial-encoded cytochrome oxidase 1 (*COX1_F2*; forward; 5'-AAA TTG TAA TCA TAA ACG CTT AGG-3' and reverse *COX1_R1*; 5'-GGC ATA ACA TTA AAT CCT AAG AA-3') was used for all samples (n = 240). For a deeper diversity and phylogenetic assessment clone libraries were constructed using the *ITS-LSU* region including the 3'-end of the 5.8S (SSU) region, the entire internal transcribed spacer (ITS2) region to the 5'-end of the LSU rDNA region (*itsD* forward;

5'- GTG AAT TGC AGA ACT CCG TG-3' and *LO* reverse; 5'- GCT ATC CTG AGR GAA ACT TCG -3'), and the plastid-coding *psbA* minicircle (*psbA* Clade D specific primers; *psbAFor_1*; 5'-GCA GCT CAT GGT TAT TTT GGT AGA C-3' and *psbARev_1*; 5'-AAT TCC CAT TCT CTA CCC ATC C-3'; LaJeunesse & Thornhill 2011) were used on a subset of test samples (n = 24 and n = 8 respectively). PCR amplifications for each marker were conducted according to the following conditions, with a product volume of 50 µL constituting: 5.0 µL of 10x PCR Buffer (Bioline), 2.0 µL of $MgCl_2$ (2 mM), 1 µL of each primer (10 mM), 1 µL (2.5 mM of each dATP, dCTP, dGTP, and dTTP), 0.2 µL of Hotstart Immolase *Taq* polymerase (Bioline Incl., London, UK), 1 µL of DNA, and 39 µL of sterile water. PCR amplification for *COI* and $psbA^{ncr}$ initiated at 95ºC for 10 min, followed by 40 cycles at 94ºC then 35 s at annealing temperatures of 55ºC and 56ºC respectively, 1.3 min at 72ºC, with a final extension at 72ºC for 10 min. Touchdown amplification was conducted for *ITS-LSU* as follows: denaturation at 95ºC for 10 min, 25 cycles at 94ºC then 35 s at 65ºC (reduction in annealing temperature of 0.5ºC per cycle), and 2 min at 72ºC. A further 14 cycles of 30 s at 94ºC, 35 s at 52ºC, 2 min at 72ºC, and a final 10 min extension at 72ºC. Purified *ITS-LSU* and *psbA* products were ligated into the pGEM®-T Easy vector™ (Promega), transformed into α- Select Gold Efficiency™ competent cells (Bioline), with subsequent positive inserts verified by PCR using plasmid specific primers (M13). All sequences including clone library positive inserts (8 - 12 per library), were purified with an ExoSAP-IT kit, sequenced in both directions using the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit and run on an ABI 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) at the University of Hawai'i at Mānoa, USA. All genomic DNA and PCR amplicons were visualized on 1% agarose gel.

Sequences were aligned using ClustalW2 (Thompson et al. 2002) and manually edited in Geneious Pro v.5.6.2 (Biomatters Ltd., NZ). A selection criterion of identical sequences from two or more clone libraries from independent samples was established for both *ITS-LSU* and *psbA* to minimize the effect of intragenomic variation and/or PCR artifacts on downstream analyses. All single variants were reverted to the library consensus. Phylogenetic reconstructions with comparative in house and GenBank sequences (see Table 4.2) were conducted using plugins within Geneious.

Table 4.2 Accessions of comparative sequences of *Symbiodinium* clades used in the phylogenetic analyses. Asterisks indicate the *COX1* outgroup.

Marker	*Symbiodinium* Clade	GenBank	Reference
COX1	All Clades	AY289689–AY289712	Takishita et al. 2004
COX1	**Gymnodinium simplex*	CCMP419	Santos et al. 2002
ITS	D	KC597691	Padilla-Gamino et al. Unpub.
ITS	D	AF396631	Santos et al. 2003
ITS	D1a	JN558076	Pochon et al. 2012
ITS	D1a	AJ311948	Pochon et al. 2001
ITS	D1a	AJ308900	Pochon et al. 2001
ITS	D1a	JN558080	Pochon et al. 2012
ITS	D1a	EU074897	Thornhill et al. 2007
ITS	D2	AF396627	Pochon et al. 2006
psbA	D	AB086877	Takishita et al. 2003
psbA	D	AB086878	Takishita et al. 2003
psbA	D	AB086863	Takishita et al. 2003
psbA	D	JQ043586	LaJeunesse & Thornhill 2011

Phylogenetic inferences for the markers *ITS-LSU* and *psbA* were constructed using the neighbour joining (NJ) (Jukes & Cantor 1969) and maximum likelihood (ML) (PHYML 2.1.0; Guindon & Gascuel 2003) methods with 1000 bootstrap replicates (Felsenstein, 1985) and unrooted circle trees constructed with the latter (ML). Phylogenetic inference for *COX1* was constructed using MrBayes 2.0.5 (Huelsenbeck & Ronquist 2001) in addition to NJ and ML, rooted with the dinoflagellate *Gymnodinium simplex* (Lohmann) Kofoid & Swezy 1921. Maximum likelihood (ML) phylogeny was conducted using the best-fit model (JC) of nucleotide substitution as selected in jModelTest 2 (Darriba et al. 2012) using the Akaike Information Criterion (AIC). Bayesian inference (BI) phylogeny was made with a JC69 substitution model and burn-in of 100,000.

Multivariate Analyses of Integration

Investigations of trait integration were conducted using routines within the PRIMER-E v6.1.12 statistical package (Clarke & Gorley 2006), with PERMANOVA+ v1.02 extension (Anderson 2001). To identify phenotypic trait integration on a functional level as a consequence of developmental (V_E), environmental (V_G) or an interaction of the two (V_{GxE}) processes, an initial multivariate correlative ($p < 0.05$) approach was conducted as a Draftsman plot. Trait 'subsets'

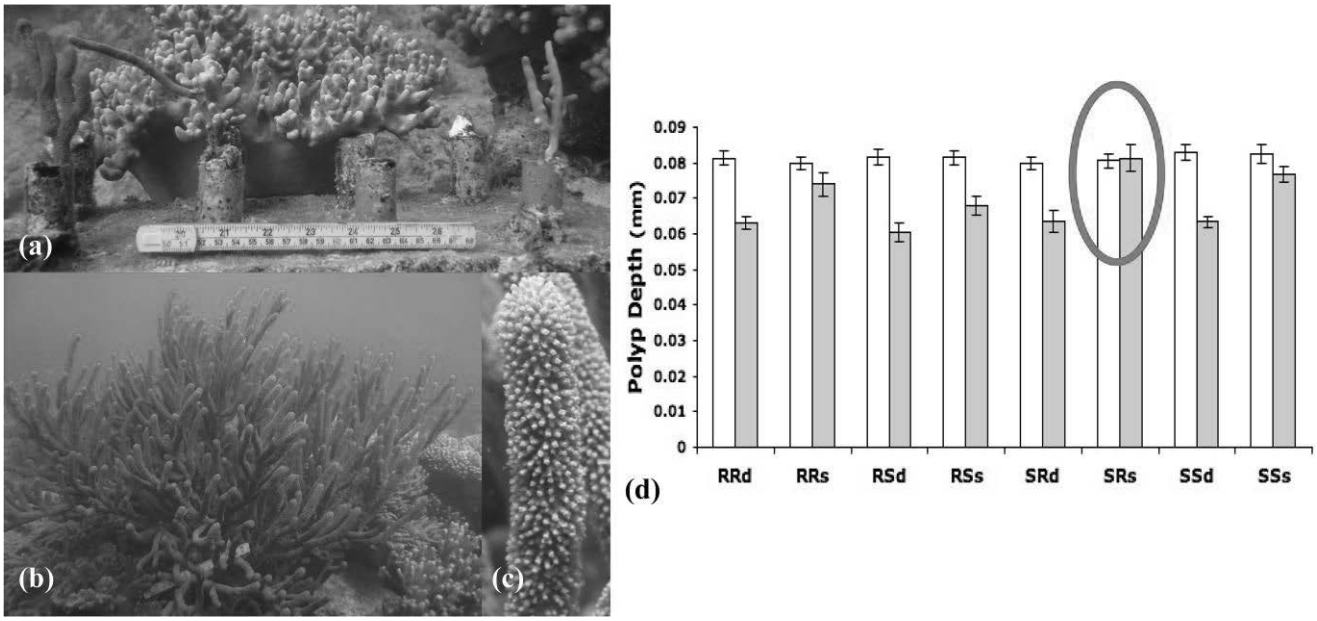

Figure 4.5. *Isis hippuris* colonies of (a) damaged transplant block clippings from Ridge 1 to Sampela at 3 m depth, (b) an adjacent resident, and (c) a close up of large pink externally brooded eggs and extended yellow polyps. (d) Mean (± SE) polyp depth (mm) from test colonies between 2010 (white bars) and 2011 (grey bars). Red ring highlights no change in polyp depth in transplanted colonies compared to all other test colonies.

or 'phenotypic modules' (Magwene 2001b) were subsequently selected on the basis of conditionality through correlation for downstream analyses. Reaction norms were constructed for all phenotypic traits tested and partitioned into the aforementioned phenotypic modules: colony, polyp, sclerite and optical dynamics. Phenotypic trait data were log transformed (correcting for positive association) and 'zero-adjusted' Bray-Curtis resemblance matrices constructed (Clarke et al. 2006b) for tests of plasticity vs. adaptation between and within the two study sites: Ridge 1 and Sampela. Sites were segregated by clone origin and multi-factorial models with 9999 permutations (PERMANOVA; Anderson 2001) were performed with reduced trait dimensionality visualised in the constrained canonical analysis of principal coordinates (CAP; Anderson and Willis 2003). Topological equivalent traits were identified using similarity percentages (SIMPER; Clarke 1993) and displayed as a vector overlay on the CAP ordination. Specific traits within and between delimited subsets were then modelled for phenotypic integration primarily in response to irradiance; environmental influence was investigated using nonparametric multivariate regression (McArdle & Anderson 2001) with the *DISTLM*forward routine (Anderson 2003). Based on a Euclidean distance matrix, all raw environmental variables (Table 4.1) were normalised and significance tested using 9999 permutations (Anderson 2001).

Table 4.3. *Isis hippuris* test colony annual mortality and survival. Values calculated out of 24 test clippings per treatment. Asterisk indicates likely reduction in test colony numbers due to a human disturbance event; revised values out of 16 in brackets.

Treatment	Mortality		Survivorship rate
	n	%	
Ridge (Source)			
[RRs] Ridge - Ridge shallow	1	4.2	0.958
[RRd] Ridge - Ridge deep	0	0	1.000
[SRs] Ridge - Sampela shallow*	11(6)	45.8(37.5)	0.583(0.625)
[SRd] Ridge - Sampela deep	2	8.3	0.917
Sampela (Source)			
[SSs] Sampela - Sampela shallow	3	12.5	0.875
[SSd] Sampela - Sampela deep	2	8.3	0.917
[RSs] Sampela - Ridge shallow	2	8.3	0.917
[RSd] Sampela - Ridge deep	1	4.2	0.958

All variables, with the exception of latitude and longitude, were entered into the statistical models as raw values. Values were edited visually with significant outliers removed.

4.3 RESULTS

4.3.1 Comparative Phenotypic Traits

A total of 38 phenotypic traits were assessed on all test colonies; 17 at the colony level, 5 at the polyp and canal level, 8 at the sclerite level, and 8 optical parameters including chlorophyll fluorescence (Tables 4.4 - 7). Of the 192 test colony clippings transplanted, 171 were recovered in 2011 and standardized to 5 colonies per test block for all subsequent trait assessments. Overall 431,761 phenotypic measurements/counts were made (2010 & 2011). Survivorship between the two sites was considerable with 86.5% and 90.6% for Ridge and Sampela clones respectively and no signs of bleaching or predation observed. Only a single block (Sampela shallow transplanted from the Ridge, Figure 4.5) was damaged due to accidental human interference such that it was excluded from further analyses. The greatest test colony mortality was at Sampela (Table 4.3) specifically those transplants from Ridge 1 (SRs), which even with the exclusion of the damaged block, still had the lowest survivorship across all tests. Nevertheless, surviving colonies appeared healthy, probably by being elevated from reef competition for 12 months. The bivalve *Pteria* cf. *tortirostris* Dunder 1848 often associated with gorgonians, was present on shallow (SSs = 2; SRs = 2) and deep (SSd = 2; SRd = 6) clippings at Sampela and a single observation at Ridge (RSd = 1).

Table 4.4. *Isis hippuris* macro-morphological (colony level) traits of reciprocal transplant and control colony clippings sourced from Ridge 1. Test codes defined in Figure 4.2.

Phenotypic Trait	Ridge 1 (mean ± SE)							
	RRs(10)	**RRs(11)**	**RRd(10)**	**RRd(11)**	**SRs(10)**	**SRs(11)**	**SRd(10)**	**SRd(11)**
Clipping (cm)								
[H] Height	10.62 ± 0.45	13.45 ± 0.59	10.25 ± 0.41	13.36 ± 0.7	10.12 ± 0.36	12.50 ± 0.48	11.48 ± 0.47	13.67 ± 0.54
[W] Width	3.03 ± 0.25	4.98 ± 0.43	2.61 ± 0.20	4.20 ± 0.35	2.79 ± 0.30	4.26 ± 0.40	3.06 ± 0.25	3.75 ± 0.30
[B] Base	0.55 ± 0.03	0.78 ± 0.09	0.54 ± 0.03	0.61 ± 0.04	0.60 ± 0.05	0.86 ± 0.10	0.57 ± 0.03	0.64 ± 0.05
[TBL] Total Branch Length	51.83 ± 4.24	76.55 ± 6.6	46.35 ± 3.11	65.49 ± 4.7	48.47 ± 4.58	79.60 ± 6.25	47.28 ± 2.97	59.89 ± 4.83
[ML] Mother Length	8.20 ± 0.43	9.96 ± 0.49	7.90 ± 0.42	9.88 ± 0.62	8.06 ± 0.43	9.40 ± 0.60	8.59 ± 0.53	9.90 ± 0.64
[MW] Mother Width	1.18 ± 0.17	1.82 ± 0.09	1.03 ± 0.15	1.28 ± 0.16	1.09 ± 0.16	1.72 ± 0.12	1.14 ± 0.14	1.42 ± 0.17
[DL] Daughter Length	2.85 ± 0.17	3.84 ± 0.21	3.17 ± 0.17	3.86 ± 0.20	3.26 ± 0.22	4.14 ± 0.31	2.83 ± 0.17	3.44 ± 0.18
[DW] Daughter Width	0.34 ± 0.02	0.38 ± 0.017	0.35 ± 0.03	0.33 ± 0.01	0.33 ± 0.02	0.38 ± 0.03	0.35 ± 0.02	0.35 ± 0.01
[Bu] Bud #	3.27 ± 0.6	4.13 ± 0.55	3.07 ± 0.64	4.33 ± 0.53	2.58 ± 0.65	3.8 ± 0.52	3.33 ± 0.59	3.0 ± 0.58
[TB#] Total Branch #	13.67 ± 1.48	18.6 ± 1.54	12.13 ± 0.91	16.07 ± 1.24	12.5 ± 1.45	16.67 ± 1.72	12.67 ± 1.15	13.67 ± 1.17
[DB#] Total Daughter Branch #	10.53 ± 1.16	14.47 ± 1.3	9.27 ± 0.68	12.47 ± 0.86	9.58 ± 1.10	13.1 ± 0.8	9.80 ± 0.85	10.6 ± 0.84
[MB#] Total Mother Branch #	3.13 ± 0.38	4.13 ± 0.42	2.87 ± 0.40	3.60 ± 0.51	2.92 ± 0.43	3.56 ± 0.36	2.87 ± 0.34	3.07 ± 0.37
[*c*] MB#:TB#	4.76 ± 0.38	4.69 ± 0.32	5.15 ± 0.60	5.60 ± 0.77	4.72 ± 0.40	4.75 ± 0.34	4.85 ± 0.41	4.93 ± 0.40
[*r*] Ramification rate	0.268 ± 0.117		0.258 ± 0.060		0.205 ± 0.060		0.230 ± 0.109	
[PA] Projected Area	32.24 ± 3.04	67.34 ± 6.97	27.11 ± 2.62	57.0 ± 6.68	28.46 ± 3.5	53.05 ± 5.21	35.11 ± 3.14	51.71 ± 4.84
[PBA] Projected Branch Area	43.03 ± 7.09	86.98 ± 10.24	34.0 ± 5.48	56.15 ± 8.65	38.09 ± 7.10	88.69 ± 9.08	38.16 ± 5.2	57.29 ± 8.31
[Po] Porosity %	87.02 ± 0.26	86.70 ± 0.12	87.03 ± 0.26	89.82 ± 0.80	87.03 ± 0.26	83.64 ± 0.18	87.03 ± 0.26	89.33 ± 0.12

Table 4.5. *Isis hippuris* macro-morphological (colony level) traits of reciprocal transplant and control colony clippings sourced from Sampela. Test codes defined in Figure 4.2.

Phenotypic Trait	Sampela (mean ± SE)							
	SSs(10)	**SSs(11)**	**SSd(10)**	**SSd(11)**	**RSs(10)**	**RSs(11)**	**RSd(10)**	**RSd(11)**
Clipping (cm)								
[H] Height	11.37 ± 0.26	12.9 ± 0.54	11.54 ± 0.34	13.53 ± 0.5	11.31 ± 0.34	12.71 ± 0.49	11.83 ± 0.37	13.90 ± 0.56
[W] Width	2.82 ± 0.15	3.52 ± 0.25	2.92 ± 0.20	3.50 ± 0.28	2.32 ± 0.16	3.18 ± 0.39	2.79 ± 0.23	3.49 ± 0.41
[B] Base	0.63 ± 0.02	0.77 ± 0.08	0.61 ± 0.03	0.65 ± 0.05	0.65 ± 0.04	0.97 ± 0.18	0.67 ± 0.04	0.72 ± 0.11
[TBL] Total Branch Length	54.06 ± 3.94	68.51 ± 7.12	49.28 ± 3.34	70.92 ± 4.22	49.07 ± 5.06	68.03 ± 8.0	47.65 ± 4.01	69.67 ± 7.0
[ML] Mother Length	8.97 ± 0.49	10.23 ± 0.53	8.82 ± 0.52	10.42 ± 0.55	9.68 ± 0.47	9.48 ± 0.6	10.09 ± 0.50	11.42 ± 0.55
[MW] Mother Width	0.98 ± 0.17	1.20 ± 0.02	0.98 ± 0.18	1.33 ± 0.20	0.75 ± 0.02	1.16 ± 0.02	0.79 ± 0.18	1.45 ± 0.02
[DL] Daughter Length	3.93 ± 0.22	4.57 ± 0.26	4.14 ± 0.23	4.85 ± 0.26	4.18 ± 0.26	4.69 ± 0.28	4.18 ± 0.24	4.60 ± 0.28
[DW] Daughter Width	0.44 ± 0.04	0.41 ± 0.03	0.36 ± 0.03	0.34 ± 0.02	0.34 ± 0.02	0.31 ± 0.02	0.36 ± 0.02	0.32 ± 0.02
[Bu] Bud #	1.8 ± 0.34	2.27 ± 0.66	1.33 ± 0.42	2.67 ± 0.59	2.2 ± 0.47	2.21 ± 0.52	1.53 ± 0.36	2.8 ± 0.73
[TB#] Total Branch #	11.6 ± 1.01	15.64 ± 2.7	9.53 ± 0.80	12.6 ± 1.23	11.4 ± 2.16	13.8 ± 1.8	9 ± 0.62	13.2 ± 1.28
[DB#] Total Daughter Branch #	9.33 ± 0.81	11.8 ± 2.47	7.33 ± 0.61	10.27 ± 0.73	9.27 ± 1.71	10.0 ± 1.1	7.27 ± 0.48	10.6 ± 1.0
[MB#] Total Mother Branch #	2.27 ± 0.30	3.00 ± 0.47	2.2 ± 0.26	2.73 ± 0.32	2.13 ± 0.48	3.8 ± 1.04	1.73 ± 0.21	2.6 ± 0.34
[*c*] MB#:TB#	5.91 ± 0.56	5.78 ± 5.67	4.92 ± 0.51	4.86 ± 0.45	5.8 ± 0.33	5.01 ± 0.37	5.78 ± 0.45	5.83 ± 0.90
[*r*] Ramification rate	0.200 ± 0.081		0.146 ± 0.082		0.142 ± 0.083		0.146 ± 0.082	
[PA] Projected Area	31.90 ± 1.65	44.19 ± 4.94	33.49 ± 2.19	47.72 ± 4.48	26.26 ± 1.83	40.77 ± 5.45	33.12 ± 3.08	49.82 ± 6.97
[PBA] Projected Branch Area	42.15 ± 7.20	61.25 ± 12.53	36.73 ± 7.42	64.45 ± 11.13	43.03 ± 7.99	58.91 ± 12.81	32.81 ± 8.2	62.50 ± 6.41
[Po] Porosity %	88.90 ± 0.14	87.81 ± 0.23	88.90 ± 0.14	86.66 ± 0.18	88.90 ± 0.14	86.62 ± 0.21	88.90 ± 0.14	86.41 ± 0.19

Table 4.6. *Isis hippuris* micro-morphological traits of reciprocal transplant and control colony clippings sourced from Ridge 1 and Sampela. Test codes defined in Figure 4.2.

Phenotypic Trait	Dimensions (mean ± SE)									
	Ridge 2010	2011				Sampela 2010	2011			
		RRs	RRd	SRs	SRd		SSs	SSd	RSs	RSd
Micromorphology (mm)										
[PD] Polyp Density (cm^{-2})	87.13 ± 2.60	94.63 ±3.07	94.43 ± 6.98	88.56 ± 5.74	95.33 ± 6.03	100.21 ± 5.32	100.77 ± 4.17	102.74 ± 6.61	93.91 ± 4.65	89.15 ± 7.00
[Pd] Polyp Depth	0.081 ± 0.00	0.074 ± 0.004	0.063 ± 0.002	0.081 ± 0.004	0.064 ± 0.003	0.081 ± 0.001	0.077 ± 0.002	0.066 ± 0.002	0.068 ± 0.002	0.060 ± 0.003
[pD] Polyp Diameter	0.035 ± 0.00	0.031 ± 0.002	0.023 ± 0.001	0.033 ± 0.003	0.018 ± 0.001	0.030 ± 0.001	0.030 ± 0.001	0.021 ± 0.001	0.033 ± 0.001	0.029 ± 0.001
[ID] Inter-polyp Distance	0.052 ± 0.00	0.06 ± 0.002	0.060 ± 0.003	0.058 ± 0.003	0.063 ± 0.003	0.055 ± 0.001	0.060 ± 0.002	0.060 ± 0.002	0.057 ± 0.002	0.057 ± 0.003
[Cd] Canal Diameter	0.02 ± 0.00	0.020 ± 0.002	0.018 ± 0.001	0.019 ± 0.002	0.017 ± 0.001	0.02 ± 0.001	0.018 ± 0.001	0.017 ± 0.001	0.018 ± 0.001	0.019 ± 0.002
Sclerites (mm)										
[CL1] Club Length 1	0.073 ± 0.001	0.072 ± 0.001	0.074 ± 0.002	0.074 ± 0.001	0.073 ± 0.001	0.068 ± 0.001	0.069 ± 0.001	0.069 ± 0.001	0.071 ± 0.001	0.069 ± 0.001
[CW1] Club mean Width 1	0.021 ± 0.000	0.022 ± 0.000	0.021 ± 0.001	0.022 ± 0.001	0.021 ± 0.001	0.019 ± 0.000	0.018 ± 0.001	0.020 ± 0.001	0.021 ± 0.001	0.019 ± 0.001
[CL2] Club Length 2	0.072 ± 0.000	0.067 ± 0.003	0.068 ± 0.002	0.071 ± 0.001	0.071 ± 0.001	0.068 ± 0.002	0.068 ± 0.001	0.069 ± 0.001	0.069 ± 0.001	0.068 ± 0.001
[CW2] Club mean Width 2	0.033 ± 0.000	0.034 ± 0.001	0.032 ± 0.001	0.033 ± 0.001	0.033 ± 0.001	0.031 ± 0.001	0.030 ± 0.001	0.031 ± 0.001	0.031 ± 0.001	0.030 ± 0.001
[CaL] Capstan Length	0.115 ± 0.004	0.117 ± 0.003	0.116 ± 0.003	0.1143 ± 0.003	0.110 ± 0.002	0.100 ± 0.002	0.104 ± 0.003	0.107 ± 0.002	0.104 ± 0.002	0.104 ± 0.002
[CaW] Capstan mean Width	0.073 ± 0.001	0.072 ± 0.002	0.072 ± 0.002	0.070 ± 0.002	0.067 ± 0.002	0.066 ± 0.001	0.066 ± 0.001	0.065 ± 0.001	0.066 ± 0.001	0.063 ± 0.002
[SL] Spindle Length	0.165 ± 0.003	0.176 ± 0.004	0.170 ± 0.004	0.174 ± 0.005	0.165 ± 0.003	0.161 ± 0.003	0.169 ± 0.004	0.168 ± 0.004	0.171 ± 0.005	0.163 ± 0.005
[SW] Spindle Width	0.067 ± 0.001	0.075 ± 0.002	0.073 ± 0.002	0.071 ± 0.002	0.069 ± 0.002	0.063 ± 0.001	0.063 ± 0.001	0.062 ± 0.001	0.069 ± 0.002	0.065 ± 0.002

Table 4.7. *Isis hippuris* optical parameters of reciprocal transplant and control colony clippings sourced from Ridge 1 and Sampela. Test codes defined in Figure 4.2.

Phenotypic Trait	Dimensions (mean ± SE)				
	Ridge 2010	2011			
		RRs	RRd	SRs	SRd
Optical Parameters					
[ZD] Zooxanthellar Density (cells cm^{-2}) $x10^6$	6.71 ± 0.33	5.07 ± 0.20	3.39 ± 0.94	4.98 ± 0.58	3.22 ± 0.19
[MI] Mitotic Index %	1.93 ± 0.16	1.36 ± 0.17	1.92 ± 0.91	2.81 ± 0.51	2.48 ± 0.32
[Zd] Zooxanthellar mean Diameter (µm)	7.449 ± 0.027	7.767 ± 0.026	7.834 ± 0.027	8.164 ± 0.027	7.859 ± 0.029
[SA:V] Zooxanthellar SA:V	0.813 ± 0.003	0.779 ± 0.003	0.775 ± 0.003	0.741 ± 0.003	0.771 ± 0.003
[*a**] Chl *a* Specific Absorption (m^{-2} mg chl *a*)	0.030 ± 0.005	0.033 ± 0.007	0.028 ± 0.001	0.046 ± 0.005	0.040 ± 0.004
[*A*] Chl *a* Absorbance (µg cm^{-2})	13.72 ± 1.01	12.28 ± 1.88	14.25 ± 3.52	6.34 ± 0.85	6.67 ± 0.61
[CZ] Chl *a* Absorbance (pg $cell^{-1}$)	2.19 ± 0.17	2.38 ± 0.35	4.06 ± 0.27	1.58 ± 0.31	2.21 ± 0.25
[ΔF/Fm'] Light Adapted Yield	0.520 ± 0.013	0.564 ± 0.006	0.550 ± 0.006	0.579 ± 0.007	0.587 ± 0.006

Phenotypic Trait	Dimensions (mean ± SE)				
	Sampela 2010	2011			
		SSs	SSd	RSs	RSd
Optical Parameters					
[ZD] Zooxanthellar Density (cells cm^{-2}) $x10^6$	5.47 ± 0.20	5.06 ± 0.52	3.05 ± 0.20	5.00 ± 0.43	3.27 ± 0.31
[MI] Mitotic Index %	2.57 ± 0.17	2.38 ± 0.24	1.82 ± 0.29	1.77 ± 0.27	1.90 ± 0.30
[Zd] Zooxanthellar mean Diameter (µm)	7.912 ± 0.028	8.213 ± 0.027	8.184 ± 0.027	8.303 ± 0.025	8.127 ± 0.027
[SA:V] Zooxanthellar SA:V	0.764 ± 0.002	0.737 ± 0.002	0.739 ± 0.002	0.728 ± 0.002	0.744 ± 0.002
[*a**] Chl *a* Specific Absorption (m^{-2} mg chl *a*)	0.037 ± 0.003	0.026 ± 0.003	0.026 ± 0.003	0.020 ± 0.002	0.027 ± 0.009
[*A*] Chl *a* Absorbance (µg cm^{-2})	10.93 ± 0.87	11.15 ± 0.51	10.41 ± 0.42	14.38 ± 1.83	10.21 ± 0.85
[CZ] Chl *a* Absorbance (pg $cell^{-1}$)	2.04 ± 0.15	2.37 ± 0.27	3.50 ± 0.27	3.12 ± 0.44	3.37 ± 0.31
[ΔF/Fm'] Light Adapted Yield	0.530 ± 0.012	0.537 ± 0.007	0.527 ± 0.006	0.589 ± 0.005	0.580 ± 0.006

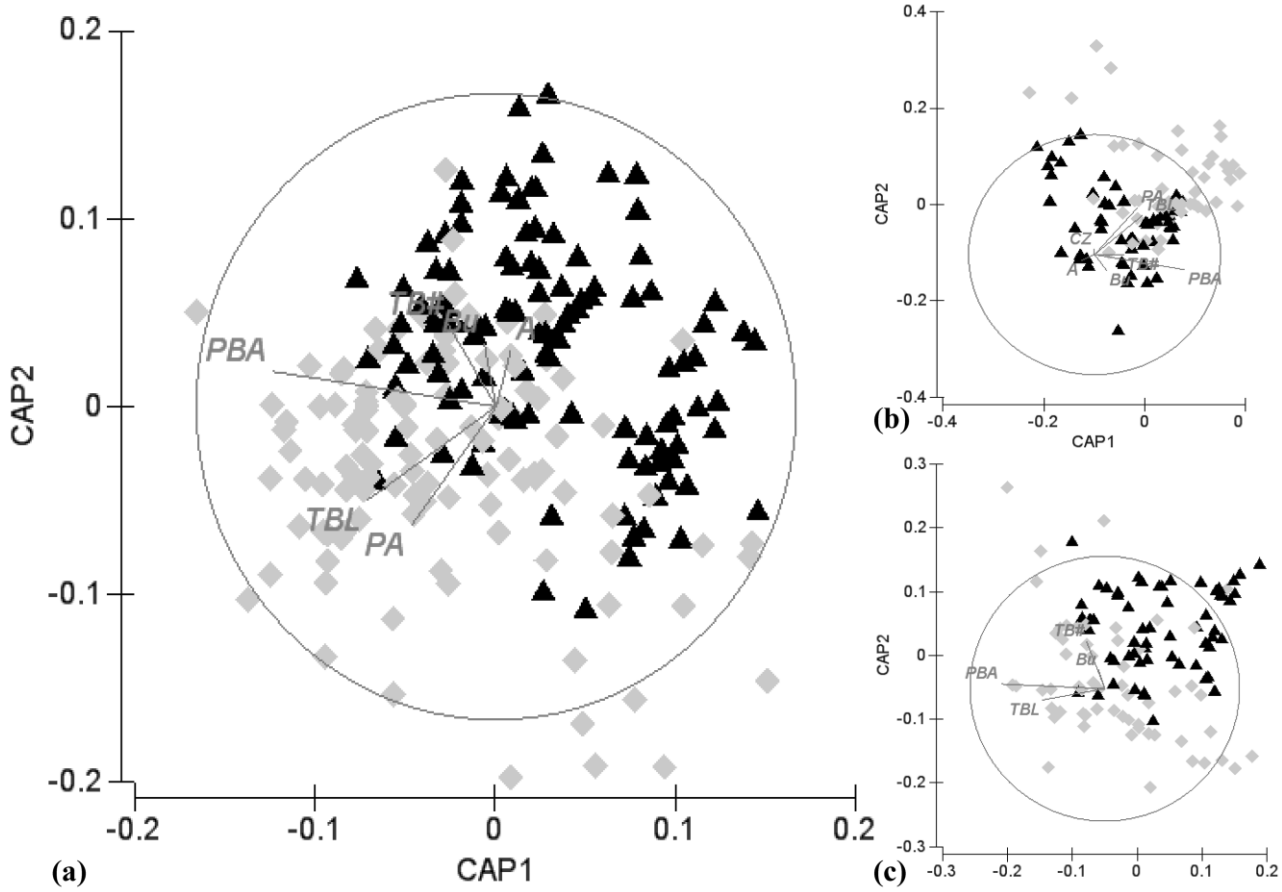

Figure 4.6. CAP ordinations of (a) all *Isis hippuris* phenotypic traits and tests, with the same model repeated for (b) the Ridge and (c) Sampela. Test labels 2010 (▲) and 2011 (◆); see table 4.4 - 7 for vector trait codes.

Results from the full PERMANOVA model across all tests revealed annual, site and depth differences with no interaction effects (Table 4.8; Figure 4.6). Principle traits overlaying such differences were at the colony (PBA 13%, Bu 9%, PA, TBL & TB# 6% dissimilarity) and chl *a* absorbance levels (*A* 6% dissimilarity). Models segregated by site show that differences in depth and chl *a* irradiance response [*A*] from the full model, were specifically attributed to the Ridge (Table 4.8; Figure 4.6a,b). Ridge dissimilarities between depth and site averaged 11% for PBA and 7% for Bu, PA and *A*, whereas only the annual difference was significant at Sampela primarily being at the colony branching level (PBA 14%, Bu 9%, TB# 6%, TBL 5% dissimilarity). These results imply that growth and chl *a* absorbance efficiency maintain fixed and plastic responses of the holobiont phenotype for Sampela and Ridge source colonies respectively. To further investigate this, the same statistical model was parsed into each phenotypic module (colony, polyp, sclerite and optical dynamics) testing for both within and between factor variance (Table 4.8).

Table 4.8. PERMANOVA of *Isis hippuris* test colony phenotypic traits. Repetitive analyses results on full (all tests and traits) and parsed models between sites (Ridge, Sampela) and phenotypic modules (colony, polyps, sclerites and optical parameters). Note, only interactions with a significant effect are presented. **P* < 0.01, ***P* < 0.001, ****P* < 0.0001, ns, not significant. Y, S and D, denotes year, site and depth respectively.

Source	All Tests			Ridge			Sampela		
	df	SS	pseudo-*F*	*df*	SS	pseudo-*F*	*df*	SS	pseudo-*F*
All Variables									
Year	1	1410.2	33.292***	1	799.05	22.427***	1	720.5	14.659***
Site	1	471.27	11.126***	1	76.658	2.1515^{ns}	1	54.257	1.1039^{ns}
Depth	1	1115.66	2.7304*	1	1117.25	3.2908*	1	74.286	1.5114^{ns}
Total	225	11522		107	4711.1		117	6349.5	
Colony									
Year	1	1962.5	32.364***	1	1066.8	22.868***	1	934.14	12.391**
Site	1	706.51	11.651**	1	43.754	0.93791^{ns}	1	84.12	1.1158^{ns}
Depth	1	104.27	1.7195^{ns}	1	131.4	2.8166*	1	76.697	1.0173^{ns}
Total	225			107	6045.6		117	9462.8	
Polyps									
Year	1	26.016	4.3663*	1	21.475	4.0458*	1	14.972	2.2393^{ns}
Site	1	18.603	3.1222^{ns}	1	0.5288	0.09625^{ns}	1	13.532	2.0238^{ns}
Depth	1	7.501	1.2589^{ns}	1	4.1891	0.78922^{ns}	1	5.17	0.77325^{ns}
Total	225	1371.9		107	564.74		117	788.14	
Sclerites									
Year	1	110.62	8.4485***	1	70.468	5.0004*	1	47.065	3.7412*
Site	1	443.85	33.899***	1	15.792	1.1206^{ns}	1	11.916	11.916^{ns}
Depth	1	26.615	2.0327^{ns}	1	14.884	1.0562^{ns}	1	14.029	14.029^{ns}
Total	225	3456.9		107	1544.3		117	1474.4	
Optical Parameters									
Year	1	1190.7	29.61***	1	790.94	18.661***	1	785.07	23.491***
Site	1	203.81	5.0682*	1	365.71	8.6282**	1	40.362	1.2077^{ns}
Depth	1	249.62	6.2075*	1	200.95	4.7411*	1	121.26	3.6284^{ns}
YxS	1	383.72	9.5422**	1	335.96	7.9264**	1	53.075	1.5881^{ns}
YxD	1	324.82	8.0775**	1	241.63	5.7008*	1	143.49	4.2936*
SxD	1	53.949	1.3416^{ns}	1	30.831	0.7274^{ns}	1	34.472	1.0315^{ns}
YxSxD	1	36.672	0.91195^{ns}	1	-11.222	-	1	2.4987	0.074766^{ns}
Total	225	11201		107	6150.8		117	4856.7	
Optical Parameters: 2-Factor Model									
Site	1	203.81	4.2269**	1	365.71	6.8263**	1	40.362	0.98718^{ns}
Depth	1	249.62	5.1772*	1	200.95	3.751*	1	121.26	2.9658*
Total	225	11201		107	6150.8		117	4856.7	

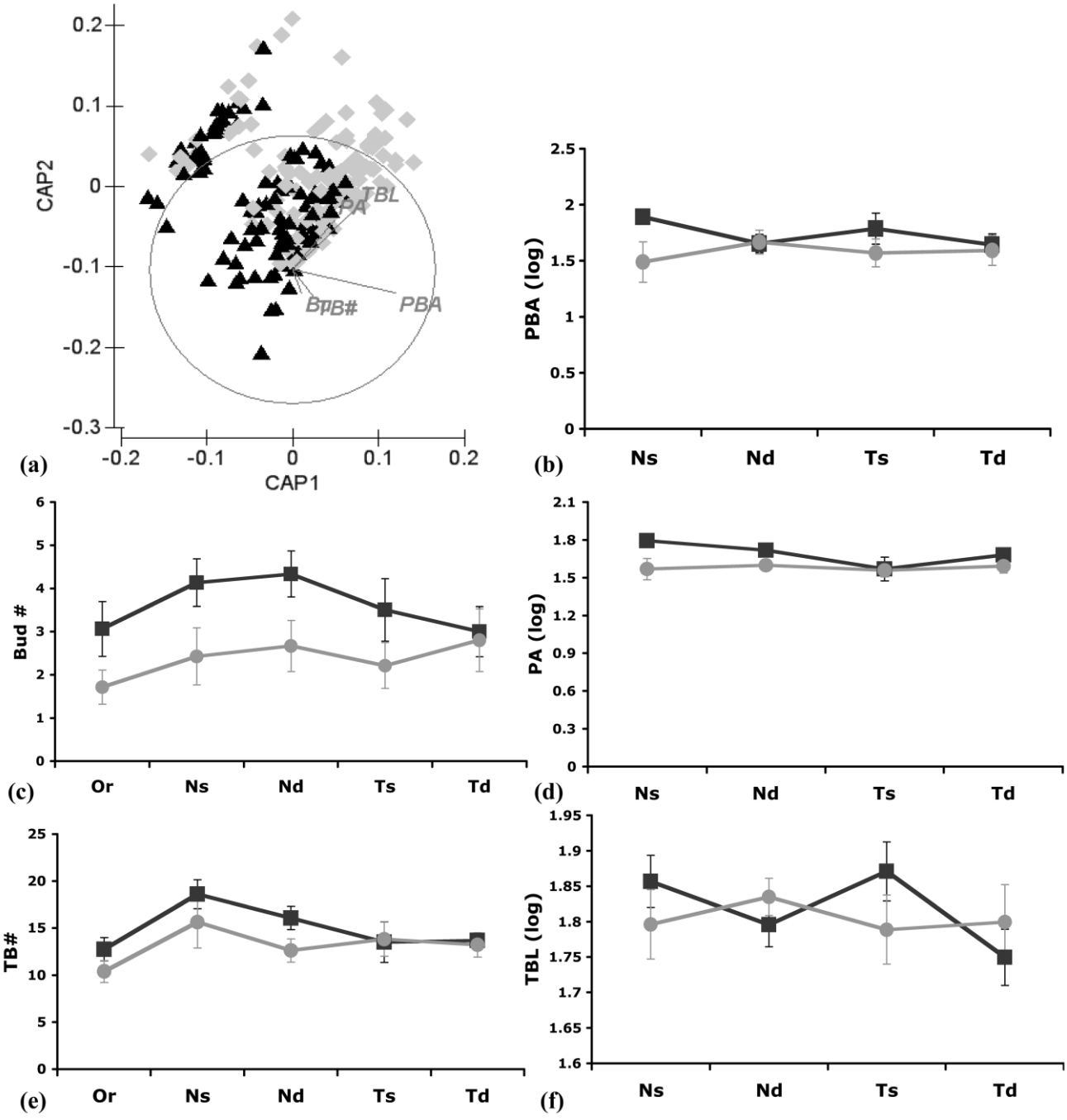

Figure 4.7. *Isis hippuris* morphological traits with: (a) CAP ordination of all test traits. Reaction norms of annual growth (adjusted for positive association, see equation 1) for branching parameters between 2010 - 2011 with (b) the projected branch area [PBA], (d) the projected colony area [PA], (f) the total branch length [TBL]. Reaction norms of (c) bud number and (e) total branch number [TB#]. Factor level labels 2010 (▲) and 2011 (◆), Ridge (—■—) and Sampela (—●—), and Or, origin; Ns, native shallow; Nd, native deep; Ts, transplant shallow; Td, transplant deep.

Colony Morphometrics

Colony growth was one of the principal drivers of annual site differences (see Table 4.4 - 5 & 4.8), typically greater for Ridge colonies with a notable reduction at depth (Figure 4.7b, f). Correction for positive association through annual growth (Eq.1; Figure 4.7) reveals both growth rate and colony form was relatively preserved in Sampela colonies compared to those from the Ridge. Initial bursts in branching and bud number are likely attributed to increased resource allocation to colony growth and form (Figure 4.7c, e). Reaction norms reveal V_G and V_E variance, particularly for budding and less so for total branch number. Total branch length accounted for the variability in projected branch area, mirroring the pattern of greater to reduced branching intensity for Ridge source colonies with increased depth (as a function of light intensity). To a far lesser extent was the opposite true for Sampela, increasing branch length with increased light attenuation, highlighting a clear V_{GxE} interaction between the two morphotypes (Figure 4.7b, f). Projected area (PA) remained constant for Sampela yet varied slightly for the Ridge, likely a reflection of differential growth; finally, V_G existed between both source colonies with environmentally induced plasticity at Sampela shallow for Ridge colonies (Figure 4.7d). Trait dissimilarities were similar however for both sites (PBA 15% and 18%, Bu 11% and 12% for Ridge [also with PA 9%] and Sampela with TB# & TBL at 7%) often juxtaposing each other as with the case of PBA and TBL (V_{GxE}). Branching trait integration probably explains such differences, particularly facilitating Ridge colony plasticity relative to increased water flow and differential light availability. Branching trait integration was also evident in the parameter *c*, which remained constant, with no evidence for dissimilarity between tests in alignment with previous studies (Table 4.4 - 5; Sánchez 2004). Branch width was not as independently influential as branch length and number, but was reflected in PBA. Taken together, within phenotypic module integration between branching traits was evident in the differential morphotype responses to environmental change.

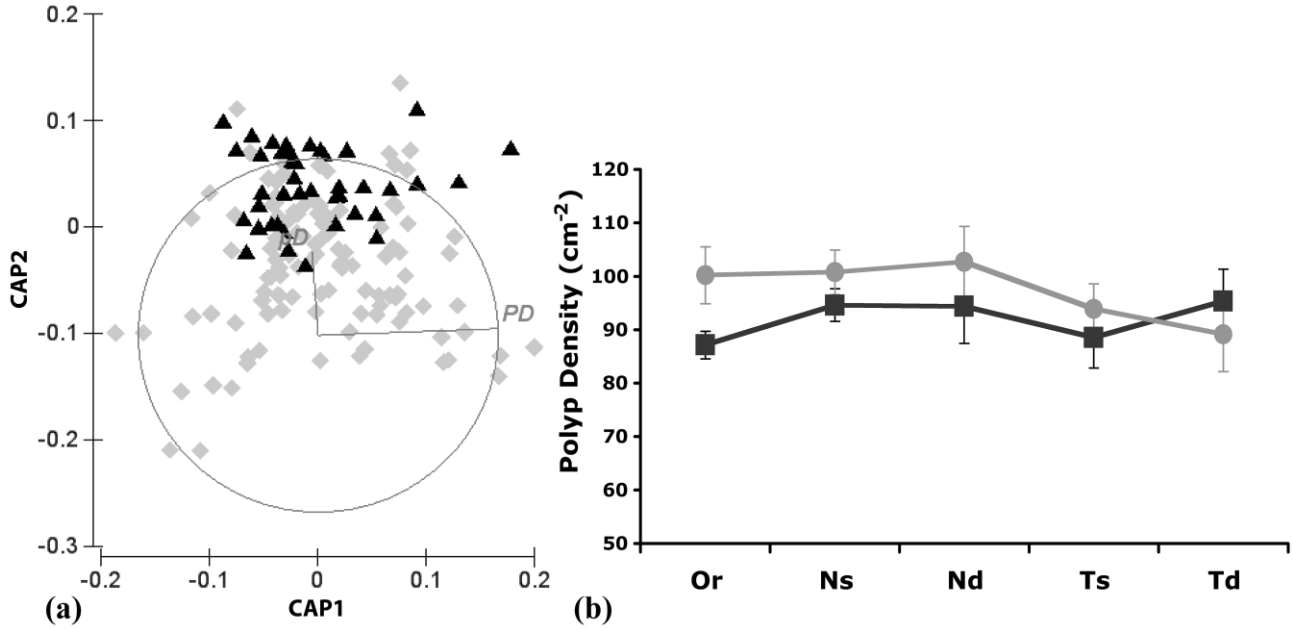

Figure 4.8. (a) CAP ordination of *Isis hippuris* polyp dimensions with (b) reaction norms of polyp density as the most variable trait observed. Factor level labels 2010 (▲) and 2011 (◆), Ridge (■) and Sampela (●), and Or, origin; Ns, native shallow; Nd, native deep; Ts, transplant shallow; Td, transplant deep.

Polyps

Little variation in polyp dimensions was observed with only an annual significant difference at the Ridge (Table 4.8). On average there was 85% dissimilarity due to polyp densities in Ridge colonies irrespective of a clear reduction in polyp density in Sampela transplants at the Ridge (Figure 4.8b; Table 4.8). Thus, there appears to be a similar response to depth by both source colonies at the opposite environment suggesting additional nutritional factors are of influence as well as a V_{ExG} interaction. Furthermore, both polyp density and diameter reduced at depth specifically at the Ridge, but with only minimal influence on the analyses for the latter (pD; Table 4.6). Polyp depth was considerably reduced in nearly all but one of the transplant blocks (Figure 4.5). This block was omitted from the remainder of the analyses due to disturbance (see above), however it is noteworthy that two of the three remaining clippings were externally brooding pink eggs also observed in the surrounding native reef colonies (Figure 4.5), but not present on any other reef within the area, only Sampela. Dissection also revealed the presence of eggs within polyps of 2 control colonies (see SSs polyp depth; Table 4.6) in 2011, yet eggs were regularly encountered within source colony polyps in 2010 with no site preference suggesting resource allocation to growth not reproduction in 2011.

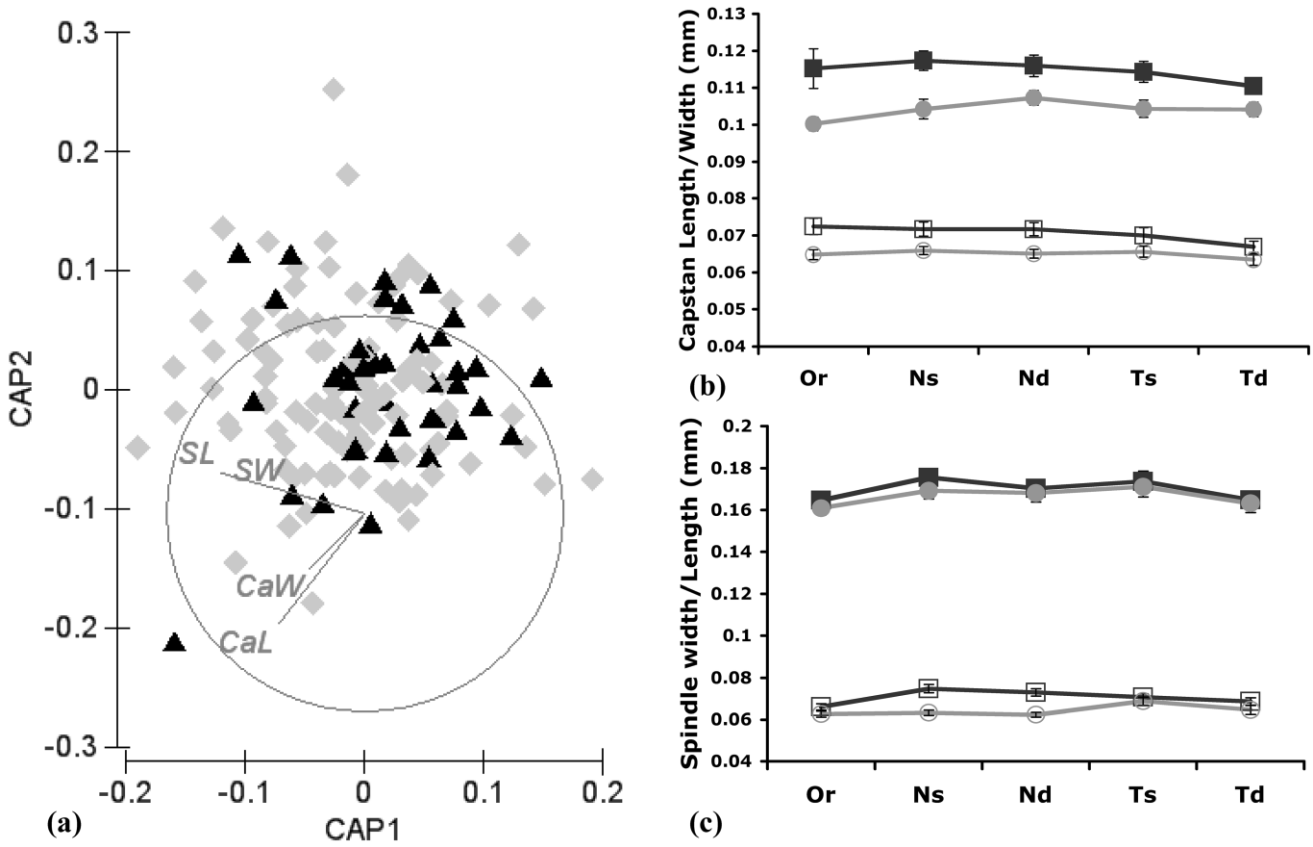

Figure 4.9. *Isis hippuris* annual sclerite size distribution across all tests. (a) CAP analyses of sclerite variation with promine epresented as reaction norms for (b) capstan, and (c) spind ns. Sclerite (s 6) and test codes are 2010 (▲) and 2011 (◆), Ridge (■, □, ●, ○) and Sampela (■, □, ●, ○), and Or, origin; Ns, native shallow; Nd, native deep; Ts, transplant shallow; Td, transplant deep.

R[SL] S[SL] R[SW] S[SW] R[SL] S[SL] R[SW] S[SW] Ns Nd Ts Td Td

Sclerites

Variations in sclerite dimensions were primarily in coenenchyme surface capstans and subsurface spindles, with dissimilarities of SL 24 and 29%, CaL 19.5% and 17%, SW 13% and 12%, CaW 13 and 10% for Ridge and Sampela respectively (Figure 4.9). A consistent decrease in sclerite size with reduced irradiance was observed for all tests, most markedly with spindle length and within colonies particularly from the Ridge (Figure 4.9b, c). Most notable, however, was the consistency in reduced size in all sclerites from Sampela suggesting V_G and V_E in both cases.

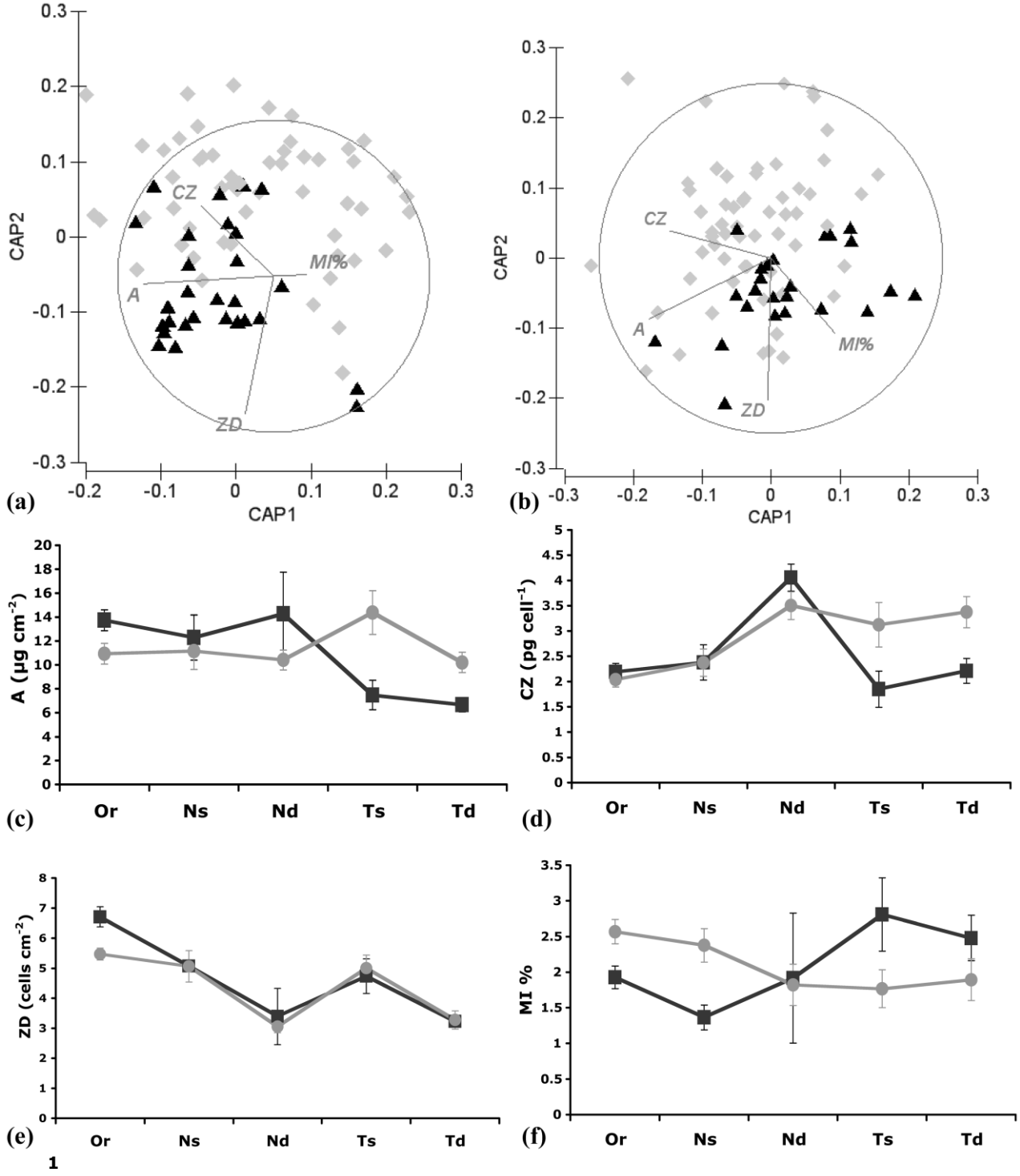

Figure 4.10. Optical parameters from test colonies with CAP constrained ordinations of (a) Ridge and, (b) Sampela. Reaction norms of principal driver traits including chl *a* absorbance normalised to (c) surface area, (d) per zooxanthellar cell, (e) zooxanthellar density and (f) mitotic index. Test codes are: deep (▲) and shallow (◆), Ridge (■) and Sampela (●), and Or, origin; Ns, native shallow; Nd, native deep; Ts, transplant shallow; Td, transplant deep.

Optical Parameters

The influence of light-dependent phenotypic responses was most apparent on Ridge source colonies (Table 4.8; Figure 4.10). Significant main effects of both full and site-specific optical

trait PERMANOVA models were countered by interactions between year x depth for both sites in addition to year x site for the Ridge (Table 4.8). This suggests that the differences between source colony responses to environmental change were variable; neither adjusting to the same magnitude nor in the same way when placed in the opposite environment (Figure 4.10). Pairwise differences were significant between sites in both years ($P \leq 0.01$), depth in 2011 ($P < 0.0001$), and depth within sites ($P \leq 0.04$). Naturally, pairwise comparisons revealed no difference between depths in 2010 considering all source colonies were selected at shallow optical equivalent depths (Figure 4.3d). Therefore, a two-factor model was constructed, omitting the factor 'year'. This confirmed pairwise comparisons for depth at both sites on transplantation, but no difference within Sampela colonies suggested a consistent response to reduced irradiance by depth irrespective of location or colony source (Table 4.8). Average dissimilarities across all tests in alignment with CAP vector overlays were *A* 30% and 27%, *CZ* 24% and 24.5%, MI 18% and 21%, and ZD 22% and 21% for the Ridge and Sampela respectively. Chl *a* concentration [*A*] increased with irradiance being consistently lower in Sampela colonies until transplanted to the shallow Ridge and *vice versa*, with a stronger response from Ridge colonies to Sampela (Figure 4.10c). This would suggest a V_{GxE} interaction response between *I. hippuris* holobionts across the two sites. Chl *a* per cell [*CZ*] increased with depth in both cases but also dropped considerably in Ridge transplants to Sampela and increased from Sampela to Ridge at both depths (Figure 4.10d).

Overall, reaction norms demonstrate magnitudinal differences indicating V_G and V_E responses between holobionts to environmental change. Zooxanthellae cell density [ZD] drove differences between depth but not site, both morphotypes responding similarly to their environments indicating a V_E and V_{GxE} interaction, i.e., plastic response (Figure 4.10e). Cell division was greater at Sampela irrespective of colony origin, being most dramatic in Ridge colonies (Figure 4.10f). Furthermore, zooxanthellar cell size was slightly larger at Sampela irrespective of treatment: average 8.2 µm and 7.8 µm for Sampela and Ridge respectively. Zooxanthellae SA:V was inversely related to cell density at the Ridge providing greater diffusion efficiency. Coupled with pigment concentration and density, this provides no evidence for self-shading through packing in test colonies from the Ridge at depth. Zooxanthellae SA:V in Sampelan colonies however, were relatively unresponsive to environmental change with consistently larger cells (Table 4.7). Chl *a* specific absorption [*a**] appeared to have little effect on Sampela

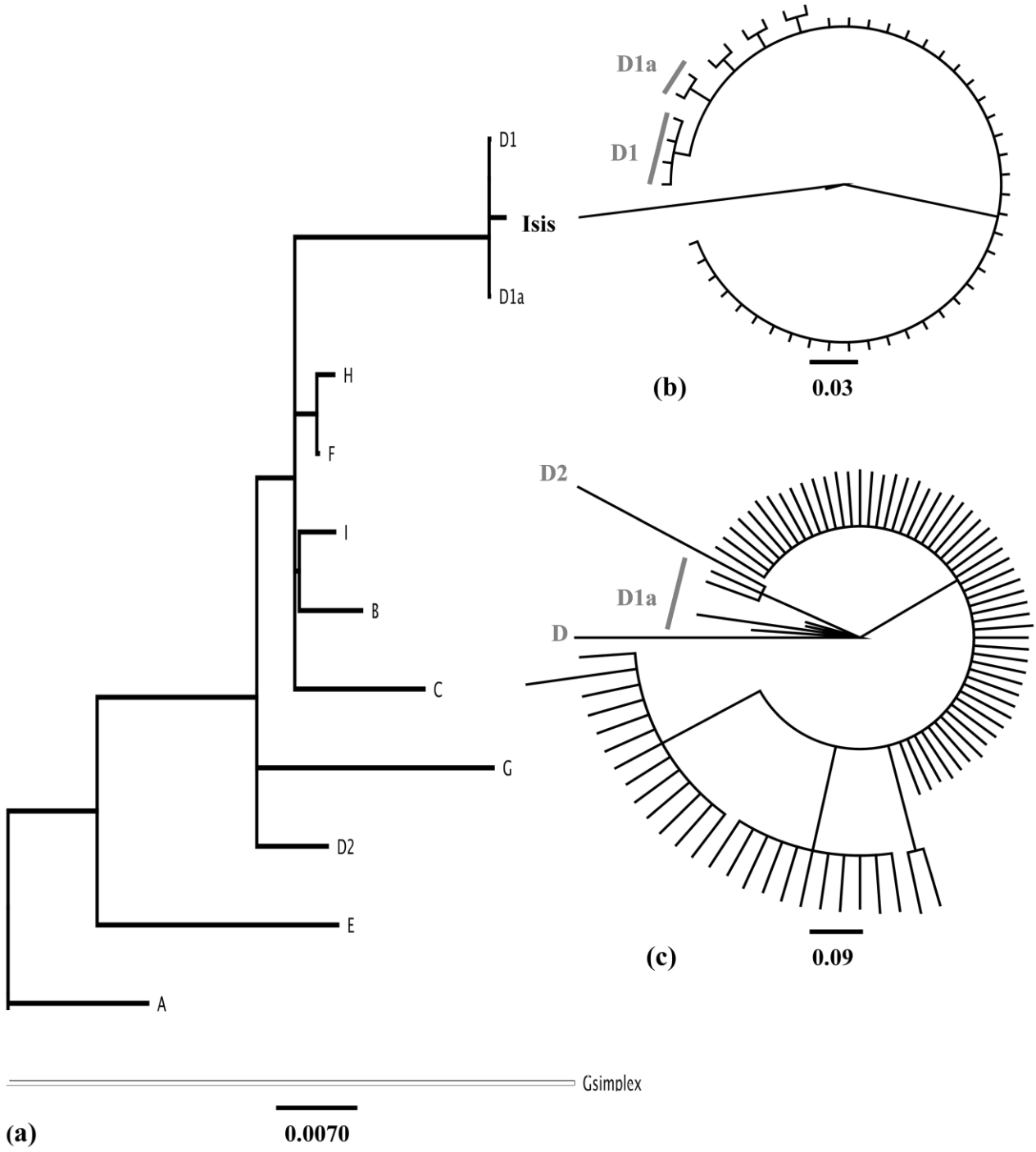

Figure 4.11. Phylograms of *Symbiodinium* clades based on neighbour joining (NJ), bayesian inference (BI), and maximum likelihood (ML) analyses (1000 bootstrap) of the (a) mitochondrial-encoded cytochrome oxidase *COX1* region (Takabayashi et al. 2004), unrooted trees (NJ/ML) of (b) the plastid-coding *psbA* minicircle, and (c) the internal transcribed spacer (ITS) region. Red bars and letters indicate clade D GenBank sequences. Scale bars correspond to base changes per site.

colonies decreasing with depth at the Ridge, the latter also the case with Ridge colonies plus a consistent increase on transplantation to Sampela for both depths (Table 4.7). Effective quantum yield [ΔF/Fm'] in light-adapted (*in situ*) colonies revealed an increase in those transplanted to and from Sampela with only a slight decrease at the native depth (Table 4.7).

Taken together, in virtually all optical traits the response to transplantation was greater and/or more variable in colonies from the Ridge, further corroborating an increased plastic response of the Ridge holobiont to environmental change compared to a somewhat more restrained and often contrasting response within Sampelan colonies.

Environmental Parameters

With no annual differences between measured abiotic traits all DISTLM*forward* analyses were performed on within (depth) and between site differences based on reduced test models as outlined in Table 4.8. Results comparing all phenotypic traits within and between sites showed that light ($K_{d(PAR)}$), water flow and turbidity explained 69.2% of test colony differences. Segregation by source colony site revealed that water flow, light ($K_{d(PAR)}$) and turbidity explained 57.5% for Ridge colony differences and that sediment, light ($K_{d(PAR)}$) and water flow explained 69.5% for Sampela source colonies. These patterns were consistent for all phenotypic module and integrated trait analyses.

Symbiodinium Genetic Analyses

Symbiodinium were identified using the mitochondrial marker *COX1*, rDNA ITS2, and the plastid-coding *psbA* minicircle. A total of 186 *COX1* sequences were recovered for phylogenetic analyses with previously published results (Table 4.2; Figure 4.11). Without exception all sequences were a novel strain of D1a, differing by only a single base pair from previously published results (Takishita et al. 2004; Figure 4.11). Of the 80 ITS2 cloned sequences recovered, there included no less than 7 for each test, a total of 9 haplotypes with 1 - 6 bp difference between them and 1-11 bp difference from previous studies (Table 4.2), and thus also novel. Sequenced clones from the *psbA* marker totaled 46 with 4 or more clones for each test and 5 haplotypes 1 - 2 bp apart with 1 - 3 bp difference from previous work (D1a, Takishita et al. 2003). No patterns of annual, site or depth specificity were present between haplotypes for either marker. Of the three gene regions and methods utilized in this study, there was no evidence of symbiont shuffling within the *I. hippuris* holobiont across all tests.

Table 4.9. Phenotypic trait correlation table with Ridge above the diagonal and Sampela below. Values indicate: ≥ 0.7 very strong positive correlation, 0.4 to 0.69 strong positive correlation, 0.20 to 0.39 weak to moderate positive correlation, -0.19 to 0.19 negligible, -0.20 to -0.39 weak to moderate negative correlation, -0.4 to -0.69 strong negative correlation, ≥ -0.7 very strong negative correlation. Trait codes see Tables 4.5-7.

	TB#	**TBL**	**Bu**	**PA**	**PBA**	**PD**	**SL**	**SW**	**CaL**	**CaW**	***A***	***CZ***	**ZD**	**MI%**
TB#	1	**0.739**	**0.395**	**0.576**	**0.735**	-0.140	0.062	0.126	-0.004	-0.013	**-0.280**	-0.192	-0.054	0.017
TBL	**0.721**	1	**0.285**	**0.693**	**0.865**	0.004	**0.350**	**0.395**	0.119	0.057	**-0.224**	-0.113	-0.103	-0.055
Bu	**0.339**	**0.279**	1	**0.319**	**0.309**	-0.102	0.156	0.116	0.002	-0.067	**-0.232**	-0.166	-0.045	0.019
PA	**0.476**	**0.733**	**0.222**	1	**0.632**	-0.100	**0.238**	**0.401**	0.080	-0.123	**-0.281**	-0.054	**-0.274**	-0.099
PBA	**0.756**	**0.904**	**0.256**	**0.607**	1	-0.031	0.112	**0.248**	-0.002	0.004	**-0.245**	-0.167	-0.059	0.024
PD	-0.166	-0.103	-0.029	-0.166	-0.095	1	**0.226**	0.089	0.065	0.007	-0.048	-0.061	-0.003	0.017
SL	**0.303**	**0.434**	0.196	**0.264**	**0.349**	**-0.203**	1	**0.689**	**0.440**	0.198	-0.081	-0.075	-0.002	-0.061
SW	**0.313**	**0.387**	0.119	**0.265**	**0.330**	-0.067	**0.703**	1	**0.350**	**0.332**	-0.020	0.065	-0.128	**-0.208**
CaL	0.071	0.077	**0.230**	0.127	0.009	-0.066	0.154	0.155	1	**0.482**	0.145	0.030	0.124	**-0.225**
CaW	-0.021	0.027	0.126	0.083	-0.039	-0.003	**0.331**	**0.345**	**0.559**	1	0.142	0.075	0.062	0.090
A	0.148	**0.209**	0.006	-0.011	**0.212**	**0.275**	-0.060	0.172	**-0.218**	-0.078	1	**0.751**	**0.207**	-0.110
CZ	0.171	**0.318**	0.047	0.164	**0.286**	**0.224**	-0.040	0.095	-0.098	-0.087	**0.714**	1	**-0.487**	-0.067
ZD	-0.073	**-0.209**	-0.073	**-0.262**	-0.158	0.036	-0.024	0.072	-0.127	0.027	0.192	**-0.548**	1	-0.061
MI%	-0.120	**-0.230**	-0.032	-0.156	**-0.203**	-0.036	-0.007	-0.087	-0.092	-0.096	-0.124	**-0.280**	**0.244**	1

Multivariate Analyses of Integration

Multivariate analyses revealed 14 prominent phenotypic traits within and between *I. hippuris* morphotypes that drive the differences between tests; PBA, Bu, PA, TBL, TB#, PD, CaL, CaW, SL, SW, *A*, ZD, *CZ*, and MI. These traits were isolated and re-modeled confirming their distributional influence (year: pseudo-*F* 24.111/16.21, $P < 0.0001$ Ridge and Sampela; with additional site; pseudo-*F* 3.001, $P < 0.03$ and depth: pseudo-*F* 3.7882, $P < 0.01$ for the Ridge), as were the remaining traits as a likely indication of canalised integration confirming magnitudinal trait differences within and between the two morphotypes (site: pseudo-*F* 13.084,

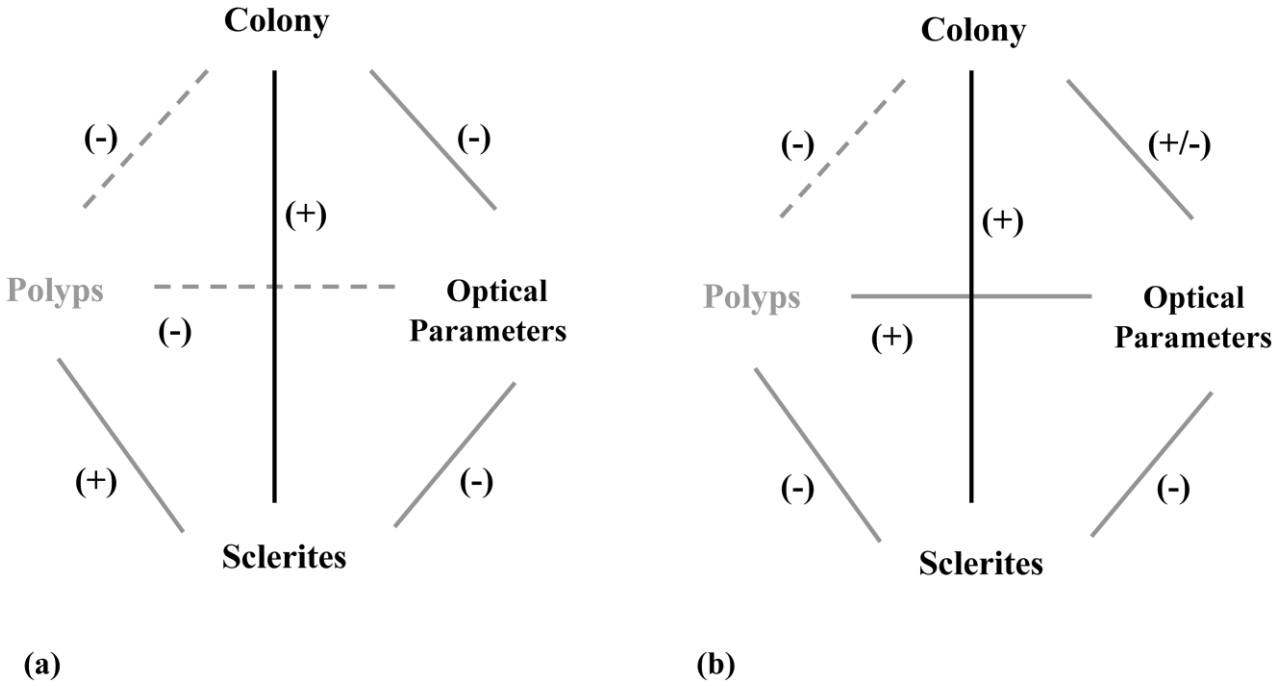

Figure 4.12. Phenotypic module integration models of *Isis hippuris* morphotypes from (a) Ridge, and (b) Sampela. Line key: (▬) very strong to strong correlation, (▬) weak to moderate correlation, (▬ ▬) and grey lettered modules are negligible, (+/-) positive/negative correlation.

$P < 0.0001$). Tests of within and between trait integration, which act to delimit *I. hippuris* morphotypes across sites, were modelled through covariance matrices (Table 4.9) corroborating vector values throughout the analyses. Phenotype module (colony, polyps, sclerites, and optical parameters) vertices were all significantly correlated within and between other modules with the consistent exception of polyps (Figure 4.12; Table 4.9). Polyps were the most weakly correlated trait, particularly at the Ridge, with those present typically responding differently, if at all, between the two sites/morphotypes to environmental change (polyps to sclerites and optical parameters). A similar scenario existed between colony responses and optical parameters with pigment densities positively correlated at Sampela. Colony level traits were moderately well correlated with sclerites regards both sites. Sclerites had a weak negatively correlation with chl *a* pigmentation [*A*] at Sampela and mitotic index [MI] at the Ridge, yet doubtful that such associations have any meaning in terms of integration. Typically stronger in Sampela chl *a* cellular pigment density [*CZ*] was positively correlated with [*A*] yet both having a weak negative association with ZD, suggestive of self-shading. Generally the majority of correlations between phenotypic modules were weak with some in contrast which tends to suggest a lack of coordination in development and growth between the two morphotypes and limited integration between phenotypic modules.

4.4 DISCUSSION

Previous research revealed that *I. hippuris* morphotypes within the WMNP were genetically segregated, strongly suggesting ecological divergence between healthy and anthropogenically impacted reefs (Chapter 3). Here, differential responses to environmental change illustrate phenotypic plasticity in healthy reef morphotypes, with reduced trait variability in colonies from the impacted reef, suggestive of genetic differentiation (V_G) or genotype-by-environmental interactions (V_{GxE}) between most traits. Specifically, colonies parsed annually primarily due to growth and source colony differences. With the exception of optical traits, all bathymetric differences were at the Ridge at the colony and chl *a* absorbance levels. Irrespective of test, Sampela clones did not differ significantly between sites or depth; again with the exception of optical traits largely driven by chl *a* absorbance levels. Most striking however was the lack of polyp, and to a lesser extent, sclerite differentiation between tests expressing trait canalisation particularly at the primary module level (polyps *sensu stricto*). Optical trait parameters were highly variable particularly in Ridge clones, yet *Symbiodinium* cladal type remained unchanged across all test colonies indicative of both plasticity capacity yet under some degree of host control. Trait integration was largely at the within phenotypic module level indicative of residual influences (e.g., developmental, trophic, and the cumulative effect of low variable traits) within and between traits. Such patterns coupled with known genetic differences and low dispersal properties (Chapter 3) make pertinent two notions; that *I. hippuris* morphotypes have 1) a high plasticity capacity as seen in Ridge colonies which, 2) over time through continual anthropogenic encroachment is resulting in incipient ecological divergence gradually leading to phenotypic canalisation at the module level in Sampela colonies. Therefore, two genetic and phenotypically divergent lineages partition at opposite ends of an environmental cline largely driven by differential light attenuation, hydrodynamics and poor dispersal, all likely exacerbated by anthropogenic influence at Sampela itself semi-lagoonal.

4.4.1 Isis hippuris *Plastic or Fixed?*

Results for the first objective demonstrate clearly that *I. hippuris* morphotypes within the WMNP show differential phenotypic responses in contrasting reef environments. Tests of local adaptation through reciprocal transplant experiments suggest phenotypically plastic colonies from the Ridge and incipient ecological divergence in Sampelan residents, the latter relatively insensitive to environmental change. Such patterns basically indicate that *I. hippuris* is inherently plastic, yet at Sampela may possibly have become functionally overwhelmed through prolonged anthropogenic disturbance leading to a breakdown in evolutionary capacitance (accumulated cryptic genetic variation). Fixation of adaptive cryptic variation over that of

random mutations, lead to genetic assimilation of the adaptive phenotype through epigenetic heritability. Separate adaptive fitness peaks through ecological niche specialization start to emerge. Peaks become further reinforced by the actions of pleiotropy, linkage disequilibrium or concerted evolution (Sánchez & Lasker 2003, Naidoo et al. 2013) as well as a reproductive strategy that overrides processes such as genetic drift and migration in an inherently plastic phenotype over time (Levene 1953, Hereford 2009, Blanquart et al. 2013). Thus, local adaptation at Sampela enhances diversity whereby selection in a novel environment may eventually supersede other evolutionary forces should conditions persist.

Resilience to environmental change will naturally be reflected in a population's ecology and survival, with habitat-dependent phenotypes showing immigrant inviability on transplantation (Prada & Hellberg 2013). *I. hippuris* test clones from Sampela were robust to environmental change, suggesting that a robust phenotype may be evolving in, and to, an anthropogenically compromised environment. This scenario has been observed in other taxa (e.g., Barshis 2009, Bellantuono et al. 2012), whereby low cost adaptation is reflected in weak or absent functional trade-offs (Hereford 2009). Conversely, greater plasticity as seen in Ridge residents leads to functional trade-offs on transplantation to degraded shallow reefs irrespective of accidental human interference. Thus, even when controlled for competition through block elevation and clipping distance, *I. hippuris* phenotypes from the Ridge have greater habitat dependency for shallow clear water reefs, exhibiting less survivorship under environmental change. Divergence in trade-off necessity between morphotypes, therefore provide further evidence for the onset of ecological speciation (Rundle & Nosil 2005) with Sampela residents relatively resilient to environmental change.

Plasticity capacity is shaped by natural selection leading to genetic stability through genetic assimilation in a stress-induced phenotype and may further lead to genetic incompatibility with non-native *I. hippuris* corals. However, this idea appears unlikely at this point, due to the presence of eggs on disturbed transplanted clones at Sampela from the Ridge. Test colonies were externally brooding pink eggs, also observed in the surrounding native colonies, but not present on either test control or colonies within the area. This suggests the presence of waterborne exudates inducing coordinated reproductive activity between colonies only at Sampela and that such exudates remained functional at the induction level. However, post zygotic isolation between source and transplanted colonies is still not out of the question, warranting further investigation. Nonetheless, it may be interpreted that resources in the clippings on the disturbed block, were allocated to reproduction for survival whereas all other

test colonies were in a state of juvenile growth – note that the presence of eggs in 2010 source colonies discounts the notion that all were male. Time to sexual maturity in these animals is unknown, however it is not unlikely that sexual maturity is typically limited to ~20 cm in height, previous years being dedicated to growth as seen in other gorgonians (e.g., Brazeau & Lasker 1990, Coma et al. 1995, Beiring & Lasker 2000) in order to establish the colony.

4.4.2 Isis hippuris *Holobiont Responses*

For the second objective, tests of divergence through local adaptation using reciprocal transplant experiments, demonstrated differential physiological responses of *I. hippuris* morphotypes within the WMNP in contrasting reef environments. Annual growth accounted for multivariate model differences, yet when adjusted for positive association, revealed clear colony (viz., branching) plasticity at the Ridge particularly across bathymetry. Reductions in colony porosity, branch articulation (TB#, Bu#), and chl *a* absorptance (*A*, *CZ*) with increased branch length primarily in transplants to Sampela maximizes light capture, reducing self-shading as well as sediment settlement typical of low light and water flow environments (Kawaguti 1943, Shaish et al. 2006). Functional integration at the morph-optical trait level is only moderately correlated, however, opposed between *I. hippuris* morphotypes. Similarly, polyp density was only weakly correlated with optical traits at Sampela, with a slight reduction on transplantation to the Ridge, which may be concomitant with reduced allochthonous food sources and/or differential light availability. Low polyp densities are typically encountered in low light (as a function of depth) environments (West et al. 1993, Kim et al. 2004, Prada et al. 2008, Prada & Hellberg 2013), possibly a regulatory mechanism to reduced resource availability. Overall, fine-scale differences within polyp and canal dimensions, particularly at depth, with concomitant variations in zooxanthellae and pigment densities, are further indicative of dynamic shifts in resource reliance at the optical and colony level facilitating the additive effect of polyps (module *sensu stricto*) as a growth response and primary resource facilitator.

Colony growth and form are dependent on feeding strategy, with the same genotype often showing differential resource allocation patterns (hetero/phototrophic capacity) in different environments (Sebens 1997, Poorter & Nagel 2000, Weiner 2004). The relative dependency of phototrophy and heterotrophy in *I. hippuris* morphotypes in the different environments have, however, likely shifted without significant changes in endosymbiont or, as in the case of Sampela, phenotype. Previous work revealed species specificity in octocoral heterotrophic food source with *I. hippuris* from the Seychelles and the Great Barrier Reef only feeding on bacterioplankton and curiously low photosynthetic rates (Sorokin 1991). This implies minimal

resource translocation, yet results presented here are in agreement with bio-optical and bio-physical values for other cnidarian taxa (e.g., Falkowski & Dubinsky 1981). Moreover, holobiont metabolism can be profoundly affected by light (Baker et al. 2011), sedimentation (Riegl & Branch 1995) and nutrient enrichment (Risk et al. 2009), which often act in exacerbated concert (e.g., Baker et al. 2011).

The sclerites of gorgonian corals are suggested to be most susceptible to selection through environmental change (Bayer & Stefani 1987, West et al. 1993, West 1997, Prada et al. 2008; but see Skoufas 2006). Here, only subtle variations in coenenchyme surface and subsurface capstans and spindles respectively occurred within the test period. Notably, the sclerites of the Sampelan clones remained consistently smaller in size reducing further at the Ridge. A decrease in calcification through reduced photosynthate translocation may account for this growth reduction in Sampela transplants to the Ridge, in addition to a greater former reliance on heterotrophic feeding through nitrogenous and allochthonous sources at Sampela. Utilization of various nitrogenous sources (e.g., nitrate, nitrite, ammonium) can also increase zooxanthellae densities (Muscatine et al. 1989, Fagoonee et al. 1999) and subsequent fixed carbon production through increased photosynthetic efficiency (Koop et al. 2001, Lesser et al. 2010). Furthermore, nitrogenous compounds stimulate calcification with or without light (Crossland & Barnes 1974). Thus the mutual exchange of photosynthetic carbon and nitrogen (whether from the host and/or eutrophic sea water) likely act synergistically in the formation of skeletal elements and growth, which may account for reduced sclerite growth when removed from high nutrient waters in adapted *I. hippuris* phenotypes. Therefore, assessing energy apportionment within and between *I. hippuris* morphotypes, endosymbionts, and the environment using comparative stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) would greatly elucidate energy transfer roles and thus, differential trophic allocation as a mechanism of phenotypic plasticity and divergence to environmental change.

The consistency in reduced sclerite size of and to Sampela may be host regulated, enhancing the internal light field under reduced pigment density in order to maintain overall absorption efficiency (Schlichter et al. 1986, Stambler & Dubinsky 2005, Enríquez et al. 2005). Dynamic stability was further established in Sampela colonies with evident adjustments in Ridge transplants to Sampela. Moreover, the effective quantum yield ($\Delta F/Fm'$), expressing the variation in PSII photochemical efficiency under ambient light (*in situ;* the capacity to use the absorbed light energy), was relatively invariable with slight increases in colonies transplanted to and from Sampela. Assessing light-adapted fluorescent yield is on an immediate time scale and

does not depict reaction centre dynamics which may be disrupted mirroring the >50% pigment loss in Ridge transplants to Sampela (see Dove et al. 2006). Therefore, to assess light absorption efficiency and utilization (= photosynthesis), *in situ* rapid light response vs. irradiance (PAR) curves would reveal if morphotypes differentially modify non-photochemical quenching, maintaining constant photoacclimatory operating efficiency in their respective environments (Hennige et al. 2008).

All test colonies exhibited identical patterns in zooxanthellae density, specifically reductions with increased bathymetry irrespective of site. This suggests regulation through irradiance and anatomical-specific carrying capacity particularly considering a concomitant reduction in colony growth and area with depth, also previously noted in scleractinians for seasonal irradiance (Fitt et al. 2000, Warner et al. 2002) and nutrient shifts (Fagonee et al. 1999). Typically, photoacclimatory responses to heterogeneous irradiances involve variations in chl *a* concentrations at the cellular level (Falkowski & Dubinsky 1981, McCloskey & Muscatine 1984, Porter et al. 1984, Fitt & Cook 2001). In this study, chl *a* absorbance efficiencies (*A*, *CZ*) were positively correlated. Characteristic increases in light absorption efficiency via greater pigment density at depth (McCloskey & Muscatine 1984, Porter et al. 1984, Enriquez et al. 2005, Lesser et al. 2010) were evident only in Ridge residents, the inverse being true in Sampela clones, further homogenised at the cellular level indicative of photoadaptation over time. Interestingly, however, Ridge transplants to Sampela revealed clear photoacclimatory responses to variable irradiance levels through both optical and morphological trait adjustment. Increased branch and chl *a* per cell densities are typical photoacclimatory responses to increased light attenuation in Scleractinia (Falkowski & Dubinsky 1981, McCloskey & Muscatine 1984, Porter et al. 1984), yet the opposite was largely true for these gorgonian Ridge transplants. A combination of reduced branch density and > 50% reduction in chl *a* concentration indicate stress responses to increased irradiance. But a reduction and maintenance in chl *a* densities per cell in shallow and deep test colonies respectively, concomitant with increased total branch length and overall reduced articulation suggest stress responses to increased irradiance are either masked or exacerbated by elevated nutrient levels and sedimentation at Sampela. Nevertheless, absorbance through pigment density at Sampela may indicate the onset of genetic accommodation demonstrated by an evolutionary shift in slope elevation of the reaction norm (Aubin-Horth & Renn 2009). Photoacclimatory control within the *I. hippuris* holobiont may therefore lie to some degree with the host particularly considering enhanced physiological performance in free-living *Symbiodinium* (e.g., Enríquez et al. 2005). However, photoacclimation in effective antennae-absorption is relatively conserved across certain

Symbiodinium cladal types, modifications present at the biophysical level (A - B, F; Hennige et al. 2009). Whether this is the same for clade D variants is unclear requiring investigations into PSII light curve interception to elucidate if endosymbionts within *I. hippuris* morphotypes differentially modify non-photochemical quenching to maintain constant photoacclimatory operating efficiency in alternate environments (see Hennige et al. 2008). The dynamic interplay between host-symbiont regulatory roles leading photoacclimatory capacity within the holobiont is fascinating (reviewed by Yellowlees et al. 2008, Davy et al. 2012, Fay & Webber 2012, Lesser et al. 2013) yet outside the scope of this study.

Photoacclimation combining optical and morphological traits can be seen in the increase of a^* in Ridge clones when transplanted to Sampela. Here, results strongly suggest that multi-scattering of the local light field and enhanced incident radiation capture were facilitated through the intricate geometry of the sclerites and axis. This down regulation of light absorption efficiency through reduced pigment density (Enriquez et al. 2005), coupled with increased zooxanthellae cell division (MI) is indicative of increased nutrient load and heterotrophic uptake (Fitt & Cook 2001, Davy et al. 2012). The generally small (*cf.* Wilkerson et al. 1988) zooxanthellae cell size and SA:V in Ridge colonies compared to Sampela started to increase in size on transplantation, emulating that of Sampela residents. Such subtleties maybe supportive of more variable trait responses, or may simply reflect increased intracellular space through reduced symbiont cell density at Sampela. Cell division and size vary with depth and coral taxa, previously negatively correlated (Wilkerson et al. 1988); yet the opposite is the case in *I. hippuris*. *Symbiodinium* cells between host morphotypes were consistently 5% larger within the Sampela holobiont but reduced to < 1.4% difference due to an increased cell size in Ridge transplants to Sampela, suggesting non-heritability within the Ridge. However, heritability or the onset of is unlikely in Sampela clones - contrasted with those noted in Jamaican scleractinians (Schoenberg & Trench 1980) - considering the invariable *Symbiodinium* cladal types across all test taxa. Taken together, Sampela clones showed a repetitive level of dynamic stability in optical and overall phenotypic traits across all tests, delimiting them from Ridge colonies. Photoacclimatory responses at the physiological and morphological level facilitated Ridge colonies at Sampela, likely unaccustomed to high nutrient, sedimented and low water flow environments further exacerbated by variable irradiance levels interacting with the light absorbing and scattering properties of the water itself (Stambler & Dubinsky 2005) and its elevated particulates (Porter 1976).

4.4.3 Endosymbiont Specificity

Resource allocation structure within the *I. hippuris* holobiont maximises fitness through physiological optima set by a novel *Symbiodinium* clade D1a. Technological advances reveal multiple cryptic endosymbiont types providing acclimatory 'flexibility' to environmental change in 39 scleractinian species (Silverstein et al. 2012 see also Putnam et al. 2012). Yet clear coral-algal specificity within the *I. hippuris* holobiont was evident with little genetic variation across highly variable (ITS2, *psbA*) and relatively conserved (*COX1*) molecular markers. Host-symbiont specificity is not uncommon in Octocorallia (Goulet & Coffroth 2003, van Oppen et al. 2005, Goulet 2006, Goulet et al. 2008, Baker & Romanski 2007) and has been attributed to an assumption of reduced autotrophic dependence within this group (Baker & Romanski 2007, but see Sorokin 1991). As stated, relative trophic structure is undetermined in *I. hippuris* colonies within the Wakatobi, however harbouring putatively stress tolerant clade D1a *Symbiodinium* (Jones et al. 2008, LaJeunesse et al. 2009, Stat & Gates 2011, but see Abrego et al. 2008) likely adds to the holobiont biological success, host-symbiont phylotype specialists apparently the most resistant to environmental stress (Putnam et al. 2012). Contrary however, is the currently perceived opportunistic (Stat & Gates 2011) or parasitic (Sachs et al. 2011, Lesser et al. 2013) nature of clade D. Tests of *Symbiodinium* diversity and reciprocal metabolic pathways within the holobiont using next generation sequencing and predictive modeling (PRMT; Larsen et al. 2011a) would determine both cryptic symbiont communities and metabolic interactions that structure holobiont trophic adaptations across environmental gradients. Resulting metabolic hypotheses may then be addressed through metabolic profiling, providing strong insights into integral metabolic links within the coral-*Symbiodinium* symbiosis, further elucidating nutrient cycling and the role of endosymbiont(s) in response to environmental change (Larsen et al. 2011b).

4.4.4 Isis hippuris *Trait Integration*

In order to test the theoretical framework of local adaptation leading to divergent selection through the action of environmental perturbation, the suite of phenotypic traits considered were summarised into phenotypic modules with topological equivalent traits driving the overall distributions determined. In the final objective, significant trait integration was greatest within, yet low between, phenotypic modules, likely indicative of residual errors (V_{error}) through developmental, trophic, and the cumulative effect of low variable traits. These factors as well as optical dampers such as mycosporine-like amino acids (MAAs; Gates et al. 1995, Lesser & Farrell 2004) and green fluorescent proteins (GFP; Dove et al. 2006) may also contribute to the residual variance (Hageman et al. 1999) between phenotypic correlates. Furthermore,

developmental covariance between traits, coupled with the action of pleiotropy can lead to covariance of seemingly unrelated traits. Tests of conditional dependence/independence (partial correlation coefficients; Magwene 2001, Sánchez & Lasker 2003, Wille & Bühlmann 2006) may yield insight into the relative integration within and between phenotypic traits and how they associate under environmental change. However, as evident in tables 4.4 - 7, 4.9 and respective phenotype module figures, the collective effect of each trait likely pull together even in synergy to determine the overall phenotype at any one point in time and space (Blanquart et al. 2013). Thus, an integrated trait cascade effect may act in unison – it is of interest that omission of primary traits on the same multivariate modules yield identical results. Hence, such traits may act as facilitators, by-products of the main adaptive traits or an interaction of the two, the former consequential of the latter (Gould & Lewontin 1979). In sum, trait integration was typically higher within Ridge sourced colonies indicating greater plasticity through integration – a necessary component for rapid responses to environmental change. Yet phenotypic invariability was evident in Sampela clones pre-adapted to variable light and high nutrient loads.

It is essential to distinguish between the 'primary adaptive' and 'by-product' traits; a relatively low/invariant trait may in fact be the adaptive framework of which an organism is constructed. In the case of *I. hippuris* – and gorgonians *per se* - it is clear that polyps are the primary adaptation, considered canalised with only their variable profusion indicative of environmental influence. Phenotypic characters such as the coenenchyme (soft tissue), sclerites, and axis etc. have in fact originated from the polyp wall, are by-products and subsequent facilitators of the primary modules, the polyps and thus coined 'exaptations' (evolved not for their original utility; Gould 1997). Yet selection acts on plasticity capacity (Kaandorp & Kübler 2001) resulting in divergent phenotypes often evident at the phenotypic level with favoured (often facilitative/by-product) traits the subject of evolutionary change (Kawecki & Ebert 2004). It can therefore be considered that by the interaction of light, nutrient and hydrodynamic regime, branching dynamics are simply 'exaptive' (Gould 1997) or the platform for polyps, the primary adaptation. The developmental influence of polyps on the colony therefore, should be the research target for processes of evolutionary change. Theoretical supposition aside, evolutionary changes on the phenotypic level are nature's manifesto of how human influences are affecting evolutionary change, which in itself will continue to persist irrespective of our presence.

4.5 CONCLUSION

Reciprocal transplant experiments across contrasting reef sites within the WMNP, strongly suggest plasticity capacity and incipient divergence in phenotypic traits for Ridge and Sampela

I. hippuris morphotypes respectively. *I. hippuris* morphotypes present at the Ridge and in previously sampled locations within the region (Chapter 3) are therefore, the ancestral type. Tests of coalescence would provide validation to this hypothesis (Puritz et al. 2012). Patterns of divergence suggested also by immigrant inviability, are likely determined through prolonged anthropogenic disturbance underpinned by reproductive strategy, which overrides processes such as genetic drift in an inherently plastic phenotype (Levene 1953, Hereford 2009, Blanquart et al. 2013). Resource allocation structure within the holobiont likely reaches differential physiological optima for each *I. hippuris* type, which maximises fitness in their respective environments through morph-optical trait integration. Both morphological and physiological photoacclimatory responses confirm mechanistic adjustments to maintain such fitness optima. Multivariate models reveal polyp dynamics being largely canalised and determinate traits; the density and size of which controlling resource acquisition and capacity (in terms of endosymbiont density). Investigations into the relative dependence on autotrophy *verses* heterotrophy leading to resource allocation change within the *I. hippuris* holobiont would undoubtedly confirm mechanisms of both biological success and incipient divergence consequential of genetically assimilated phenotypes to environmental change. It is proposed that *I. hippuris* morphotypes are in a state of ecological divergence and act as viable indicators on the effects of burgeoning anthropogenic encroachment on mechanisms of biodiversity and reef health within the Coral Triangle.

CHAPTER 5: GORGONIANS IN THE WAKATOBI MARINE NATIONAL PARK, INDONESIA: WHAT CAN THEY TELL US ABOUT EVOLUTIONARY PROCESSES IN ENVIRONMENTAL CHANGE?

5.1 CONTEXTUAL SUMMARY

The Wakatobi Marine National Park (WMNP), SE Sulawesi, Indonesia, comprises possibly the most biodiverse marine ecosystems on the planet with gorgonian corals epitomising such diversity. Central within the Coral Triangle, the WMNP's human population and inherent exploitations are as diverse as its natural resources. In fact the natural laboratory this region presents, encompasses natural and anthropogenic interactions bestowed upon the researcher as a haven for discovery and local collaborations akin to those by the marine fauna and flora itself. This work clearly demonstrates the utility of gorgonian corals as conservation indicator taxa through straightforward ecological assessment and experimentation, specifically across environmental clines.

As conspicuous members of any marine community, the modular nature of gorgonians continues to baffle scientists. Still, unresolved questions of plasticity, divergence, or homoplasy hamper species delimitation and biodiversity assessments, particularly on coral reefs throughout the Indo-Pacific (van Ofwegen 2004) despite their high regional abundance and diversity (Tomascik et al. 2004). The present work substantiates these notions, whereby 197 species and morphotypes from 42 genera, and 12 families within all currently accepted suborders were recognised from the shallow waters of the WMNP, increasing with depth inviting tests of refugia (Chapter 2). Meticulous morphological differentiation is not, however, indicative of non-interbreeding taxa, and may in fact represent differential phenotypic responses to environmental heterogeneity.

Assessment of gorgonian abundance and diversity across environmental gradients within the WMNP revealed a clear loss of gorgonian diversity relative to increased sedimentation and reduced light associated with anthropogenic disturbance. Zooxanthellate and azooxanthellate taxa were clearly parsed between depth and reef health, the former more tolerant to anthropogenic perturbation than the latter. Notably, the two distinct morphotypes of the zooxanthellate isidid *Isis hippuris* Linnaeus 1758, were highly abundant across environmental clines: long-branched bushy colonies on degraded reefs, and short-branched multi/planar colonies on healthy reefs. Such morphological differentiation may be a consequence of high

plasticity capacity of the *I. hippuris* holobiont, two previously diverged species, or anthropogenically driven incipient ecological divergence on degraded reefs. Morphological and molecular results reveal unsatisfactory assignment of *I. hippuris* morphotypes to previously described alternatives (*Isis reticulata* Nutting 1910, *Isis minorbrachyblasta* Zou, Huang & Wang 1991), further suggesting incipient ecological divergence through clear haplotype division between sites of differing reef health (Chapter 3). Multivariate analyses consistently revealed light availability, sedimentation and water flow as significant explanatory variables for morphotype differences, suggesting a dynamic interplay between *I. hippuris* morphotypes and their environment. To further assess potential mechanisms of divergence and survivorship in alternate reef habitats, a reciprocal transplant experiment (RTE) for one year yielded insightful results (Chapter 4). Firstly, reduced survivorship of healthy reef morphotypes on degraded reefs implied the onset of lineage segregation through immigrant inviability. Secondly, multivariate analyses revealed differences were attributed to *I. hippuris* morphotype origin, with phenotypic responses to environmental change typically plastic in colonies from the healthy site, whereas those from the degraded site were relatively insensitive to change. Prominent phenotypic traits were at the morphological and bio-optical levels integrated to maintain functional optima, ultimately influenced by resource availability and acquisition. Thirdly, that such optical responses were not attributed to endosymbiont diversity or shuffling, with all test colonies possessing a novel clade D1a *Symbiodinium* throughout. Residual error (V_{error}) unaccounted for by measured phenotypic traits, invite tests of energy transfer roles within and between the holobiont including biophysical photoacclimatory responses assessing functional thresholds to environmental stress.

This research aimed to investigate gorgonian responses to environmental change within the WMNP, SE Sulawesi, Indonesia. Collectively, this was achieved, challenging notions of ecological importance, taxonomic validity, and the overall effects of anthropogenic encroachment on mechanisms of plasticity and divergence within the coral holobiont. Key issues as a consequence of this work include tests of 1) deep-reef refugia, 2) priority systematics, and 3) mechanisms of ecological divergence and physiological assessment exploring intrinsic and extrinsic interactions that may define the host-symbiont relationship.

5.2 BIODIVERSITY & REFUGIA

Gorgonian corals within the WMNP exhibited high species diversity and abundance particularly on healthy coral reefs (Chapter 2). Of the 51 genera and 14 families recognised for shallow-water gorgonians in the Indo-Pacific (Fabricius & Alderslade 2001), 42 genera and 12 families

were present within the WMNP, comprising all higher order groups. Coral reef biodiversity is considered at its peak within the Coral Triangle, most notably in the Indo-Malay-Philippine (IMP) region (Stehli & Wells 1971, Veron et al. 2009, 2011, Bellwood et al. 2012, Briggs & Bowen 2013, Gaither & Rocha 2013). The origin of such diversity remains the source of much intrigue and investigation with various hypotheses being proposed. Firstly, the Centre of Origin hypothesis predicts the IMP as a centre of speciation where species disperse marginally (Ekman 1953). Secondly, the Centre of Overlap hypothesis predicts species diversity to be a consequence of dispersal overlap in all directions from numerous biogeographic provinces (Woodland 1983). Thirdly, the Centre of Accumulation hypothesis predicts that peripheral speciation through dispersal is extended unidirectionally by prevailing currents into the IMP (Ladd 1960), and finally, the Centre of Survival hypothesis whereby species are buffered by extinction in contrasting peripheral locations (Paulay 1990). All likely pull in unison, with differential responses of marine taxa and biodiversity feedback between hypothesised models, attributing to the current high biodiversity (Bowen et al. 2013). Yet how such hypotheses stand in the face of anthropogenic impact accelerating and/or exacerbating natural processes of environmental change is unknown. Furthermore, such hypotheses are almost solely based on reef fish and scleractinian corals (see Bellwood & Hughes 2001 but now see Bowen et al. 2013, Sanciangro et al. 2013) advocating latitudinal gradients of species richness driven by the universal currency of energy availability (Gaston & Spicer 2004, Evans et al. 2005). In its simplicity, most biodiversity may be a synergistic combination of increased habitat (Sanciangro et al. 2013) and energy/resource availability (Rohde 1992). Yet how these hypotheses relate to gorgonian corals without greater sampling effort is unknown, further exacerbated by a paucity of knowledge on gorgonian reproductive strategies throughout the Indo-Pacific, casting doubt on dispersal ability, range size and subsequent taxonomic assignment. Nevertheless, comparative global coral diversity primarily lies within the Octocorallia, comprising estimates of 64% (3400 species) compared to that of Scleractinia 27% (1450 species; Williams & Cairns 2013). Moreover, gorgonian corals are phylogenetically older than the Scleractinia (Lindstrom 1978, Bengtson 1981, Cope 2005, Stolarski et al. 2011). Therefore, as one of the most diverse invertebrate groups in benthic marine ecosystems, typically conservation 'flagship' species (Tinsley 2005, Linares et al. 2008, Cerrano et al. 2010) in many regions, it is curious that gorgonians are generally overlooked on coral reefs within the Indo-Pacific.

In the WMNP, gorgonian diversity increases with depth, almost exclusively by azooxanthellate taxa (Chapter 2). Such diversity, colony size and number of recruits were observed to

Figure 5.1. Distribution of Indo-Pacific tropical gorgonians found at shallow (0 – 39 m; green spots) and mesophotic (40 – 200 m; blue spots) depths. Data sources: USNM, AMNH, BPBM, NIWA, Nutting (1910a - e, 1911), Stiasny (1937, 1940), Mai-Bao-Thu & Domantay (1970, 1971), Muzik & Wainwright (1977), Colin et al. (1986), Goh & Chou (1994, 1996), Paulay et al. (2003), Fabricius et al. (2007), Rowley (2013; Chapter 2).

continually increase to depths way beyond 60 m (pers. obs.). This pattern is consistent with other Indo-Pacific regions where benthic communities become dominated by gorgonian corals

at 40 - 200 m (Figure 5.1; Marshall Islands, Colin et al. 1986; Palau, Mariana Islands, Paulay et al. 2003; Palau, Fabricius et al. 2007; Great Barrier Reef, Australia, Bridge et al. 2012; Philippines 2013, Rowley unpublished data). Deeper reefs ("mesophotic coral reefs" [MCEs] or the "twilight zone") are posited to act as refugia against disturbances as well as a haven for larval source pools (Glynn 1996, Reigl & Piller 2003, Bongaerts et al. 2010). Most importantly, such deep reef refugia are hypothesised to harbour reef components largely unaffected by geological sea-level change during times of markedly reduced shallow water habitats (Helm & Schülke 2003; RL Pyle, BW Bowen & J Copus pers. comm.). Increasing evidence reveals deep reefs possess unique communities with less than 50% overlap of shallow taxa (Pyle 1988, 1990, Macintyre et al. 1991, Bongaerts et al. 2010). In the WMNP, gorgonian diversity increased with depth, with numerous taxa distributed from 5 > 60 m, a pattern consistent with records from other Indo-Pacific regions (USNM, BPBM, BNHM, Nutting 1910a - e, 1911, Stiasny 1937, 1940, Mai-Bao-Thu & Domantay 1970, 1971, Paulay et al. 2003, Fabricius et al. 2007).

Overlapping taxa across bathymetry are ideal targets for tests of resilience and comparative divergence through local adaptation within certain genera, as well as 'twilight' habitat specialists. Interestingly, azooxanthellate gorgonian genera such as *Acanthogorgia* Gray 1857, *Annella* Gray 1858, *Bebryce* Philippi 1841, *Ellisella* Gray 1858, and *Villogorgia* Duchassaing & Michelloti 1862 span remarkable depths (5 ≥ 1000 m) across their distributional range. Yet within-group polyphyly across bathymetry (deep-water monophyly typically disrupted by shallow-water taxa; McFadden et al. 2006, Pante et al. 2012, Chapter 3) calls for further systematic assessment (e.g., coalescence; Puritz & Toonen 2011, Puritz et al. 2012b), with polyphyletic groups either a consequence of convergent evolution or deep divergence.

The question of shallow reefs becoming seeded by deep reefs is determined by species-specific responses to intrinsic (e.g., reproduction, settlement, development, symbioses) and extrinsic (e.g., hydrodynamics, light, temperature, sedimentation, water quality) factors (Fabricius et al. 2007), as well as evolutionary processes such as speciation, extinction and dispersal (Mora et al. 2003*).* Determining how and whether certain taxa are habitat specialists or bathymetric migrators remains to be elucidated, undoubtedly unveiling key evolutionary mechanisms facilitating survival over geological time. Moreover, assessing evolutionary principles on taxa inhabiting mesophotic reefs may shed light on the origin of deep-sea and shallow-water taxa. Before the opening of the Drake Passage ~25MYA (late Eocene) and the onset of the thermohaline circulation, much of the deep oceans were decidedly warmer and often anoxic (Chase et al. 1975, Scher 2006). Thus, the deep-sea gorgonians we know today would have migrated and evolved from shallower depths with huge depth ranges still apparent (e.g., > 4000 m range in deep-sea Chrysogorgiidae; USNM records 2013). Naturally, such benign marine environments invite deep-sea specialists such as *Metallogorgia melanotrichos* Wright & Studer 1889, with just a single haplotype across its geographic range of three oceans (Pante et al. 2012), similarly with its associate ophiuroid within the Atlantic (Cho & Shank 2010). Conversely, in the shallows, environmental heterogeneity selects high plasticity capacity that may become fixed at functional optima (as suggested in *Isis hippuris* at Sampela in Chapter 4), leading to restricted geographic range size and endemicity (e.g., Calosi et al. 2008, 2010).

Clearly, a high number of gorgonian taxa are widely distributed throughout the Indo-Pacific is present, particularly at mesophotic depths (Figure 5.1). Such unique communities may be relatively consistent throughout the Indo-Pacific and particularly prevalent within the Philippines (Figure 5.1) consisting of over 7100 islands (Williams & Chen in review) likely acting as habitat stepping-stones for dispersal within and between this and other regions. This,

coupled with ecoclimatic stability within the Coral Triangle, provides testable hypotheses for the distinct gorgonian diversity as shown in the WMNP and other regions (Colin et al. 1986, Paulay et al. 2003, Fabricius et al. 2007, Bridge et al. 2012, this study). Nevertheless, many regions require greater sampling efforts across bathymetry using advanced rebreather diving technology (e.g., Pyle 1996, Pyle et al. 2008) in order to begin addressing mechanisms of diversity and refugia. However, tests of diversity and evolution without historical investigation are inconclusive (Gould 1997, Rohde 1999), further requiring tests of coalescence.

5.3 SYSTEMATICS IN THE SEA

Ecological and taxonomic investigations indicate much work has yet to be completed to achieve effective gorgonian taxonomic assignment, in terms of conservation biodiversity assessments. Nevertheless, indicator taxa can and have been teased out as surrogates for reef component responses to environmental change (Chapter 2). In the case of *Isis hippuris*, gaining an historical perspective in the context of a structure-function relationship with its environment may provide insight into evolutionary divergent mechanisms between morphotypes (see Chapter 4). It is interesting, however, that phylogenetic analyses reveal that the Isididae is polyphyletic with the addition of its type species *I. hippuris*, and therefore seemingly unrelated (Chapter 3; Figure 3.7). The jointed bamboo-like axis (Figure 5.2a, b) that unites the family may simply be a relictual anachronism consequential of geological processes over time, yet phylogenetic analyses corroborate the early taxonomic suggestion that the characteristic jointed axis of the Isididae is in fact a convergent trait. Again, tests of phylogenomic coalescence using next generation sequencing (NGS) would differentiate evolutionary mechanisms acting between members within the Isididae; testing the evolutionary origin of the axis as either 1) deep divergence or, 2) convergent evolution. However, what would be the purpose of a jointed axis in the deep ocean when structurally there is no significant requirement for hydrodynamic flexibility in such a benign environment? A relictual anachronism therefore seems innately plausible with the evolution of flexibility through ecological necessity in shallow and steep wall environments, the latter often subject to high nutrient deep-water upwellings as seen at the healthy Ridge site in the WMNP (Gieskes et al. 1988). Even though unknown, the stratigraphic history may therefore evolve from either the high hydrodynamics on shallow reefs and/or refugial depths (e.g., 40 – 200 m), which then migrate through bathymetric expansion in response to resource competition, predation, random or specific settlement cues, encompassing any number or combination of ecological or developmental processes. Whatever the scenario, survival requires an individual and/or species to have the capacity to respond, and thus, not be entirely passive to its

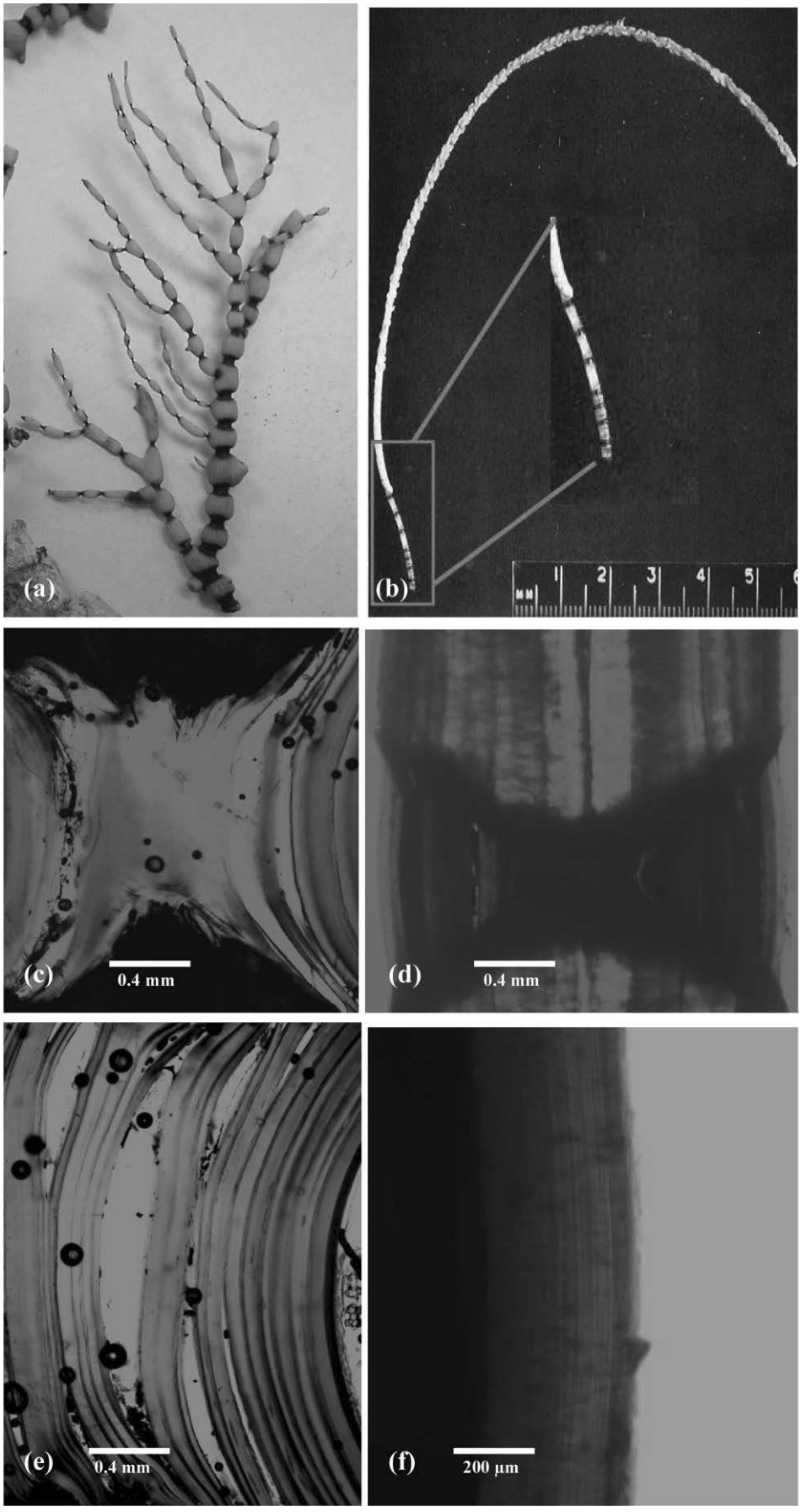
(a)
(b)
(c)
0.4 mm
(d)
0.4 mm
(e)
0.4 mm
(f)
200 µm

Figure 5.2. (Previous page) Isididae jointed axis comparison between *Isis hippuris* Linnaeus 1758 and *Circinisis circinata* Grant 1976 of the (a, b) denuded colony, (c, d) horny proteinaceous (gorgonin) node, and (e, f) node fibres.

environment, which is particularly true for those taxa which are sessile (Cossins et al. 2006). Migration to the shallows necessitates functionally advantageous traits to increases in light, hydrodynamics and temperature, including shorter more flexible axis joints (Figure 5.2c,e; Helm & Schülke 2003) and photosynthetic endosymbionts as seen in *Isis hippuris*. Conversely, deep-water migration particularly in the advent of the thermohaline circulation (Chase et al. 1975, Scher 2006), facilitates expansion into benign environments whereby flexible widely spaced and fibrous proteinaceous horny nodes become taught and robust (Figure 5.2d, f) and often reinforced with calcareous material (Grasshoff & Zibrowius 1983, Helm & Schülke 2003). Clearly, isidids are functionally adapted to differential hydrodynamic regimes across bathymetry, however this does not explain the significant polyphyly within the family.

The consistent phylogenetic incongruence at the subordinal and family-levels (McFadden et al. 2006, Pante et al. 2012, Sanchez et al. 2003b) seemingly lacks functional divergence to environmental change. Fixed traits when redundant (such as a jointed axis in the deep-ocean) may well be maintained through continuous sea level changes over geological time; bet hedging with insurance if you will. Furthermore, a loss and re-gain of traits may occur through the differential expression of genes in response to environmental change over the millennia. It lacks empirical sense to have the repetitive evolution of multiple traits in the absence of any functional cause, however. For example, the fused scleritic composition of the calcareous internodes of *I. hippuris* contrasted with the non-scleritic axis of deep-sea isidids, characteristic of the Calcaxonia *per se*, is another logical trait differentiation parsing the Isididae across bathymetry (Figure 5.3). Clearly a scleritic axis would permit greater flexibility in high water flow environments, however numerous deep-sea taxa, namely of the Scleraxonia (e.g., *Corallium* Cuvier 1798 [see Figure 5.3b], *Paragorgia* Milne-Edwards 1857, *Anthothela* Verrill 1879) possess this same trait. Furthermore, the only exception to the Holaxonian axis composition of a horny axis supported by non-scleritic calcareous material including a central core is the Keroeididae Kinoshita 1910, notable again for its scleritic axis. Bathymetric distribution within the Keroeididae are again akin to previous adaptation to high flow shallower waters with subsequent bathymetric migration to safer depths e.g., *Keroeides* Studer 1887 of 51 > 650 m, similarly with *Corallium* 96 > 2500 m. Henceforth, such patterns provide target

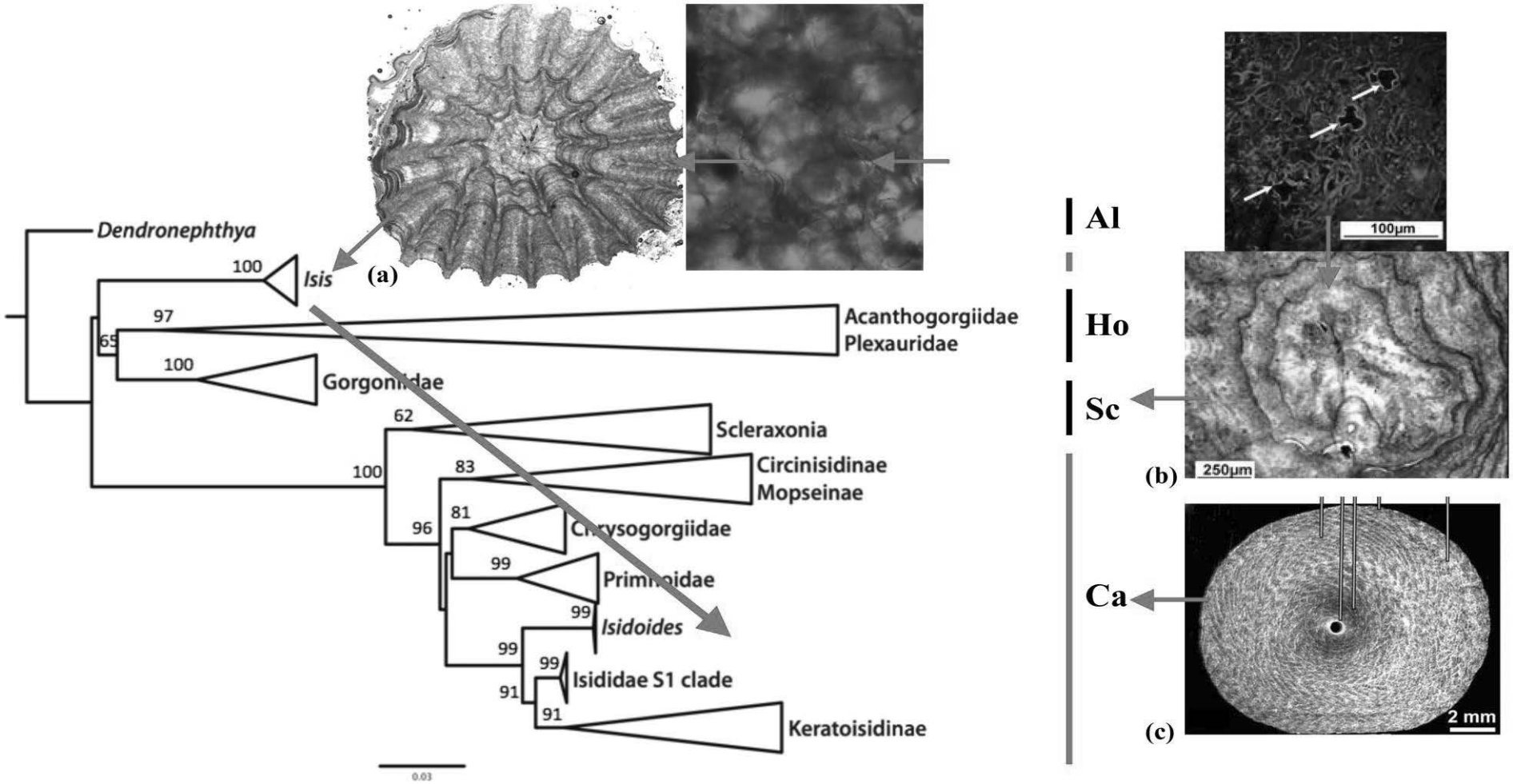

Figure 5.3. Phylogenetic reconstruction based on maximum likelihood (ML) analyses of the mt MutS (1800 bp) region (Heestand-Saucier *et al. in* prep.,). Axis cross-sections of (a) *Isis hippuris* with inset showing outline of capstan radiate composition, (b) *Corallium rubrum* with inset showing sclerite outline (white arrows; Debreuil et al. 2011), and (c) *Keratoisis* sp. (Noé & Dullo 2006). Branch numbers represent ML support. Letters Sc = Scleraxonians, Ca = Calcaxonians, Ho = Holaxonians, and Al = Alcyoniinans. Large red arrow depicts *Isis* separation to the rest of the Isididae (also seen in Figure 3.7).

taxonomic groups and traits (e.g., the axis) to further investigate functional divergence or convergence within gorgonians as a consequence of environmental and/or climatic change.

Finally, it is noteworthy that *I. hippuris* is consistently phylogenetically situated next or close to members of the Alcyoniina (Figure 3.7, 5.3), a prominent soft coral group. Suggestions of the evolution of a central axis from the soft corals may be discarded, however, through the fossil record revealing primitive gorgonians possessing a solid axis that becomes jointed coinciding with habitat change (Helm & Schülke 2003). Water energy is therefore considered the most selective agent for gorgonian corals (Langer 1989), further substantiating the notion of mesophotic refugia distributing bathymetric migrators which themselves evolve through local adaptation into habitat specialists.

Evidently there is a need for phylogenetic reconstruction exploiting recent advances in next generation sequencing and bioinformatics coupled with radiometric dating of fossil skeletal material (Abbey et al. 2013, Nelson et al. 2013). Targeting gorgonian taxa across specific bathymetric ranges, will inevitably shed light on both phylogenetic and evolutionary processes within the Octocorallia.

5.4 MECHANISMS OF ECOLOGICAL DIVERGENCE

Fitness enhancement through plasticity capacity produces phenotypic novelty in response to environmental change, particularly in modular marine organisms, ultimately leading to enhanced biodiversity over time (Levene 1953, Blanquart et al. 2013). Here, in order to test the theoretical framework of local adaptation leading to divergent selection through the action of environmental perturbation, a suite of phenotypic traits were considered and parsed into phenotypic modules (Figure 4.1; Chapter 4). Results suggested inherent plasticity capacity as evident in healthy reef morphotypes, yet through the continual action of anthropogenic disturbance on a semi-lagoonal reef, local adaptation has led to incipient divergent selection in *I. hippuris* within the WMNP. This can be depicted through a ‘sliding scale’ of phenotypic evolution between *I. hippuris* morphotypes in response to environmental change (Figure 5.4). The biological success of *I. hippuris* is therefore likely due to its pliable modular nature combined with a dynamic symbiotic association maintaining functional optima. Thus, further reinforcement through low reproductive dispersal and asexual fragmentation with trait fixation at the population level, plasticity capacity may then lead to genetic stability in a stress-induced phenotype with genetic incompatibility in the former, but further lead to enhanced phenotypic resilience. Whether such patterns are recapitulated throughout the *I. hippuris* distributional range remains to be

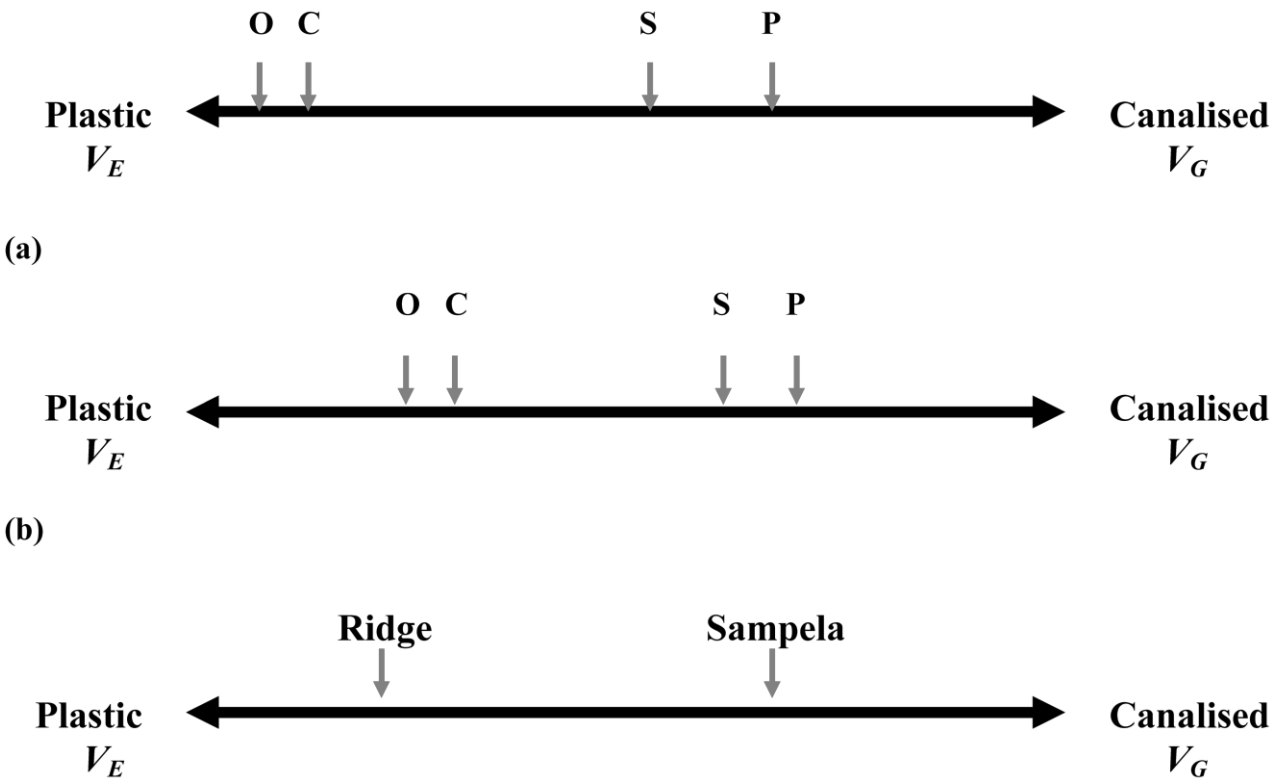

Figure 5.4. Sliding scale of phenotypic evolution within and between *Isis hippuris* morphotypes across habitat gradients within the WMNP, Indonesia. (a) Ridge (healthy site) morphotype responses to environmental change; (b) Sampela (impacted site) morphotype responses to environmental change, and (c) summary evolutionary trait development suggested as a consequence of plasticity capacity at the Ridge and incipient divergence through genetic assimilation of beneficial traits on degraded reefs. Red arrows depict phenotypic module position along the sliding scale of phenotypic evolutionary continuum between environmentally sensitive (V_E) to insensitive (V_G). Codes: C, colony; P, polyps; S, sclerites; O, optical parameters.

elucidated. Nonetheless *I. hippuris* can clearly be considered as a significant indicator of reef health, with a view for two species delineation in light of tests on reproductive isolation ascertained through cross fertilisation, and subsequent coalescence (time to divergence, as seen in Asteroidea, Puritz & Toonen 2011, Puritz et al. 2012b).

Restricted gene flow in the face of anthropogenic disturbance likely acts at the trophic level. Polyps are the primary phenotypic module (*sensu stricto*) for resource acquisition through the proportional variability of heterotrophy *verses* autotrophy. In each *I. hippuris* morphotype, polyp dynamics were relatively constrained compared to other traits and consistently so in Sampela

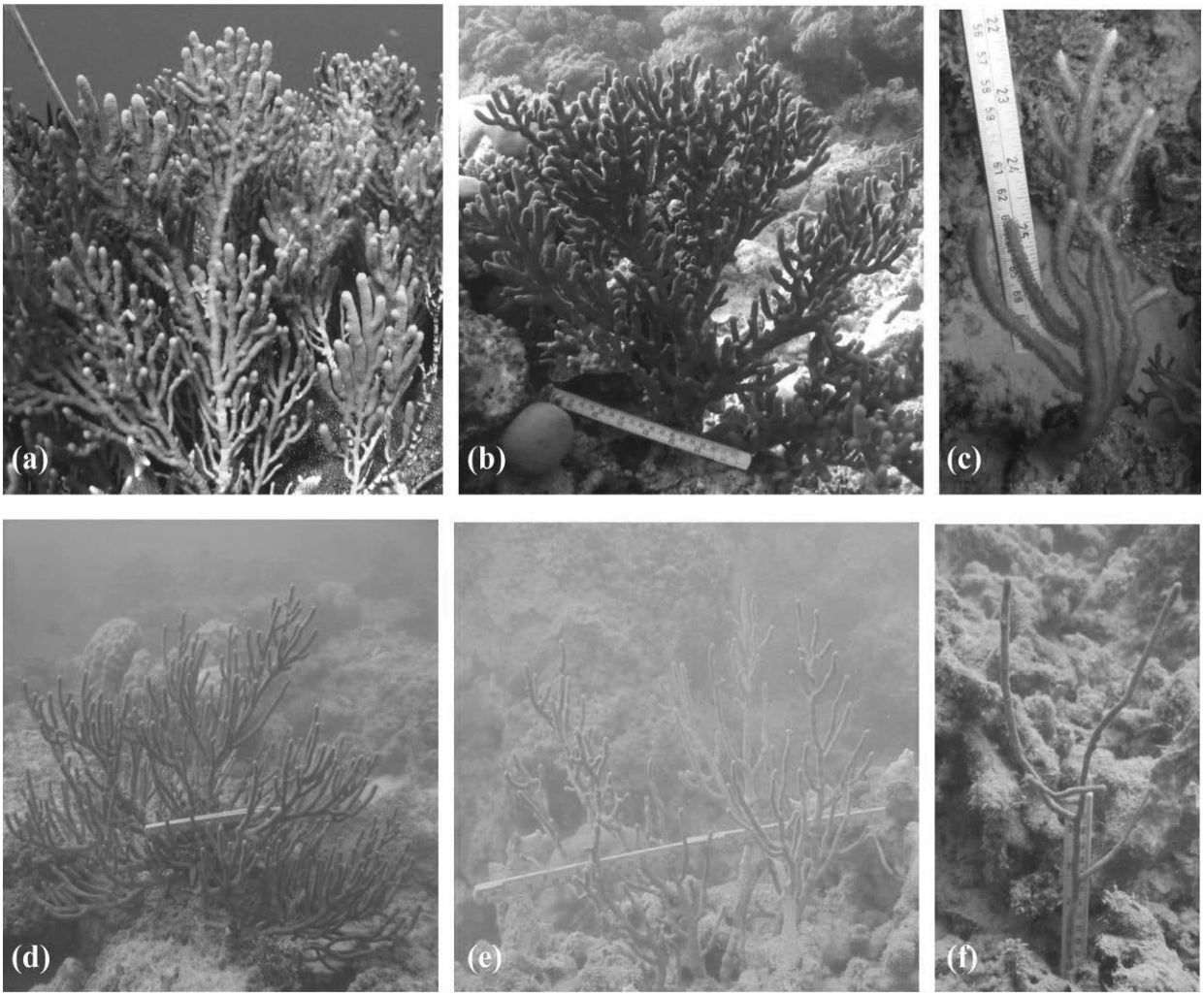

Figure 5.5. *Isis hippuris* morphotypes across bathymetry from the Ridge at (a) 2 m, (b) 6 m, (c) 14 m. Sampela at (d) 2 m, (e) 6 m, (f) 10 m.

colonies (Figure 5.4a, b). Therefore, photoacclimatory responses at the morphological and physiological level facilitate mechanistic adjustments maintaining resource acquisition by the polyp within the *I. hippuris* holobiont (Chapter 4). Resource, thus fitness optima in contrasting reef health environments would inevitably be reflected in photosynthetic efficiency through the non-photochemical quenching and electron transfer of PSII (Hennige et al. 2008). Moreover, phenotypic variability in native colonies across bathymetry (Figure 5.5) would therefore reveal differential photoacclimatory responses not accounted for by light-adapted yield alone, to differential environmental regimes.

Phenotypic responses across environmental gradients such as bathymetry are not unknown, particularly in zooxanthellate gorgonians (e.g., West et al. 1993, Kim et al. 2004, Prada et al. 2008, Prada & Hellberg 2013; Figure 5.5). In figure 5.5, *I. hippuris* colonies exhibit clear morphological plasticity with decreased light availability. However, this response may be due to

a differential photoacclimatory capacity between sites, which may further reinforce ecological divergent mechanisms between morphotypes. Tests of electron transfer efficiency through photosystem reaction centres would provide a much more elaborate explanation of photosynthetic responses and efficiency, and can be achieved via *in situ* rapid light response curve analyses with additional considerations for the photosynthetically useable radiation (PUR; MacIntyre et al. 2002, Suggett et al. 2007, Hennige et al. 2008) available to the individual at any one time. Furthermore, even though multi-marker molecular analysis strongly suggested symbiont specificity in *I. hippuris* irrespective of test or sampling site (Chapter 4), Rowley et al. (2011) discovered colonies that were occasionally associated with *Symbiodinium* clade C on degraded reefs at depth (Figure 5.6). It was concluded in Chapter 4 that the tight association with a novel type within the putatively stress tolerant clade D *Symbiodinium* strongly accounted for the biological success of *I. hippuris* morphotypes particularly on degraded reefs. *Symbiodinium* D cladal-types are known for their high photoacclimatory capacity, particularly in variable irradiance and highly sedimented reefs (Toller et al. 2001a, b, Baker et al. 2004, Fabricius et al. 2004, Rowan 2004, Stat & Gates 2011). However, association with *Symbiodinium* Clade C particularly at depth can also be characteristic of this clade (Rowan & Knowlton 1995, Rowan et al. 1997, Baker 2003, Chan et al. 2009, Bongaerts et al. 2010, Lesser et al. 2010). The sparse presence detected within *I. hippuris* morphotypes at depth may be either a consequence of developmental constraints or limited analytical detection methods. The mode of endosymbiont transmission is unknown for *I. hippuris*, however the additional association with a C cladal-type may be due to non-selective horizontal *Symbiodinium* acquisition by juvenile colonies leading to host selectivity as an adaptive environmentally-induced response. Tests of symbiont transmission and selection mechanisms, as well as fine scale *Symbiodinium* diversity using high resolution real-time PCR, would determine the presence of cryptic communities within individual colonies across bathymetry and reef health (Silverstein et al. 2012).

The relative contribution of light harvesting efficiency through photoacclimatory responses between *I. hippuris* morphotypes likely contributes to the residual error (V_{error}) between phenotypic traits (Chapter 4). Additional inherent contributors such as trophic interactions within and between holobiont morphotypes will inevitably lead to differential resource allocation patterns in contrasting reef environments. Therefore, shifts in resource reliance (e.g., phototrophy *versus* heterotrophy) relative to the environment would result in different

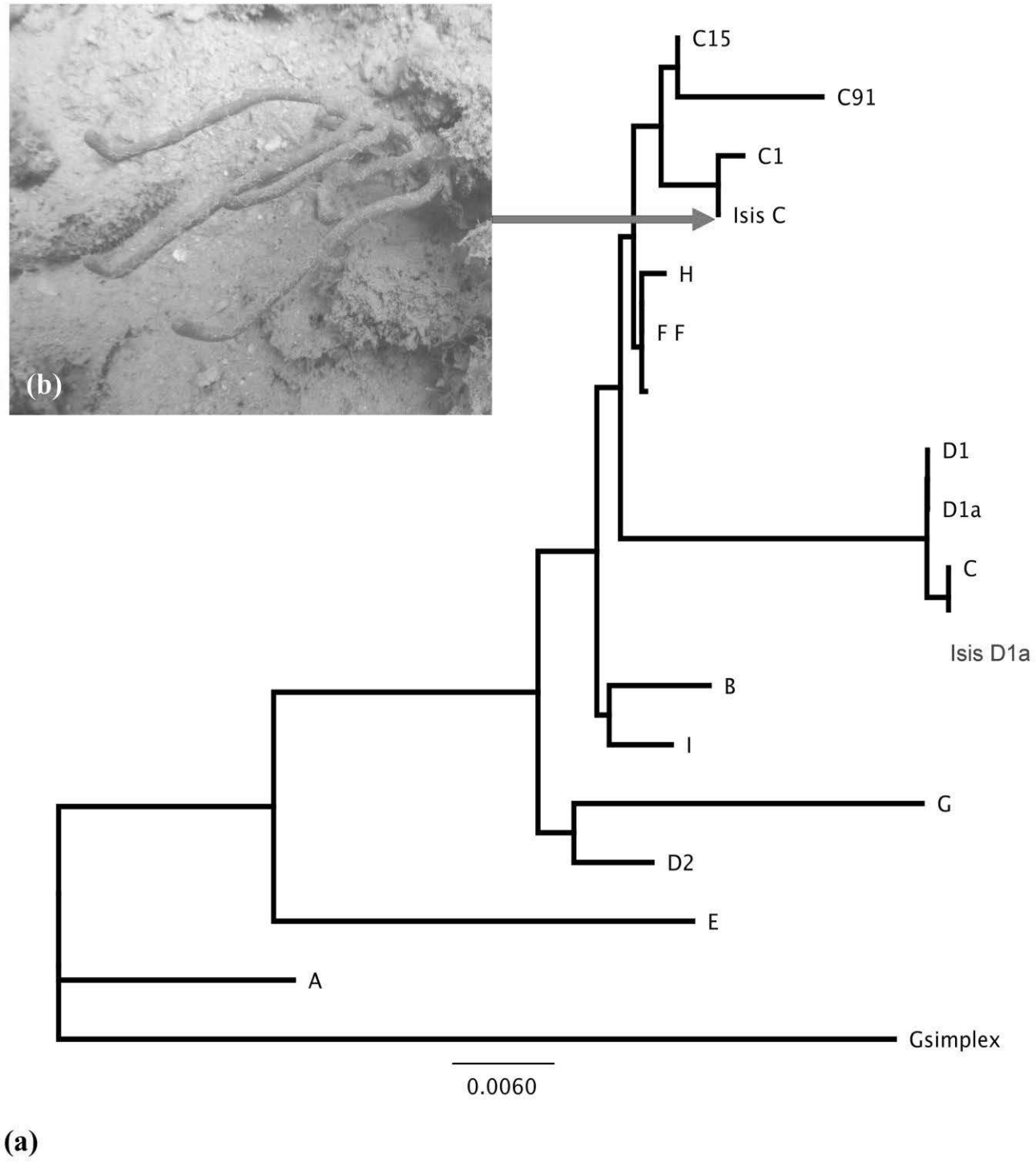

Figure 5.6. (a) Phylogenetic reconstruction of *Symbiodinium* clades within *Isis hippuris* at source depth based on maximum likelihood (ML) and bayesian inference (BI) analyses (1000 bootstrap) of the mitochondrial-encoded cytochrome oxidase *COX1* region (Takabayashi et al. 2004), rooted with *Gymnodinium simplex* (Lohmann) Kofoid & Swezy 1921. (b) Inset image of *Isis hippuris* at 12 m depth at Sampela containing clade C.

physiological optima for each *I. hippuris* morphotype, essentially working to maximise fitness in their respective environments. Here, morphological and physiological photoacclimatory responses confirm mechanistic adjustments that maintain such fitness optima between *I. hippuris* morphotypes on contrasting reefs. Assessment of trophic sources, allocation structure, and thus extrinsic and intrinsic energy transfer role(s) within and between holobiont morphotypes would inevitably provide insight into differential benthic-pelagic coupling patterns particularly on degraded reefs. Thus, mechanisms of environmentally mediated phenotypic changes in *I. hippuris* at the trophic level can be tested through; 1) coral-microbiome metabolic reciprocity, and 2) comparative energy apportionment between the host soft tissue, endosymbiont and environment via carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic analyses.

Firstly, alternative adaptive mechanisms may exist within the coral holobiont as a consequence of functional (e.g., nutrient) reciprocal metabolic pathways between the host and its microbiome (associate microbial ecological community). Tests of coral holobiont reciprocity through metabolic interactions using next generation sequencing (NGS) approaches, would further elucidate nutrient cycling between contrasting environments. Therefore, the characterisation of microbial community dynamics (e.g., species-specificity *versus* functional guilds), functional gene families and subsequent metabolic profiling through the mapping of gene expression data onto specific metabolic pathways (e.g., Larsen et al. 2011), would provide invaluable insights into integral metabolic links within the coral microbiome essentially predicted to structure holobiont trophic adaptations between environments.

Secondly, investigations into resource allocation structure within the holobiont and its environment using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic analyses, would determine if a trophic shift has occurred in response to anthropogenic disturbance, and thus, provide further corroborative evidence for environmentally mediated phenotypic responses to anthropogenic impacts. By removal of the $CaCO_3$ skeletal elements, an accurate assessment of the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic signatures in both *I. hippuris* soft tissue and algal endosymbionts has been obtained (Appendix I). Preliminary isotope results (Figure 5.7) revealed increased carbon and nitrogen levels in the degraded site morphotype and its endosymbionts compared to that of the healthy reef. What's more, comparative signatures clearly demonstrate that both host and endosymbionts at Sampela primarily obtain heterotrophic nutrients from fish sources contained in sinking particulates (POM). With a burgeoning human population of ~1600 continuously releasing waste matter onto the reef, such corroborative isotopic values are likely sourced from human fish consumption, a heavily relied upon resource

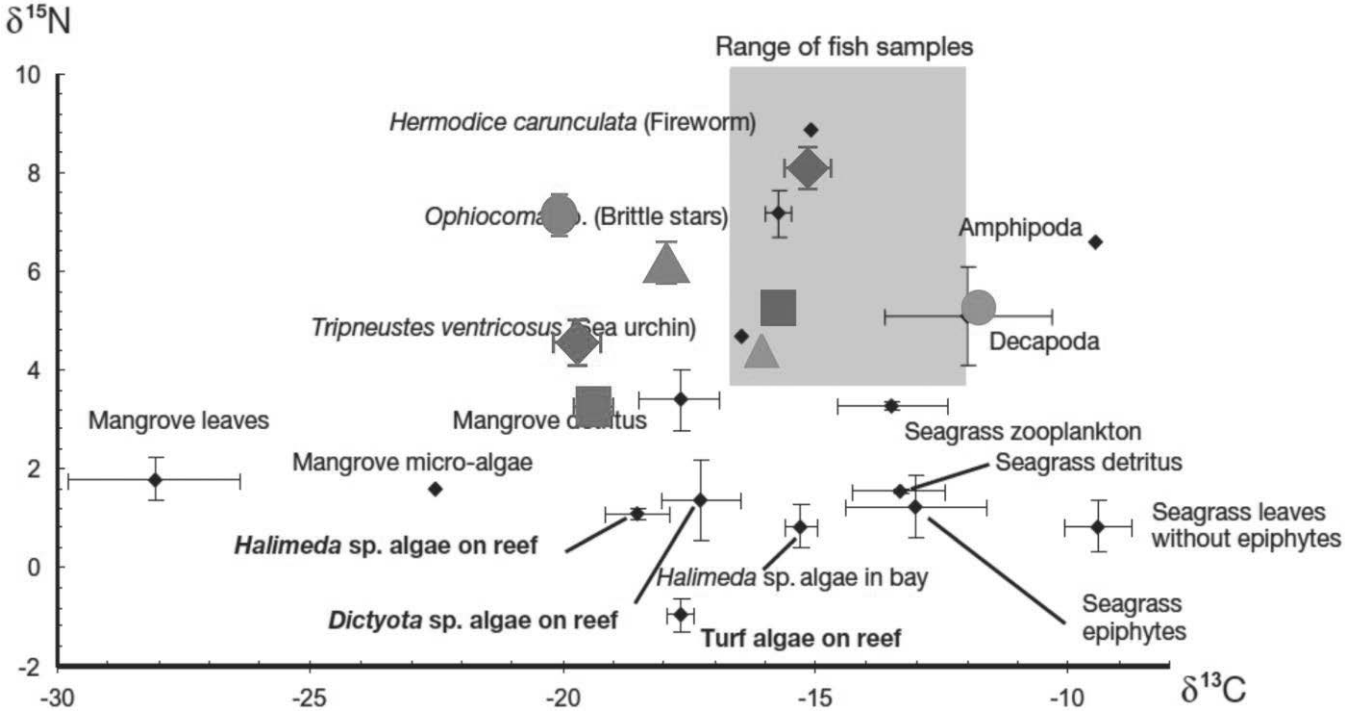

Figure 5.7. *Isis hippuris* holobiont (n = 8) and environmental (n = 72) stable isotope results (mean ± SE) from the Ridge and Sampela for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Data are superimposed on to coral reef (in bold), sea grass and mangrove (normal font) food source values with the shaded area taken from fish muscle tissue (Figure 2; Cocheret de la Morinière et al. 2003). Icons represent host soft tissue for the Ridge ( ) and Sampela ( ), and *Symbiodinium* from the Ridge ( ) and ( ). Surface sediment and plankton values for the Ridge (,) and Sampela (,) respectively.

within the community (Clifton 2013). Nevertheless, values for the Ridge (healthy site) suggest a greater reliance on phototrophy compared to allochthonous sources (Figure 5.7). It seems unlikely that light may have a significant effect on the fractionation of nitrogen as seen in the Caribbean zooxanthellate gorgonians *Gorgonia ventalina* Linnaeus 1758 and *Pseudopterogorgia americana* Gmelin 1791 between healthy and polluted reefs (Baker et al. 2011). Firstly, *I. hippuris* samples were taken at optical equivalent depths at the two sites and secondly, holobiont (host and *Symbiodinium*) isotope values were both higher and more akin to

fish levels, again suggesting alternative and greater resource supply at the degraded reef. The effect of light utilisation and trophic shifts between morphotypes could be determined through comparative $\delta^{13}C$ and $\delta^{15}N$ values coupled with photosynthetic efficiency through measuring the electron transfer efficiency of PSII via *in situ* rapid light response curves between reciprocal transplants. Henceforth, resource allocation structure will likely shift leading to compensatory

morpho-optical phenotypic responses as seen in Chapter 4. To fully investigate mechanisms of divergence at the trophic level, a thorough analysis between transplanted morphotypes (e.g., those in Chapter 4) would further elucidate resource allocation shifts and thus the role of phototrophy *versus* heterotrophy in maintaining nutritional intake under different environmental regimes.

5.5 CONSERVATION IMPLICATIONS

From the evidence presented here and throughout this research it is clear that human encroachment exacerbates or accelerates evolutionary processes within the marine environment, specifically gorgonian octocorals (Chapters 2 – 5). Undoubtedly the issue at hand is at a social and economic level, and yet the situation within the WMNP appears complex (Pilgrim et al. 2007a, b, Webber 2008, Clifton et al. 2010, Clifton 2013), with continuous reproaches manifesting social and economic repercussions that are counterproductive (Clifton 2003). Traditional folklore strategies particularly in the Bajo (sea gypsy) communities (e.g., Sampela) are increasingly shifting from subsistence to income fisheries through economic development (Pilgrim et al. 2007a). Such income fisheries discard folklore and engage in unsustainable and destructive fishing practices such as cyanide, dynamite (Pilgrim et al. 2007b) and fish fences (Exton 2010). In fact the prolific use of fish fences with a 50% decrease in mesh size has inevitably led to a marked decrease in coral reef fish abundance within the region (Exton 2010). With a concomitant increase in human population size, resource reliance and economic development, local marine resources seldom replenish with algal and/or sediment-dominated reefs, the latter increasingly the case at Sampela (see Figure 4.2b). The disturbing reality of human encroachment is acknowledged by government agencies and local communities alike, however enforcement is often favoured over community education (Clifton 2003). Well meaning in part, remedial fisheries management strategies are often implemented by conservation agencies, yet no-take zone (Unsworth et al. 2007) payoff strategies are withdrawn instilling false hope and a lack of trust in cross-cultural cooperation. It is clear that simple beneficial strategies at the local scale are productive with fisheries stock depletion ameliorated through cooperative long-term management schemes and education. Through fostering trust and cooperation with local communities in Madagascar for example, Oleson (2008) and Barnes-Mauthe et al. (2013) demonstrated that regular temporary octopus fishery closures and local community involvement both at the fisheries monitoring and education levels, led to significant increases in catch (Oliver et al. in prep.) and local income (Barnes-Mauthe et al. 2013, Oleson et al. in prep.). One can only hope that work such as this may be of some benefit to the local WMNP communities, yet in the face of human necessity and dogmatic perception it is hard to

predict and sadly out of the scope of this research. Nonetheless, gorgonian octocorals are clear indicators of reef health (Chapter 2) and biological resilience, particularly in the case of *I. hippuris* – with local adaptation at the phenotypic level likely leading to ecological divergence in sympatry (Chapters 3 & 4).

Conservation measures as a consequence of this work would undoubtedly benefit both coral reef and local village inhabitants alike. Ongoing ecological monitoring on an annual or six monthly basis, using belt transects as outlined in Chapter 2, would give an accurate assessment of gorgonian abundance and diversity across anthropogenic and bathymetric clines. Ecological assessments with concomitant abiotic parameter measurements would help identify areas of conservation concern for protection. Identifying the presence and abundance of the alternative yet conspicuous phenotype of *I. hippuris* – long-branched bushy colonies – inhabiting degraded reefs would assist in reef health assessments. Moreover, such ecological assessments coupled with trophic monitoring using stable isotope analyses of the *I. hippuris* holobiont and its environment (Figure 5.5 and Appendix I), would further provide a concise record of anthropogenic population expansion effects on coral reef habitats within the Wakatobi. Given the wide distribution of *Isis* morphotypes throughout the Indo-Pacific it is not unreasonable to propose this genus be specifically added to conservation agency annual survey lists and considered for CITES protection. This last point may seem a little severe, however, *Isis hippuris* (*sensu lato*) is still widely collected for the curios and jewellery industry (Cooper et al. 2011, Rowley pers obs.). Therefore, its presence is threatened by human encroachment and worthy of widespread monitoring awareness and protection.

Azooxanthellate gorgonian corals such as those in the deep-ocean, Mediterranean and temperate waters (e.g., *Eunicella verrucosa* Pallas 1766, *Corallium* spp. Cuvier 1798) are CITES protected and/or classified under 'vulnerable marine ecosystems' (VMEs). Deep-ocean gorgonians are a principle taxonomic group designation by the United Nations General Assembly (UNGA), safeguarded under the VME umbrella for protection against fishing activities throughout the world oceans (Rogers & Gianni 2010, Watling et al. 2011). Yet when it comes to the tropics, this highly diverse and abundant taxonomic group receives little, if any, conservation concern. This thesis highlights two clear patterns when considering azooxanthellate gorgonians on coral reefs within the WMNP (Chapter 2) and the tropical Indo-Pacific (Figure 5.1); firstly that azooxanthellate gorgonians increase in diversity and abundance with increasing depth, and yet secondly, are sensitive to anthropogenic disturbance. These are two key points of biodiversity conservation concern, ecological response patterns not uncommon to most reef taxa. However,

what sets gorgonians apart from other, in particular, benthic invertebrate groups, is their ubiquity and diversity throughout Indo-Pacific shallow and twilight reefs, most of which as yet undescribed (Bayer 1981). Gorgonians are also host to the CITES protected *Hippocampus* spp., as well as numerous species being actively exploited for commercial endeavours (e.g., Grigg 2010, Cooper et al. 2011; 21 kg of gorgonian and black coral were impounded by the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) in 2011 for the international jewellery trade (Labe L, pers. comm., 2013). Hence, gorgonian corals are a viable conservation priority group throughout the Indo-Pacific and tropics *per se*, extending and/or initiating monitoring survey initiatives to the family level assessment at the very least.

With a geological age greater than Scleractinia, coupled with increased abundance and diversity on deep reefs, it is not unreasonable to propose the success of gorgonian communities over time being due to shallow reef re-population from deeper communities in the face of global climate change. Furthermore, with gorgonian skeletal material consisting of magnesium calcite (Bayer 1973), and therefore a saturation state far less sensitive to ocean acidification than scleractinia (McCulloch et al. 2012), gorgonian corals are a prominent taxonomic group within the marine realm, acting as surrogates for local and global environmental change. So even though as humans we are unable to save habitats through their ongoing destruction, mesophotic reefs of refugia likely replenish many of their inhabitants.

5.6 CONCLUDING REMARKS

This work summarises the importance of gorgonian sea-fan corals on reefs within the WMNP, Indonesia (and the Coral Triangle *per se*); an area of huge biodiversity increasingly subject to anthropogenically-induced decline. Ongoing taxonomic investigations will undoubtedly reveal new species, evolutionary processes by which they emerged, and their relative diversity over geological time enabling predicted phenotypic, and inherent population responses to environmental change. Yet these animals, and the reefs in which they reside are on borrowed time, unless the validity of the hypothesised mesophotic reef refugia holds true. Considerable polyphyly across bathymetry within groups hold valuable insights into evolutionary mechanisms over geological time as a consequence of convergence through ecological necessity, or deep divergence. Studies on ubiquitous taxa such as the zooxanthellate isidid *I. hippuris* provide valuable insight into reef health, in addition to the processes by which ongoing environmental perturbations lead to ecological divergence on degraded reefs. For example, the biological success of *I. hippuris* within the WMNP, coupled with a strong association with the putatively stress tolerant *Symbiodinium* clade D, makes the case for environmentally robust gorgonian

species. However, the detection of *Symbiodinium* clade C at depth on degraded reefs invites tests of greater sensitivity in endosymbiont communities further reinforcing host selection control of the holobiont, and ultimately environmentally-mediated phenotypic responses to anthropogenic impacts on coral reefs. Moreover, the residual error (V_{error}) between phenotypic traits (Chapter 4) further leads to tests of divergence through metagenomic community analyses and isotopic assessment in order to elucidate mechanisms of divergence at the trophic level. Ultimately, however - and from a historical perspective - it is important to discover how traits evolved to their current utility, adaptive or exaptive, therefore as the original adaptation or the by-product (Gould 1997), which often eventually facilitates the action of the whole organism. Therefore, in order to study biodiversity in its truest form, it is necessary to know what a species is, its historical origin (Rohde 1999), evolution and hence how and why individuals and populations may respond to environmental perturbations and change. A lofty task, yet awareness brings about accurate interpretation of the quintessential biological system presented before us. Thus, investigations to further understand patterns of gorgonian ecology and biology through cross-disciplinary approaches are increasingly important in management and remedial conservation efforts.

APPENDIX I: METHODS FOR STABLE ISOTOPE ANALYSES

A:1 Host and *Symbiodinium* Separation

To determine the acclimatory capacity of the zooxanthellate gorgonian *Isis hippuris* Linnaeus 1758 between two contrasting environments, a novel analysis was successfully adapted from the hard coral literature, separating host soft tissue, algal endosymbionts and $CaCO_3$ skeletal elements (sclerites). Host soft tissue and algal endosymbionts (n = 8) were first separated through three cycles of centrifugation (5 min at 6000 g), vortexing (30 s), and resuspension in 1.5 ml FSW (0.2 μm filtered sea water). The subsequent supernatant (host slurry) was filtered through replicate 0.7 μm Whatman® GF/F (glass fiber filters, muffled at 550ºC for 3 h). The remaining $CaCO_3$/endosymbiont pellet was resuspended in 1 ml Milli-Q® deionised water (MQ-DI) with three wash cycles (as above). Residual zooxanthellae were then retained through filtration (as above). Replicate filters (GF/F x 6 per sample including host and endosymbiont acid and non-acidified filters, as well as ash free dry weight heated at 550ºC for 6 h) for each sample colony were dried (56°C for 48 h) with 3N HCL addition to one replicate set – two filters, host and endosymbiont - for comparative analyses ensuring $CaCO_3$ removal and accuracy. Replicate sample filters were folded into pre-acetone soaked 9 x 10 mm tin capsules (Costech Analytical®) for downstream δ^{13}Carbon and δ^{15}Nitrogen analyses at the University of Hawai'i at Mãnoa (see below).

A:2 SEDIMENT

Environmental $\delta^{13}C$ and $\delta^{15}N$ signature comparisons were conducted at both sample sites (Ridge and Sampela) in 2010 and 2011. Surficial sediment was sampled (n = 24) from optical equivalent depths adjacent to transplant blocks (see Figure 4.3, Chapter 4). Sediment was dried on site at 60º for 48 h and transported in muffled (550ºC for 3 h) tin foil to the University of Hawai'i at Mãnoa for further processing and analyses. Samples were ground with a marble mortar and pestle, and sieved (< 125 μm). All instruments were sequentially cleaned prior to each sample with MQ-DI, dichloromethane, methanol and acetone to minimise cross-contamination. Untreated ground samples were weighed into 5 x 9 mm tin capsules for $\delta^{15}N$ analysis. For $\delta^{13}C$ analysis, replicate ground sediment samples were acidified through the aqueous/rinse-acidification method being the most effective for high carbonate (>70%) sediment (see Komada et al. 2008, Briggs 2011, Briggs et al. in press). Hydrochloric acid (3N HCL) was slowly added to ground sediment samples in pre-weighed 50 mL centrifuge tubes until no further effervescence was detected. Acid-treated sediments underwent 3 x 10 mL MQ-DI, vortex, and centrifuge (5 min at 3000 g) wash cycles, and subsequently dried O/N at 60ºC. Dried

samples were re-weighed into 5 x 9 mm silver capsules for $\delta^{13}C$ analysis. Note: results were similar within sites (i.e., depth) and therefore pooled and presented in Figure 5.7 (Chapter 5).

A:3 PLANKTON

Water column $\delta^{13}C$ and $\delta^{15}N$ signature comparisons were conducted using vertical and horizontal plankton tows between 00:00 – 01:00 h and 07:00 – 08:00 h at both sample sites (Ridge and Sampela) in 2010 and 2011. Plankton samples (n = 48) were dried on site at 60ºC for 48 h and transported in muffled tin foil for downstream processing and analyses. Dried samples were ground and directly weighed into 5 x 9 tin capsules for $\delta^{15}N$, and silver capsules for $\delta^{13}C$ via acidification. Acidified (3 drops of 3N HCL or until effervescence ceased) replicates were dried O/N 60ºC and re-weighed prior to $\delta^{13}C$ analysis. Note: results were indeterminate within sites (i.e., vertical and horizontal) and so pooled and presented in Figure 5.7 (Chapter 5).

All δ^{13}Carbon and δ^{15}Nitrogen were determined using a Carlo Erba NA 2500 elemental analyser, interfaced via a ConFlo II to a Delta Plus mass spectrometer (Finnigan, Inc), at the University of Hawai'i at Mãnoa, USA.

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