Impacts of necrotising disease on the Endangered cauliflower soft coral *Dendronephthya australis*

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Abstract

Context: Diseases have impacted coral populations worldwide, leading to population declines and requiring active restoration efforts.

Aims: Describe population and individual impacts of necrotising disease in the Endangered octocoral *Dendronephthya australis*.

Methods: We quantified population loss and recruitment using reference photos, survey, and GPS mapping and described disease lesions using histopathology.

Key results: From December 2019 to January 2020, we observed polyp loss, necrotic lesions, and loss of large colonies of *D. australis* at Botany Bay, NSW, Aus. By September 2020 only a few scattered recruits remained, and all large colonies were lost. Histopathology of colonies sampled in January 2020 confirmed that the disease had resulted in necrosis, gastrovascular canal collapse, and internal colony integrity loss, leading to mortality. New recruits were recorded within 10 months of disease onset, and large colonies within 18 months.

Conclusions: While the necrotising disease had significant impacts on both the individual and population level, natural recruitment began quickly. As such, unlike in other populations, restoration is not currently required in the Bare Island *D. australis* population.

Implications: The extent of disease impact at the individual and population levels suggests that monitoring for lesions should be undertaken before developing conservation and restoration strategies for this species.

Introduction

Marine diseases are one of the greatest threats to marine habitat forming species in the Anthropocene, with disease having driven declines and extirpations across taxa including corals, seagrass, and macroalgae (Hughes 1994; Campbell et al. 2014; Zannella et al. 2017; Qiu et al. 2019). Disease impacts on habitat-forming species not only threaten the physical structure of these systems, but can also have bottom-up effects on a wide range of associated fauna (Hoegh-Guldberg and Bruno 2010) and can alter interactions between habitat forming species and their predators (Campbell et al. 2014). Diseases in marine habitat forming species have been more clearly documented in some groups than in others. Algal, seagrass, bivalve, and stony and gorgonian coral diseases are relatively well studied (Peters et al. 1983; Bally and Garrabou 2007; Bruno et al. 2007; Case et al. 2011; Zannella et al. 2017) while diseases in groups such as alcyonacean octocorals are poorly described (Work and Meteyer 2014; Weil et al. 2015). Unfortunately, like stony corals, octocorals are under increasing threat from climate change and disease, but disease in this group has only been relatively well-documented in gorgonian octocorals (Weil et al. 2015; Steinberg et al. 2020).

Octocorals provide invaluable ecosystem services to marine species in all climate zones and depths (Fabricius and Alderslade 2001; Steinberg et al. 2020). Invertebrates use octocorals as refuge, grazing substrate, and food (Greene 2008; Finlay-Jones et al. 2021). As many octocorals have complex branching morphologies, they are habitat for invertebrates including gastropods, ophiuroids, copepods, amphipods, and other arthropods (Bayer 1961; Muzik 1982; Wendt et al. 1985; Greene 2008; Poulos et al. 2013; Maggioni et al. 2020; Finlay-Jones et al. 2021). Octocorals also support vertebrate species, providing important shelter for seahorses and a food source for butterflyfish (Lourie and Randall 2003; Pratchett 2007; Harasti et al. 2014). In temperate Australia, habitats dominated by the Endangered octocoral *Dendronephthya australis* have been found to have higher biodiversity value than surrounding sand, seagrass, and sponge garden habitats (Poulos et al. 2013); and in the Great Barrier Reef, fish diversity increased with increasing octocoral, but not stony coral, cover (Epstein and Kingsford 2019). As such, loss of octocorals from benthic habitats due to stressors such as disease may significantly affect mobile species biodiversity.

Histopathology can be used to characterise the cellular characteristics of lesions (Work and Meteyer 2014). Unlike many stony corals, octocorals have a pronounced inflammatory response, making

them excellent subjects for histology (Dennis et al. 2020). Gorgonian octocorals also have clearly defined granular amoebocyte immune dermal cells that show up well under hematoxylin and eosin staining (Mydlarz et al. 2008). The majority of research into octocoral diseases has focused on gorgonian sea fans and little is known about histopathology of alcyonacean octocorals (Mydlarz et al. 2008; Tracy et al. 2018, 2021; Dennis et al. 2020; Calderón-Hernández et al. 2021; but see Slattery et al. 2013). Octocoral colonies are made up of polyps connected by coenenchyme, which capture and digest food, and the gastrovascular canals and siphonophores which move water through the colony and maintain the colony shape. The gastrodermal layer lines the interior structures, and the epidermis lines the outside of the colony; between the two cell layers is the mesoglea, a layer of connective tissue and sclerites. Disruption of these structures can be indicative of predation or disease, such as visible aggregations of pathogens and/or necrotic tissues (Mydlarz et al. 2008; Work et al. 2012; Work and Aeby 2014; Raymundo et al. 2016; Tracy et al. 2018, 2021; Dennis et al. 2020; Calderón-Hernández et al. 2021). Documenting changes in tissue health and the proportions of tissue types can characterise the damage to the colony caused by disease.

The octocoral *Dendronephthya australis* has been listed as an Endangered species due to ongoing declines across New South Wales, Australia. As such, monitoring of known populations is critical for understanding further declines or potential recovery (Harasti 2016; Larkin et al. 2021a; NSW Fisheries Scientific Committee 2021). Octocoral populations can naturally regenerate after declines through recruitment of larvae from donor populations or through clonal reproduction, providing environmental conditions are suitable (Steinberg et al. 2020). If the habitat is no longer suitable for the impacted species, or if no donor populations exist for the impacted population, restoration of the habitat or species may be required (McDonald et al. 2016; Steinberg et al. 2020). For example, populations of *D. australis* in Port Stephens, NSW, are not regenerating naturally and declines have continued for several years, suggesting that restoration is needed (Harasti 2016; Larkin et al. 2021a). In fact, successful aquaculture and transplantation trials have already begun in these highly disturbed habitats (Larkin et al. 2021b, 2023a). In Botany Bay, it is unclear if populations would regenerate naturally following loss of colonies.

Here, we aimed to map the decline of *D. australis* in Botany Bay, NSW, and subsequent natural recruitment; and document the impact of field-observed lesions on individual colonies through histopathology. With this information we hope to understand the reason for the *D. australis*

population decline at Bare Island and determine whether or not assisted regeneration is needed for population recovery.

Methods

Survey and collection of Dendronephthya australis

Study sites

The sponge gardens of Bare Island in Botany Bay, New South Wales, Australia (33°59'31"S, 151°13'55"E, Fig. 1) comprise at least 14 sponge species and two octocoral species (Poore et al. 2000). Dense aggregations of the Endangered octocoral *Dendronephthya australis* are intermixed with the sponge gardens on western rock platforms that include a lower and upper platform. The study sites include these two rock platforms that make up the main Botany Bay population of *D. australis* and a search area from the platforms to 200m seaward of the rock platforms. While scattered colonies are found throughout the Bare Island area, we did not observe any other areas with aggregations. The lower platform was located at 10 metres and the upper platform was located at 8 metres depth. The lower platform perimeter measures 72.7m, and the area measures 312 square metres. The upper platform perimeter measures 94.3 metres, and the area measures 347 square metres. These platforms were chosen as the study sites as they have the largest known aggregations of *D. australis* at Bare Island.

Survey and mapping *Dendronephthya australis* colonies

Initial observations and photographs of D. australis colonies were collected during pre-survey dives at the study sites at Bare Island on 30 Jan, 6 Mar, and 19 Mar 2018, and photographs of colonies were taken. Initial lesions on D. australis colonies at the Bare Island study sites were then observed in late December 2019 (Turnbull, pers. obs) and SCUBA surveys of the platforms were conducted on 19 Jan 2020, 23 Sep 2020, 9 Oct 2020, 9 Mar 2021, and 30 Apr 2021 to document changes following that observation. Mapping was conducted along with surveys on 9 Oct 2020, 9 Mar 2021, and 30 Apr 2021 when a GPS unit became available. For further details and mapping results, see the supplemental information and figure S4. Before mapping, number of colonies on each platform was recorded but exact locations could not be determined. To ensure all colonies on both platforms

were accounted for, surveys began on the upper platform and the entire platform was swum in pairs less than one meter above the substrate, after which the edge of the platform was re-surveyed as the majority of colonies were found on the platform edges. This process was then repeated on the lower platform. Overall, surveys took approximately 40 minutes. Colonies were classified as extra small (< 5 cm tall), small (5 – 10 cm tall), medium (11 – 20 cm tall), or large (>20 cm tall). It should be noted that these corals are highly contractile with the tide, and as such "medium" and "large" size classes were difficult to differentiate between tides (Davis et al. 2015). Because retracted colonies are smaller than inflated colonies, size class bins were halved when colonies were retracted to account for semi-retracted colonies, though colonies can retract much more than this (Davis et al. 2015). To account for this, survey dives were undertaken at mid-tide, when colonies were expected to be inflated, though this expectation was not always met. Even when fully retracted, large colonies do not look like small colonies as the large number of branches are clearly visible. As such, best judgment sometime was required when determining size classes.

Impact of disease on individual coral

Sample collection

Dendronephthya australis branches were collected using 12.5 cm blunt-tipped surgical scissors and placed in individual bags. Four branches of healthy *D. australis* were collected on SCUBA from Bare Island on 6 Mar 2018, 19 Mar 2018, and five branches were collected from healthy colonies and four from diseased colonies on 19 Jan 2020. Healthy colonies were defined as those with no visible lesions and normal extension (fig. 2a), while diseased colonies were defined as those with visible missing polyps (fig. 2b) and/or visible necrosis (fig. 2c).The entirety of one small necrotic colony that was no longer attached to the benthos was also collected on 19 Jan 2020. Colonies collected on 19 Jan 2020 were used for histological analysis. Branches ranged from four to nine centimetres long. Collection causes no lasting damage to the colonies (Larkin et al. 2023a). Whole colonies were not taken as has been previously done (e.g. Corry et al., 2018) because the population of *D. australis* at Bare Island is small compared to other populations and removal of entire colonies was likely to cause harm to the population.

To prepare the samples collected on 19 Jan 2020 for histology, the branches were placed in seawater after epifauna were removed and formaldehyde was added at 10% of seawater volume. Samples remained in formalin for one month before being transferred to 70% ethanol. All healthy

samples were subsampled twice for histology – one stalk sample and one polyp sample. Damaged samples were subsampled from one to five times, as all sections of visibly damaged tissue were sampled with a margin of visually healthy surrounding tissue. All samples were decalcified in 20% w/v EDTA solution over 12 days, with the solution changed every weekday for a total of ten changes as per Wada et al. (2016). Samples were then rinsed in RO water and dried in 70% ethanol for at least two weeks as per Tracy et al. (2021). Samples were embedded in paraffin, sliced 4 µm thick, and paired serial sections were stained with Haematoxylin and Eosin (H&E) and Masson's Trichrome as per Mandelberg et al. (2016).

Structures within *D. australis* were identified by referencing Fabricius and Alderslade (2001), Mandelberg et al. (2016), and Garra et al. (2020). Histology and sub-gross histological examination were performed on a subset of samples, with one stalk and one polyp section per colony. Histology was examined on slides stained with H&E, while sub-gross histological analysis was performed on paired slides stained with Masson's Trichrome as this stain has greater colour contrast. The polyp section with the most polyps was chosen, and stalk sections were chosen at random. Gross histological examination of H&E slides was conducted to look for collapsed gastrovascular canals, expansion, attenuation, and/or sclerite proliferation mesoglea, necrosis and/or loss of the gastrodermis, epidermis, and mesentery filaments, hyperplasia of the epidermis, and discoloured cells or mesoglea.

All histological slices were analysed with the quantitative pathology and bioimage analysis program QuPath (Bankhead et al. 2017), which allows for whole slide image analysis. The program was trained on four images (two from each health state - damaged and visibly healthy) to distinguish between three tissue classes: dermal cells, mesoglea, and empty spaces left by gastrovascular canals and decalcified sclerites. The three tissue classes were different colours when stained with Masson's Trichrome – dermal cells were red, mesoglea was blue, and gastrovascular canals and sclerites were white as the spaces do not contain tissue. Regions of Interest (ROI) were defined as the tissue and all open space within the tissue so that gastrovascular canals and sclerites could be quantified while background colour outside the coral was disregarded. As QuPath could not differentiate sclerites and mesoglea by colour, sclerites were counted manually. To do so, the images were opened in QuPath with a 250x250µm^2 grid overlay. Ten random 4x4 (1x1mm^2) grid square areas were selected, with five including the sclerite dense outer layer and five excluding this area. To account for large sclerites (longer than 1mm), any sclerite with 50% or more of it's area with the grid was counted.

Differences in proportions of tissue class (dermal cells, mesoglea, and gastrovascular canals and sclerites) between health states within stalk and polyps as determined by QuPath analysis were examined using a generalised linear mixed model (GLMM) with the package glmmTMB using a Gaussian distribution (Brooks et al. 2017), residuals were checked graphically using the package DHARMa (Hartig 2020), and pairwise comparisons were made using the package emmeans (Lenth et al. 2018). The response variable (percent of the coral that was in each tissue class – dermal cells, mesoglea, and mesentery space) was log transformed for analyses of stalk slices to meet model assumptions. As there were two slices of coral per slide and multiple slices per sample, slide number was a random effect nested within slice, which was nested within the random effect of sample number. Differences in number of sclerites between damaged and undamaged colonies was examined using a GLMM using a Poisson distribution in the package lme4 (Bates et al. 2015), with colony number, area (edge or centre), and body section (stalk or polyps) included as random factors. Residuals were checked and pairwise analyses performed as above. All analyses were performed in R version 4.0.5 (2021-03-31; R Core Team, 2013). All plots were produced using the package ggplot2 (Wickham 2011).

Results

Survey of *Dendronephthya australis* colonies

Healthy *D. australis* colonies have a multi-stalked, cauliflower-like growth form with full, polyp filled crowns (Fig. 2a). Damaged colonies presented with different lesions through time. Initial lesions were characterised by whole colony retraction during mid-tide and large numbers of missing polyps. Secondary lesions were first observed on 24 Jan 2020 and were characterised by retracted colonies with large patches of dark brown or black necrotic tissue, missing branches, and necrotic holes through the centre of colonies (Fig. 2c). The black necrotic tissue dissociated from the colonies with slight water movement, and underneath the tissue was light brown against the usually healthy pink of the corals (Fig. 2c). Colonies with either lesion type are hereafter referred to as “damaged”. As gastropods are known to predate upon *D. australis* (Davis et al. 2018; Finlay-Jones et al. 2021), presence of gastropods was noted. An unknown gastropod was photographed during onset of initial lesions (Fig. 2b, inset), and several egg cowries, *Globovula cavanaghi*, were observed laying eggs on branches (Fig. S2), though no predation by the cowries was noted.

Survey results are presented in Table 2, Figures 3a,b, and Figures S3-S5. Before 24 Jan 2020, photographs were taken incidentally to sample collection and the number of colonies photographed is reported in Table 2, and the photographs presented in Figures S3 and S4, but density is not calculated as the photographs likely underestimate the full population. For mapping results please see the supplemental information.

Impact of disease on individual coral

Histology

Results of histological examination of ten H&E stained slides of visually healthy (Fig. 4a-f, i) and seven slides of damaged (Fig. 4g,h,j-l) specimens are presented in Table 2. No signs of bacterial aggregation, fungal filaments, or other pathogens were noted on histological sections. As *Dendronephthya australis* is aposymbiotic, Symbiodiniaceae were not examined during histology.

Sub-gross histology

In all sub-gross histology figures, the gastrodermis, epidermis, and other dermal cells are stained red, while the mesoglea is stained blue (Fig. 5, Fig. S1). Stalk and polyp tissue composition significantly differed between the class of tissue and the interaction between health state and tissue class (ANOVA, $p < 0.05$), but not health state alone (ANOVA, $p > 0.05$). All tissue classes (mesoglea, dermal cells, and empty space left by gastrovascular canals and sclerites) were significantly different between damaged and visibly healthy individuals (ANOVA, $p < 0.05$). For stalk and polyp slices, there was a significantly higher proportion of mesoglea in damaged than visibly healthy individuals, and significantly fewer dermal cells or empty space (Fig. 6c,d, Table 3). There was no significant difference in the number of sclerites between damaged and undamaged colonies (Table 4).

Discussion

We documented a population decline associated with the appearance of necrotic lesions and subsequent recruitment in the Endangered cauliflower soft coral, *Dendronephthya australis* at Bare Island, Botany Bay, Australia. Polyp lesions were recorded in December of 2019, followed by larger, necrotic lesions that extended into the trunk of colonies in January 2020, and no large colonies were surveyed on the study sites by September 2020. While disease may have played a role in the

observed declines, other factors, including bushfires, could also have been the cause. Lesions were associated with changes in the structure of the colonies, including collapsed gastrovascular canals, expanded mesoglea, and significant necrosis. After loss of all large colonies, natural recruitment of *D. australis* was recorded within 10 months of the onset of disease and large colonies were recorded within 18 months, suggesting that natural recruitment and growth rates of *D. australis* are quite high at Bare Island. Unfortunately, little is known about diseases and their consequences in this Endangered species. We found that disease is a serious threat to *D. australis*, suggesting that disease monitoring should be undertaken during survey of this species. Development of interventions may need to become a management priority if further disease and loss is discovered.

Dendronephthya australis declines and recovery

While systematic surveys were not conducted prior to observation of lesions at Bare Island, collection of distal branches had been ongoing since 6 March 2018. During this time, the population appeared stable, with dense aggregations across both platforms as exemplified in Figure 3. Unfortunately, all large colonies at Bare Island were lost between December 2019 and September 2020. December 2019 and January 2020 were part of an El Niño cycle in Eastern Australia, which saw high temperatures, low rainfall, coral bleaching in the Great Barrier Reef, and severe bushfires in NSW that impacted estuarine benthic habitats (Barros et al. 2022; Gissing et al. 2022), including shifting benthic communities (Bracewell et al. 2023), and may have affected the estuarine habitats at Bare Island. Population loss has also occurred in other estuaries; notably two populations in Port Stephens, NSW, Australia (Seahorse Gardens and Pipeline) declined by 96% and 73%, respectively, between 2009 and 2015 and these declines are ongoing (Harasti 2016; Larkin et al. 2021a). Though the Bare Island population is relatively small, declines here suggest that colonies outside the main population centre in Port Stephens are also vulnerable to disturbances and should be included in monitoring efforts. In our study, the declines do not appear to be caused by physical smothering or damage from boating equipment as has been recorded previously (Harasti 2016; Larkin et al. 2021a). Instead, losses resulted after lesions from an unknown source. Sclerite proliferation can occur around predation lesions (Calderón-Hernández et al. 2021), but no evidence of proliferation was found in this study. In addition no pathogens were noted on histological sections, suggesting that the disease was either caused by a non-infectious agent, or the infectious agent is not observable on light microscopy and a higher resolution technique, such as electron microscopy, could possibly identify the cause. Additionally, the declines coincided with the Black Summer bushfires of 2019,

304 which significantly impacted estuarine environments and could have impacted *D. australis* in Botany
305 Bay (Barros et al. 2022; Bracewell et al. 2023). As such, *D. australis* appears to be vulnerable to
306 declines from multiple stressors across its range.

307

308 The initial lesions recorded on *D. australis* at Bare Island are consistent with previously described
309 predation lesions by fish or gastropods (Griffith 1994; Davis et al. 2018; Garra et al. 2020). Previous
310 records of predation lesions of *D. australis* have shown that colonies retract and lose feeding
311 opportunities (Davis et al. 2018) but have not led to disease as reported in this study. In addition, *D.*
312 *australis* appear to be attractive habitat for gastropods, with nubbins of *D. australis* colonised by
313 cowries within 13 days of transplantation to a novel habitat (Larkin et al. 2021b). In the tropics,
314 octocorals are often consumed by fishes, but these bites heal quickly and have not lead to disease,
315 though predation lesions have become locally necrotic (Pratchett 2007; Garra et al. 2020).
316 Conversely, gastropod predation on stony corals has been found to spread disease between colonies
317 (Nicolet et al. 2013, 2018). We did not directly observe predation on *D. australis*, with only a single
318 gastropod captured in photos of the damaged colonies and *Globovula cavanagh* observed laying
319 eggs but not actively feeding, and no fish predation observed. Previous studies have also not found
320 evidence of predation by *Globuvola cavanagh* (Corry et al. 2018; Finlay-Jones et al. 2021), though
321 another study observed predation (Larkin et al. 2021b) and other species of egg cowries are known
322 octocoral predators (Bennett 1971; Bowden et al. 1978; Coll et al. 1983; Griffith 1994). It is also
323 possible that the newly hatched cowries may predate on the octocoral tissue, though monitoring of
324 hatching and early behaviour is needed to test this. It is possible that predation made the colonies
325 more susceptible to another disturbance that was not documented such as a major storm or flood
326 event, or that the colonies were stressed due to early necrotising disease which made them
327 vulnerable to predation. It is also possible that while the lesions are consistent with predation, they
328 were early symptoms of the necrotising disease. Further work on the possible interaction between
329 predation and disease by remote camera surveys (e.g. Losey et al. 1994), regular surveys of colonies
330 and possible predators to detect population spikes and better documentation of *D. australis*
331 response to predation (e.g. Raymundo et al. 2016) would significantly further our understanding of
332 the possible link between predation, disease, and population declines in *D. australis*. Finally,
333 experimental studies to understand how *D. australis* responds to trauma at the microscopic level
334 (e.g. Rodríguez-Villalobos et al. 2016) would allow for understanding of whether the lesions
335 observed were caused by predation.

336

Recruitment of new colonies to the study sites at Bare Island began quickly, with new colonies observed only ten months after the first observation of disease. Within 15 months of disease observation, large colonies were present and within 18 months, over 35 colonies had grown in the affected area. Other octocoral species recover slowly from disturbance, including the Endangered Mediterranean precious red coral *Corallium rubrum* which may take over 30 years to reach pre-disturbance population structure (Tsounis et al. 2006; Bruckner 2009; Montero-Serra et al. 2018). On the other hand, soft octocorals such as xeniids and *Carijoa* spp. grow quickly and can even become invasive (Concepcion et al. 2010; Ruiz Allais et al. 2014; Sánchez and Ballesteros 2014; Ruiz-Allais et al. 2021). Structures that appear to be developing brooding larvae were observed during gross histological analysis (Permata et al. 2000; Marlow and Martindale 2007), and *D. australis* along with other temperate *Dendronephthya* breed during the warm months and can reproduce asexually by dropping polyp bundles (Dahan and Benayahu 1997; Hwang and Song 2007; Larkin et al. 2023b). As such, The recovery in population numbers documented in our study may be due to breeding during the austral summer and/or clonal reproduction. Interestingly, recent work has found that *D. australis* likely broadcast spawn (Larkin et al. 2023b), suggesting that, like *Pocillopora* spp., *D. australis* may be capable of multiple modes of reproduction (Smith et al. 2019). In Port Stephens, *D. australis* release gametes at neap tide in February and March (Larkin et al. 2023b), so if colonies at Bare Island follow similar patterns then many newly recruited colonies surveyed in September of 2020 may be sexual recruits from surrounding non-affected colonies.

Impact of disease on individual corals

Damaged colonies of *D. australis* sloughed necrotic tissue during collection and histopathology found the tissue had significant dermal necrosis, collapse of gastrovascular canals, and expansion and/or attenuation of the mesoglea, all of which likely led to loss of regular function. Interestingly, one visibly healthy colony had eosinophilic hyaline membrane, which is abnormal, but was not found in any of the damaged colonies. As observed in *M. capitata* infected with white syndrome and *Sinularia* spp. infected with *Sinularia* Tissue Loss Disease (STLD; Wainwright et al. 2011; Slattery et al. 2013; Work and Meteyer 2014), structures of *D. australis* became disorganised, took on a "shredded" appearance, and dermal cells lost cohesion with connective tissues. In the field, necrosis was clearly present at the centre of infected colonies but the surrounding tissues appeared relatively unaffected. Under histology, structures of diseased *D. australis* were damaged and necrotic throughout the slice, suggesting that the disease had travelled throughout the internal structures.

Stony coral and gorgonian diseases often spread from a single point to create a lesion (Work and Aeby 2011; Work et al. 2012; Dennis et al. 2020; Sharp et al. 2020). Here, the colonies collected had advanced lesions and their point of origin could not be identified. Previously, similar necrosis and loss of epithelial and gastrodermal cells was found in the hybrid octocoral *Sinularia maxima x polydactyla,* though tissue that was adjacent to disease lesions maintained its structure (Slattery et al. 2013). Conversely, we found that the interior of colonies was significantly affected by the disease beyond the lesion site. This disease may be difficult to identify in early stages in the field as it appears to target internal structures before external ones, opposite to what has previously been observed in coral diseases.

The *D. australis* necrotising disease caused significant changes in the tissue composition of the corals that likely affected the ability of the colony to function. The collapse of gastrovascular canals, and overall loss of structure within the colonies, suggests that the disease severely impacted the ability of *D. australis* colonies to circulate water and expand polyps (Davis et al. 2015). In the most severely affected colonies, the gastrovascular canals were no longer distinguishable from other perforate structures (e.g. sclerites) and likely could not continue their role as the vascular system of the coral. As these structures are responsible for the extension of coral polyps, and *D. australis* is entirely heterotrophic, this loss could lead to starvation of the colony (Fabricius and Alderslade 2001; Davis et al. 2015; Mandelberg et al. 2016). Polyp tissues were also affected, with dermal cells in the tentacles and digestive systems losing cohesion, often to the point that polyp structures such as the actinopharynx and tentacles could no longer be distinguished. This loss of function would have impeded individual colony recovery as the corals would have been unable to feed even after eliminating the disease.

Potential for natural recovery and restoration

Dendronephthya australis is distributed in shallow, protected estuaries and bays from Jervis Bay to Port Stephens, NSW. In Port Stephens, where populations are largest, mapping and modelling of appropriate habitat found that *D. australis* prefer sandy substrates within 6.5 km of the estuary mouth in strong current, in moderate depths of 3-18 m, and with a fairly shallow seafloor slope (Poulos et al. 2016). Though a large portion of the southern shore of Port Stephens meets these criteria, *D. australis* inhabit only a small portion of habitat with appropriate conditions (Poulos et al. 2016). The habitat in Botany Bay is different to that in Port Stephens in several ways, meaning that

the model developed for Port Stephens cannot be extrapolated without modification. In Botany Bay, the colonies were found on large rocky platforms with a thin layer of algal/sediment mat on top. Attempting to remove small colonies from the substrate was not possible and it was concluded that the colonies were attached to the platform below or firmly embedded in the algal matrix. The largest aggregation of *D. australis* at Botany Bay is the one surveyed in this study at Bare Island, but scattered colonies are often encountered throughout the Bare Island area (Steinberg and Turnbull, pers. obs). In Port Stephens, all *D. australis* colonies were associated with sand, sponge, and seagrass habitats (Poulos et al. 2016), while in Botany Bay, colonies were exclusively found in sponge garden habitat. Survey of the benthic habitat requirements of *D. australis* across its range and determination of substrate attachment method in each habitat type would allow for modelling of potential *D. australis* colonies or restoration locations across its range. Root-like processes of *Dendronephthya* spp. can attach to solid objects and anchor in purely soft sediments (Barneah et al. 2002; Larkin et al. 2023a), but it is unclear if one method is preferable over the other for natural and/or assisted regeneration. Understanding the preferred habitat of this Endangered species outside of Port Stephens would greatly enhance our understanding of their ecology, and could allow for establishing populations in areas of Port Stephens that may be at lower risk for sand inundation.

Even though Botany Bay populations of *D. australis* did experience disease and all large colonies were lost, natural recruitment has already begun and, unlike in Port Stephens, the Botany Bay population likely does not currently require active restoration interventions. At present, it is unclear why the population at Botany Bay is showing signs of natural recovery while the populations in Port Stephens are not, though it may partially be due to the cause of declines. In Port Stephens, declines were caused by sedimentation and smothering (Harasti 2016; Larkin et al. 2021a), which have also severely impacted other species elsewhere (Erftemeijer et al. 2012; Jones et al. 2019), while in Botany Bay disease appears to have played an important role in declines. Understanding the differences between characteristics of the environment, *D. australis* colonies, and causes of declines, such as substrate composition, flow regimes, sedimentation rate, larval characteristics, reproductive traits, and growth rates would greatly enhance our understanding of the recovery potential of this charismatic species across its range and allow for modelling of both assisted and unassisted population recovery. Continued monitoring, especially during building and maintenance of marine infrastructure, is also critical to maintaining the health of *D. australis* populations. Overall, while active restoration interventions are not currently needed at Bare Island, disease outbreaks are expected to become more frequent under climate change (Maynard et al. 2015). As such,

understanding disease dynamics and restoration methods for this species is critical for the continued health of populations not only in Botany Bay but across temperate eastern Australia.

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Statements

Data availability statement: Data are freely available on ScienceDB https://doi.org/10.57760/sciencedb.09644 (Steinberg 2023).

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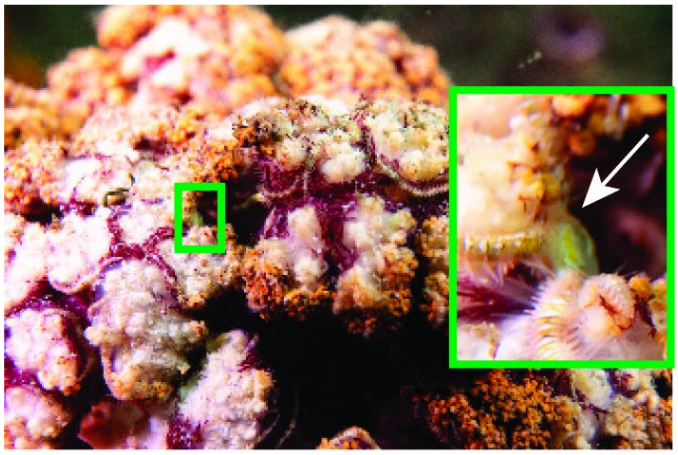
460 Figure Captions

461

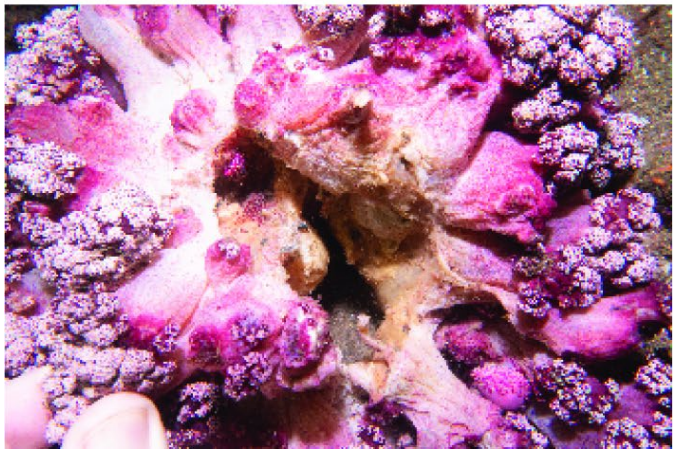
462 **Figure 1** | Map of Bare Island and adjacent beaches in Botany Bay.

a) Healthy colony 30 Jan 2018

b) Initial lesions 22 Dec 2019



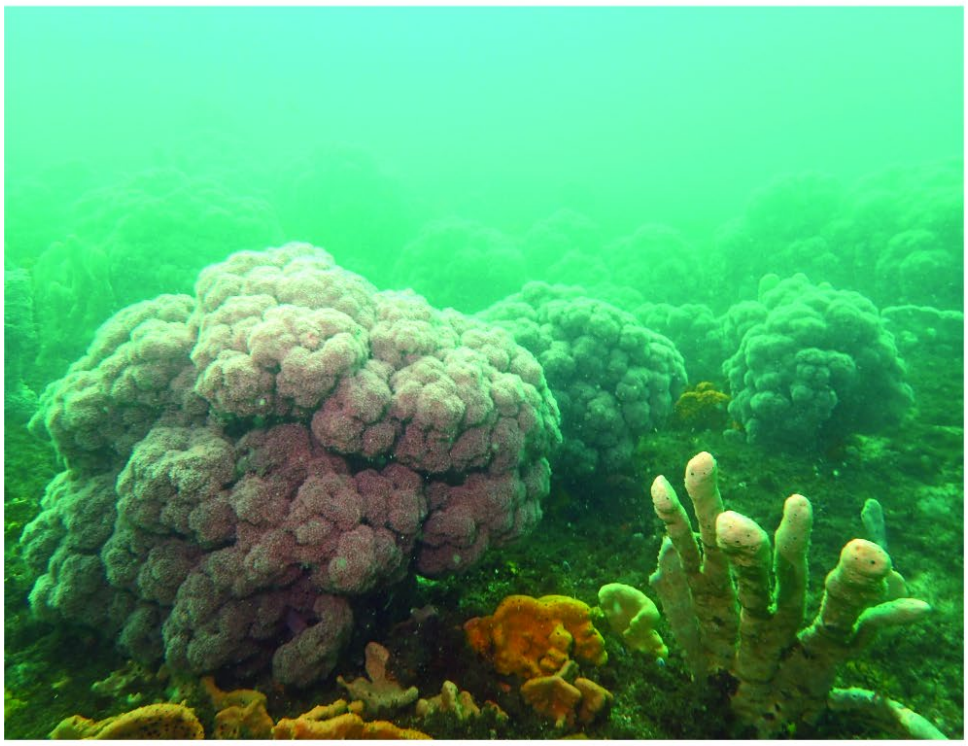
c) Secondary lesions 24 Jan 2020



463

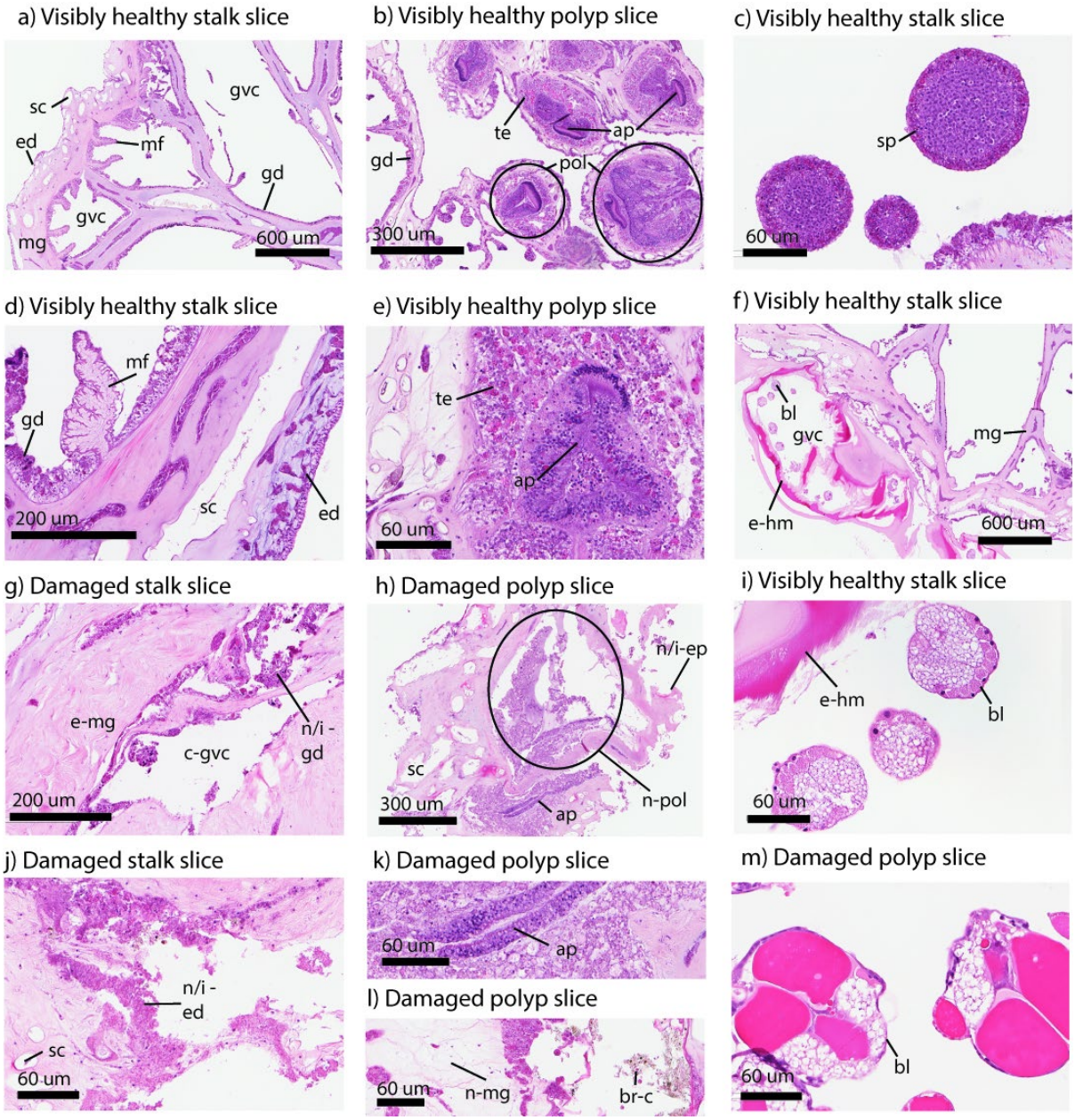
464 **Figure 2** | Progression of *Dendronephthya australis* lesions. a) healthy *D. australis* colony, b) initial
465 lesions observed on 22 Dec 2019 with closeup of gastropod inset, and c) secondary lesions with
466 characteristic colony trunk necrosis.

a) 25 September, 2018



b) 23 September, 2020

Figure 3 | Populations of *Dendronephthya australis* before (25 Sep 2018) and after (23 Sep 2020) lesions and population decline. a) A photo of a field of *D. australis* on the upper platform on 25 Sep 2018, at least 15 large colonies can be seen. b) four landscape photographs of the upper and lower platforms on 23 Sep 2020, no *D. australis* can be seen. Photographs are representative of relative abundance of *D. australis* and were not taken in the same position and orientation.



473

474 **Figure 4** | Histological examination of H&E stained slices of *Dendronephthya australis* examining
475 stalk tissue (a,d,g,j), polyp tissue (b,e,h,k,l), and reproductive tissues (c,f,i,m) at low magnification
476 (a,f), mid magnification (b,d,g,h), and high magnification (c,i,j,k,l,m). a,d) examples of healthy *D.*
477 *australis* stalk tissue, labels are as follows: mg – mesoglea, gvc – gastrovascular canal, ed –
478 epidermis, gd – gastrodermis, mf – mesentery filament, sc - sclerite. b,e) examples of healthy *D.*
479 *australis* polyp tissue, additional labels are as follows: pol – polyp, te – tentacle, and ap –
480 actinopharynx. c) Spermaries found in a visibly healthy specimen. f,i) maturing brooding larvae
481 within a gastrovascular canal of a visibly healthy colony, additional labels are as follows: bl –
482 brooding larvae, e-hm – eosinophilic hyaline membrane. Note the bright red eosinophilic hyaline
483 membrane, which is abnormal. g,j) examples of damaged *D. australis* stalk tissue. Note the expanded
484 mesoglea (e-mg), collapsed gastrovascular canals (c-gvc), and necrotic/inflammatory gastrodermis

(n/i – gd) and epidermis (n/i – ed). h,k,l) examples of damaged *D. australis* polyp tissue. Note the necrotic polyp (n-pol), the necrotic/inflammatory epidermis, necrotic mesoglea (n-gm), brown cells (br-c), and the atrophied actinopharynx, whose cells appear cuboidal as opposed to the healthy columnar configuration. m) Brooding larvae at approximately the four cell stage.

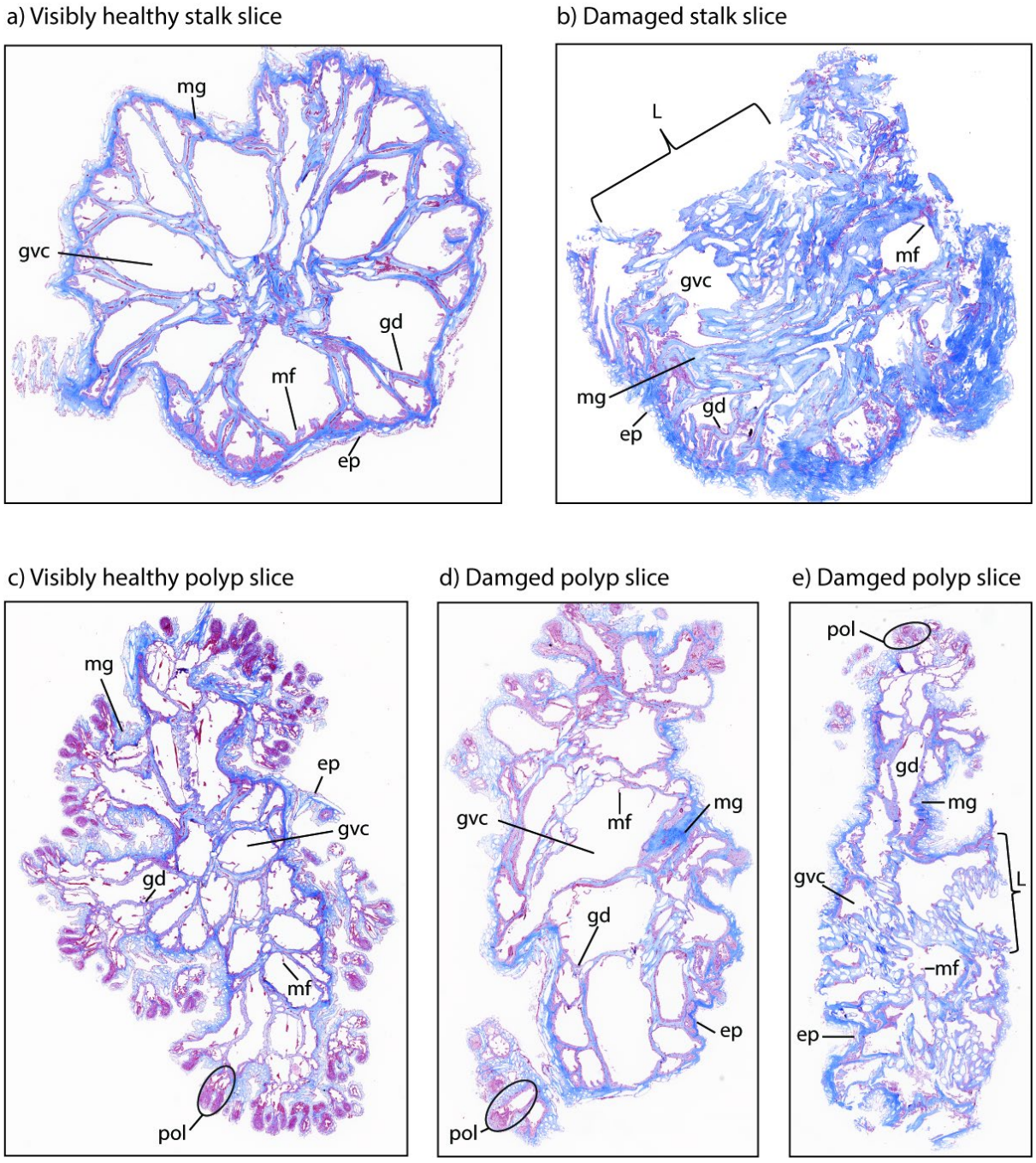


Figure 5 | Sub-gross examination of Masson's trichrome stained slices of Dendronephthya australis. a) a slice from the stalk of an visibly healthy *D. australis* branch, b) a slice from the stalk of an damaged *D. australis* branch, c) a slice from the polyps of an visibly healthy *D. australis* branch, d and e) two examples of a slice from the polyps of a damaged *D. australis* branch. Structures are

labelled as follows: pol – polyps, ep – epidermis, gd – gastrodermis , mg – mesoglea, gvc – gastrovascular canal, mf – mesentery filament.

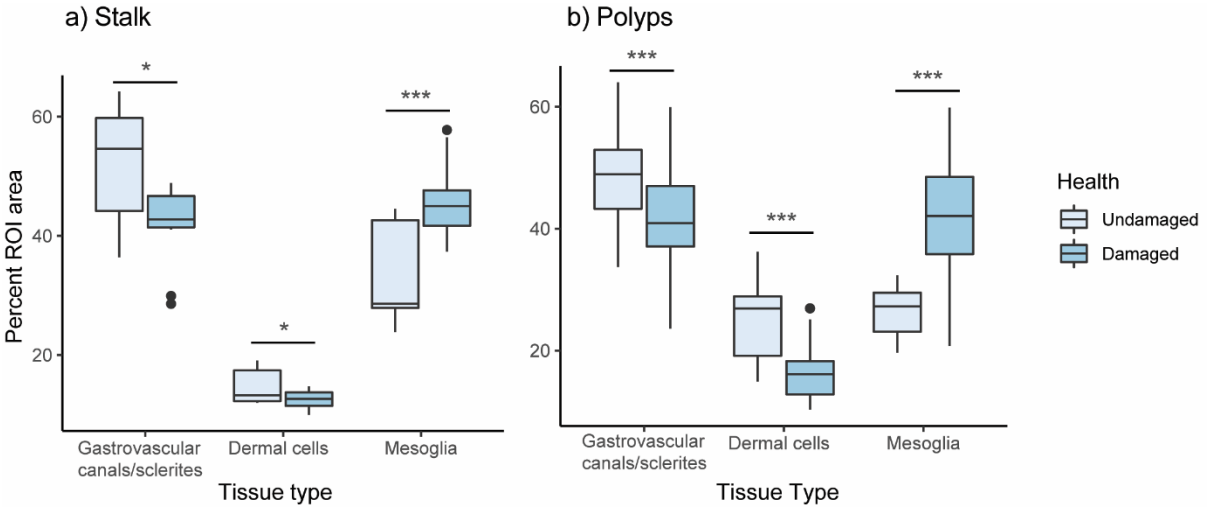


Figure 6 | Boxplots of *Dendronephthya australis* sub-gross histology analyses contrasting damaged and undamaged tissues for different tissue types. a) Percentage of region of interest (ROI) area, which includes the entire coral slice and all empty space left by gastrovascular canals and sclerites within the slice, of tissue composition of stalk slices as quantified in QuPath, and b) tissue composition of polyp slices as quantified in QuPath. Significance between visual health conditions is denoted as: * – $p < 0.05$, ** – $p < 0.005$, *** – $p < 0.0005$; all slice types and tissue types were significantly different.

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