Discovery of a host fish species for glochidia of *Westralunio carteri* Iredale, 1934 (Bivalvia: Unionoidea: Hyriidae)

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Abstract

Freshwater fishes are the usual hosts of glochidia (the parasitic larval stage) of freshwater mussels (Bivalvia: Unionoidea). *Westralunio carteri* Iredale 1934 (Carter's mussel), the only unionoid species found in the Sout West Coast Drainage Division of Australia, is endemic to the region and is currently listed as Vulnerable on the IUCN Red List, yet nothing is known of its host species. Small, white, bladder-like cysts were observed macroscopically on *Tandanus bostocki* Whitley, 1944 (freshwater cobbler) captured from the Blackwood River, Western Australia. Light microscopy of sectioned cysts revealed that they contained glochidia and these were of similar size and shape to glochidia obtained from gravid females of *W. carteri*. Glochidia were found on 40.7% of 461 *T. bostocki* examined, with a mean intensity of 10.6 cysts per infested fish. Prevalence of infestation was greater on juvenile (based on development morphology or urogenital papilla) than on adult fish. The findings represent an important step in developing conservation measures for *W. carteri* in this region.

Introduction

Freshwater mussels, of the order Unionoida, are an ancient group of bivalves found in freshwaters on all continents apart from Antarctica (Bauer & Wächtler 2001). Mussels play important roles in the functioning of freshwater ecosystems, through their biological activities, such as filter feeding, nutrient cycling and biodeposition, and by providing structural habitat and microrefugia to other benthic organisms (Strayer *et al.* 1999; Spooner & Vaughn 2008). Globally, freshwater mussels are highly imperilled, with biotic surveys in many countries demonstrating a general decline in both species richness and overall abundance (Williams *et al.* 1993; Vaughn & Taylor 1999; Strayer *et al.* 2004; Lydeard *et al.* 2008).

Unionoids are dioecious and reproduce sexually; males release sperm into the water column, which females suck in through their inhalant siphons and fertilise eggs that have migrated from the ovaries into specialised pouches in the gills known as marsupia, where the embryos develop into larval glochidia (Bauer & Wächtler 2001; Strayer 2008). Glochidia are released from marsupia, in response to disturbance or other stimuli, and if they contact a suitable host, generally a fish, may attach to the body surfaces, fins, mouth or gills (Bauer & Wächtler 2001; Strayer 2008). Following attachment, glochidia are encased in host epithelial tissue and within the epithelial cyst they undergo metamorphosis to emerge as juvenile mussels (Bauer & Wächtler 2001; Strayer 2008). To facilitate the attachment to fish, some glochidia have specialised structures (known as larval teeth) on the ventral margins of their shells. Teeth vary in morphology, but are generally hooked (Bauer & Wächtler 2001) and can often be used to identify glochidia taxonomically (Jones *et al.* 1986; Jupiter & Byrne 1997).

Eighteen species of unionoid mussels are known from Australia, all from the family Hyriidae (Walker *et al.* 2001; Graf & Cummings 2010). *Westralunio carteri* Iredale, 1934, the sole member of the genus *Westralunio* in Australia, is endemic to the South West Coast Drainage Division, where it is the only freshwater mussel found in the region (Walker *et al.* 2001; Graf & Cummings 2010). The species is currently listed as Vulnerable on the IUCN Red List of Threatened Species (IUCN 1999) and as a Priority 4 fauna (rare or near threatened or in need of monitoring) by the Western Australian Department of Environment and Conservation, under the Wildlife Conservation Act, 1950 (DEC 2010). Detailed understanding of the conservation status of *W. carteri*, and the development of conservation plans, are hampered by an almost complete lack of published information on the life history of the species, including longevity, reproductive cycle, habitat requirements and importantly, host fishes. Furthermore, of four other species of Hyriidae found in north-western and north-eastern WA, only one host fish species has been identified for glochidia of *Velesunio angasi* (Sowerby, 1867) (Klunzinger *et al.* 2010). Knowledge of host fish species, in particular, may be a crucial component of conservation planning for freshwater mussels because fishes are an obligatory part of the mussel life cycle (Haag & Warren 1998; Martel & Lauzon-Guay 2005).

Here we report, for the first time, the discovery of a host fish species, freshwater cobbler *Tandanus bostocki* Whitley, 1944 for the glochidia of *W. carteri*.

Methods

Freshwater cobbler were captured using two-winged fyke nets in four main channel sites and two tributaries in the Blackwood River (between 34.0421°S, 115.6025°E and 34.1081°S, 115.4505°E), in November 2008 as part of the study by Beatty *et al.* (2010). All fish were sexed, where possible, and measured for total length (TL), to the nearest 1 mm. Whitish, bladder-like cysts on the surface of the fish were provisionally identified as containing glochidia, and prevalence (percentage of fish infested) and intensity (number of cysts per infested fish) recorded from field examinations. Ninety five percent confidence intervals were calculated for prevalences, assuming a binomial distribution, and intensities, from 2000 bootstrap replications, using the software Quantitative Parasitology 3.0 according to methods described by Rozsa *et al.* (2000). The effect of maturity status and sex on prevalence was tested using Fisher exact tests and the effect of TL on prevalence by comparing the TL of infested and uninfested fish using analysis of variance (ANOVA). The effect of maturity status and sex (based on developmental morphology of urogenital papilla (Morrison 1988)) on intensity was tested by comparing the intensity of infestation between juvenile and adult, or male and female fish, using ANOVA. The effect of TL on intensity was tested by regression analysis.

A sub-sample of fish (n = 3) was killed in an ice slurry bath and transported to the laboratory. Cysts were examined under a dissecting microscope to determine whether they contained glochidia. Several cysts were preserved in 10% formalin in preparation for histology and scanning electron microscopy (SEM). For histology, dissected cysts were dehydrated in graded ethanols, embedded in paraffin, serially sectioned (6 µm thick) and stained with haematoxylin and eosin. For SEM, dissected cysts were dehydrated in graded ethanols, placed on a glass cover slip attached to a specimen stub, critical point dried, sputter-coated with gold, and examined and photographed in a Philips XL 20 SEM. For comparison, adult *W. carteri* (n = 2) were hand collected from the Canning River (32.1129°S, 116.0170°E), near Perth, killed in 0.01% benzocaine solution and dissected. Glochidia were transferred from gill marsupia with a probe and either examined under a compound microscope or using methods for SEM as described above.

Results and Discussion

Cysts were found on the fins, body surface and gills of *T. bostocki* (Fig. 1). Histological examination confirmed the presence of glochidia in fish cysts (Fig. 2). These were of similar size and shape to the unattached glochidia removed from specimens of *W. carteri* (Figs 3 and 4), although using morphology to identify encysted glochidia to species is difficult because the larval teeth are usually not visible (Jupiter & Byrne 1997). In unattached glochidia, these teeth appeared as two separate interlocking hooks on the ventral edges of glochidial valves (Fig. 3); somewhat similar to those described for another Australian unionoid, *Hyridella depressa* (Jupiter & Byrne 1997). Glochidial teeth function as a mechanism for attachment to host fish, and Pekkarinen & Englund (1995) found that glochidia with well-developed teeth are often attached to fins and skin rather than gills of fish. We assume that the encysted glochidia found on *T. bostocki* are *W. carteri* because this is the only unionoid species that has been described from the south-west of Western Australia (Walker *et al.* 2001; Graf & Cummings 2010).

Of 461 *T. bostocki* examined (107 males, 268 females and 86 unsexed, presumably juveniles) from the Blackwood River in November 2008, glochidial cysts were found on 40.7% (95% CI 36.3 – 45.2). Of the 107 male *T. bostocki* examined, glochidia were found on 34.6% (95% CI = 32.2 – 44.2). Of the 268 female *T. bostocki* examined, glochidia were found on 38.1% (95% CI = 25.6 – 44.4). There was no significant difference in prevalence between male and female fish (Fisher exact test, d. f. = 1, P = 0.56). Of the 86 juvenile *T. bostocki* examined,

Figure 1. (a) Glochidia cysts, appearing as white, raised areas on the caudal fin of a female *Tandanus bostocki*. (b) Individual cyst on the left dorsal side of the dorso-caudal fin of *T. bostocki*.

Figure 2. Section of a cyst (Cs), showing glochidium (G) with shell periostracum (Po), covered by fish epithelium (E), from the posterior end of the dorso-caudal fin of *Tandanus bostocki*.

Figure 3. Light microscope image of glochidium of *Westralunio carteri*; anterior-ventral view, valves open, larval teeth shown.
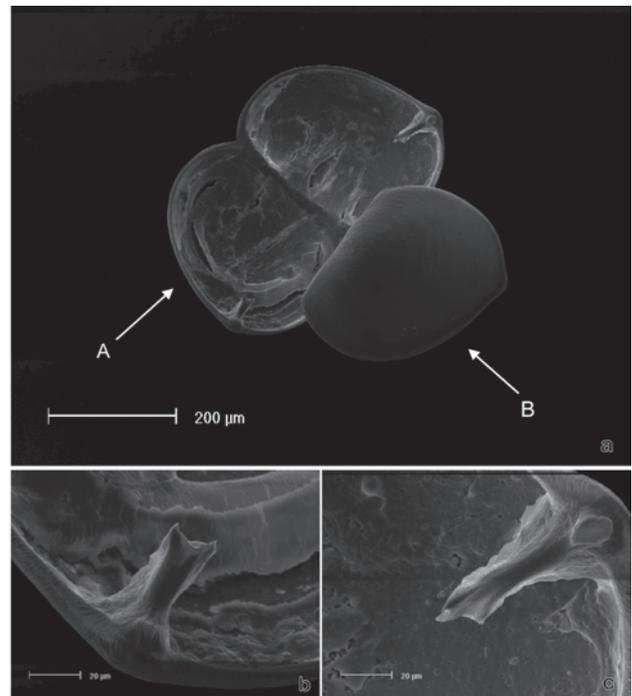

Figure 4. Scanning electron microscope (SEM) image of glochidia of *Westralunio carteri*. (a) Ventral view, valves open (A) and right valve view, valves closed (B). (b) Larval tooth of left valve, magnified image of (A). (c) Larval tooth of right valve, magnified image of (A).

glochidia were found on 57.0% (95% CI = 45.9–67.3), which was significantly greater than on adult fish (Fisher exact test, d. f. = 1, P = 0.001). Considering adult and juvenile fish separately, there were no significant differences in TL between infested and uninfested fish (for adults, F = 0.02, d. f. = 1, 374, P = 0.89; for juvenile, F = 3.29, d. f. = 1, 85, P = 0.07). Of infested fish, the intensity of infestation varied from 1–120, with a mean of 10.6 (95% CI = 9.0–12.5). There were no significant differences in intensity between male and female fish (F = 0.01, d. f. = 1, 374, P = 0.97) or between adults and juveniles (F = 2.41, d. f. = 1, 460, P = 0.12), nor were there any significant relationships between TL and intensity for adult or for juvenile fish (for adults, r^2 = 0.001, F = 0.37, d. f. = 1, 374, P = 0.54; for juveniles, r^2 = 0.01, F = 0.69, d. f. = 1, 85, P = 0.41).

Studies on other unionoid species have usually found greater prevalences of infestation on juvenile fish than on adult fish, presumably because of the development of immunological resistance in older fish (*e.g.* Bauer 1987; Hastie & Young 2001). The difference in prevalence of *W. carteri* glochidia between adult and juvenile *T. bostocki* suggests that immunological responses could also be important in this host/parasite system, although further research is required to determine the survival rates of glochidia on fish of different age classes. Variation in glochidia prevalence and intensity may also arise from habitat structure (giving rise to variations in the probability of contact between glochidia and potential hosts), fish abundance, seasonality of glochidia release, differences in mussel density and water depth (Strayer 2008).

Globally, most freshwater mussel species that have been examined, and particularly those with hooked glochidia, have been found to be host generalists, with a number of host fish species involved in the life-cycle (Haag & Warren 1998; Wächtler *et al.* 2001; Martel & Lauzon-Guay 2005; Blažek & Gelnar 2006). Although there have only been a few studies of host fish species for Australian unionoids, these have also identified multiple host species (Hiscock 1951; Atkins 1979; Walker 1981; Humphrey 1984; Widarto 1993; DPIPWE 2009; Klunzinger *et al.* 2010). It seems likely, therefore, that fishes other than *T. bostocki* may be infested with glochidia of *W. carteri* in the south-west of Western Australia, although they were not detected in a recent survey of the parasites of native and exotic fishes in the region (Lymbery *et al.* 2010). Furthermore, there are river systems within the region that contain *W. carteri* but do not contain *T. bostocki* (Morgan *et al.* 1998; Klunzinger unpublished data). Understanding the range of host fish species used by *W. carteri* in different systems, and their relative importance in maintaining the life-cycle of the species, is vital for conservation planning, because of the restricted distribution and threatened nature of many of the native fish species of the south-west of Western Australia (Morgan *et al.* 1998; Morgan & Gill 2000; Morgan 2003; Beatty & Morgan 2010).

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The status of the black bream *Acanthopagrus butcheri* (Pisces: Sparidae) population in Lake Clifton, south-western Australia

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Abstract

Lake Clifton hosts the largest living, non-marine thrombolite (microbialite) reef in the southern hemisphere. The thrombolite reef was recently listed as a critically endangered ecological community. The main threat to the ecology of the lake is increasing salinity, but other threats have also been identified, including the impact of the introduced fish *Acanthopagrus butcheri* (Munro, 1949). Samples opportunistically collected after a fish kill in 2007 indicated that *A. butcheri* in this lake experienced very low somatic growth and recruitment failure between 1995 and 2007, probably in response to hypersalinity. The evidence suggests that the *A. butcheri* population in Lake Clifton is effectively extinct. The proposed conservation strategy for Lake Clifton addresses a range of identified threats, including the eradication of *A. butcheri*. However, management action in response to this particular threat may no longer be required. The abundance of *A. butcheri* in the lake is probably very low and the population is likely to become extinct if current environmental trends continue. A dramatic reduction in growth rate after 1995 demonstrates the extraordinary growth plasticity of *A. butcheri* in response to environmental influences.

Keywords: salinity, fish kill, growth, Sparidae, thrombolite

Introduction

Lake Clifton is a small, permanently closed, coastal lake on the south-west coast of Western Australia, approximately 100 km south of Perth (lat/long S32.745°, E115.655°). The lake is part of the Peel/Yalgorup system, which was recognised as a 'Wetland of International Importance' under the Ramsar Convention in 1990. The presence of a unique thrombolite (microbialite) community in Lake Clifton was a key factor contributing to this listing. In December 2009, the thrombolites in Lake Clifton were listed as a 'critically endangered ecological community' under the Australian Commonwealth Environment Protection and Biodiversity Conservation (EPBC) Act 1999. Rising salinity in the lake, due to the inflow of increasingly saline groundwater, was identified as the greatest threat to the thrombolite community (Threatened Species Scientific Committee 2010a).

Lake Clifton was hyposaline (salinity range 8–32 gL^{-1}) in the 1970s and 1980s but has become progressively more saline since 1992 and is now frequently hypersaline (Knott *et al.* 2003; Luu *et al.* 2004; Smith *et al.* 2010). Since 2002, annual salinity has typically ranged from a minimum of 20–30 gL^{-1} in winter to a maximum of 70–80 gL^{-1} in autumn (John *et al.* 2009; Smith *et al.* 2010). Lake Clifton receives low surface flow and so the salinity of the lake is mainly a function of groundwater inflow and evaporation (Commander 1988; Davies & Lane 1996). Thrombolites in Lake Clifton have historically formed as a result of the growth of cyanobacteria *Scytonema* sp., which are dependant on a constant discharge of fresh to brackish groundwater directly into their habitat (Moore 1987). Hence, a shift from permanently hyposaline to permanently hypersaline conditions threatens the survival of the key microbial species responsible for thrombolite formation.

In addition to increasing salinity, a range of other threats to the thrombolite community in Lake Clifton have been identified, including physical damage to the thrombolites during feeding by *Acanthopagrus butcheri* (Munro 1949) (Threatened Species Scientific Committee 2010a). Predation by *A. butcheri* has also been suggested as a factor contributing to a decline in the diversity of macroinvertebrate fauna within the lake (Threatened Species Scientific Committee 2010a). The "control and eradication of black bream", which were stocked into the lake, is one of several Priority Actions proposed to aid the conservation of the thrombolite community (Threatened Species Scientific Committee 2010b). This action was proposed in the absence of any survey to assess the current status of the *A. butcheri* population.

Acanthopagrus butcheri is the largest fish species reported from Lake Clifton. This euryhaline species occurs in estuaries and coastal lakes across southern Australia and is highly targeted by recreational fishers across this range (Kailola *et al.* 1993). Anecdotal reports indicate that this species was first introduced to the lake by commercial fishers in 1947 and possibly re-stocked on several subsequent occasions (Dortch 2008; Dortch unpubl. data). Anecdotal reports and genetic evidence indicate the most likely sources of introduced fish are the adjacent estuaries of Peel-Harvey and Leschenault Inlet (Chaplin *et al.* 1998; Dortch 2008).

The population of *A. butcheri* in Lake Clifton was 'rediscovered' in 1996 by recreational anglers who were fishing from a boardwalk recently constructed as a

viewing platform for thrombolites. After a 2-week period of intense recreational fishing, the Department of Fisheries implemented a permanent ban on fishing in the lake. The ban was intended to protect the fish (initially thought to be an endemic population) and to prevent accidental damage to the thrombolites.

Prior to this study, information about the status of the *A. butcheri* population in Lake Clifton was derived from a sample of 100 individuals collected in November 2006 (Sarre & Potter 2000). In February 2007 a fish kill resulting in the deaths of hundreds of individual *A. butcheri* created an unexpected opportunity to collect additional samples and reassess this population.

Methods

The fish kill was first reported by members of the public on 13 February 2007. Dead fish were observed floating in shallow water around the thrombolites along the north eastern shore. Dead fish had apparently accumulated in this region as a result of a prevailing south-westerly wind. The total number of dead fish in this area were estimated to be 600–800 (A. Kidd (DEC), pers. comm.).

On 15 February 2007 a random sample of 163 partly decomposed fish was collected from a 100 m length of shoreline adjacent to the thrombolite viewing platform along the north eastern shore. The total length (TL) of each fish was measured to the nearest millimetre. Other biological data such as weight or sex was unobtainable from each fish due to the advanced state of decomposition.

Sagittal otoliths were extracted from 100 fish. A transverse section of 300 μm thickness was taken through the core of one otolith from each fish. Sections were viewed with reflected light against a black background. Age was determined by enumeration of opaque and translucent zones, which are deposited annually in *A. butcheri* otoliths (Morison *et al.* 1998). Opaque zones in *A. butcheri* otoliths are typically deposited during spring (Sarre & Potter 2000). The first complete annual 'increment' was defined as an opaque core followed by a translucent zone plus an opaque zone. Subsequent increments were defined as a translucent zone plus an opaque zone. Each otolith was assigned one of the following margin categories: 1=translucent margin, marginal increment <50% complete; 2=translucent margin, marginal increment >50% complete; 3=opaque margin. All otoliths were read once by an experienced reader and once by a second experienced reader. There was 100% agreement between these readings.

In south-western Australian estuaries, *A. butcheri* typically spawn between October and December (Sarre & Potter 1999). Therefore, an average birth date of 1 November was assigned to all fish collected from Lake Clifton in 2007.

Results and Discussion

High salinity and low oxygen have been suggested as factors contributing to the sudden mortality of black bream in Lake Clifton in February 2007 (John *et al.* 2009).

Oxygen and salinity are infrequently monitored in Lake Clifton and levels immediately prior to the fish kill were not available. However, salinity measured shortly after the kill, in February 2007, was 79 gL^{-1} (John *et al.* 2009). This is approaching the upper salinity tolerance for *A. butcheri* and likely to result in osmotic stress (Partridge & Jenkins, 2002; Hoeksema *et al.* 2006). However, high salinity does not appear to have been the primary cause of death in 2007. A period of increasing salinity, eventually reaching a lethal level, would be expected to result in an extended period of stress and probably also a protracted period of mortality. The fins and skin of dead fish were intact and did not show signs of external lesions, disease or injuries that are often seen on fish that have been subjected to a long period of extreme osmotic stress (Hoeksema *et al.* 2006). The external condition of the fish suggested that death occurred relatively rapidly. Also, all fish were at a similar stage of decomposition, suggesting that they had died at approximately the same time.

The fish kill coincided with the sudden, widespread dislodgement of large benthic microbial mats in the lake (John *et al.* 2009). Dead fish and pieces of microbial mats, up to 50 cm in diameter, were observed floating together among the thrombolites. The decomposition of these mats may have created hypoxic zones and led to the sudden asphyxiation of fish. Hence, asphyxia was likely to be the primary cause of death in Lake Clifton in February 2007.

The total lengths of dead *A. butcheri* collected from Lake Clifton in February 2007 ranged from 235 to 328 mm, with an average length of 271 + 14 (+ s.d.) mm. All fish were estimated to be 11 years old and therefore spawned in late 1995.

The mean length-at-age of fish collected in 2007 was substantially lower than that predicted by von Bertalanffy growth parameters derived from fish collected from Lake Clifton in 1996 (429 mm TL at 11 years of age) (Sarre & Potter 2000) (Fig. 1). In 1996, the *A. butcheri* population in Lake Clifton exhibited a relatively fast growth rate, which was significantly higher than growth measured in three other south-western Australian estuaries (Moore River, Walpole/Nornalup Inlet, Wellstead Estuary) and slightly higher than growth
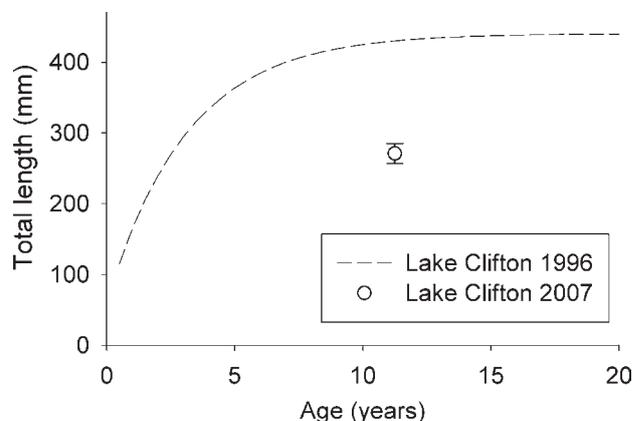

Figure 1. Length-at-age of *Acanthopagrus butcheri* collected from Lake Clifton in 1996 (represented by von Bertalanffy growth curve fitted by Sarre (1999)) and length-at-age (mean + s.d.) of fish collected from Lake Clifton in 2007.

measured in the Swan Estuary (approximately 90 km to the north of Lake Clifton) (Sarre & Potter 2000). The magnitude of the decline in growth in Lake Clifton between 1996 and 2007 indicated that all of the fish collected in 2007 had experienced slow growth over numerous years. Overall, these observations suggest that environmental conditions in Lake Clifton were relatively favourable to the growth of *A. butcheri* prior to 1996 but were unfavourable after 1996.

Previous observations of differences in growth rates among estuaries have provided evidence of the plasticity of growth by *A. butcheri* (Sarre & Potter 2000). These differences are environmentally driven and do not reflect genetic differences between populations (Partridge *et al.* 2004). The decline in growth of *A. butcheri* in Lake Clifton between 1996 and 2007 further demonstrates the extraordinary growth plasticity of this species in response to environmental factors.

Acanthopagrus butcheri can tolerate a wide range of salinities, from 0 to at least 60 gL^{-1}. Juveniles and adults typically display signs of osmotic stress, including reduced growth, external lesions and mortality at salinities above 60 gL^{-1}, although adult fish have been observed in the wild at salinities of up to 114 gL^{-1} (Partridge & Jenkins 2002; Hoeksema *et al.* 2006). The extremely slow growth of *A. butcheri* collected from Lake Clifton in 2007 suggests that these individuals may have experienced prolonged periods of osmotic stress and spent a substantial part of their life at salinities above 60 gL^{-1}.

Other factors could also have potentially affected the growth rate of *A. butcheri* in Lake Clifton. In particular, increased salinity may have altered the composition of the invertebrate community in the lake, forcing a change in the diet of *A. butcheri*. However, the stomach contents of fish sampled in 1996 were dominated (40% by volume) by *Capitella capitata* (Polychaeta), which was abundant in Lake Clifton sediments in the late 1990s (Sarre *et al.* 2000; Konishi *et al.* 2001). *Capitella capitata* is extremely euryhaline (Geddes & Butler 1984) and is likely to have remained abundant despite recent hypersalinity. Therefore, the dominant prey item consumed by *A. butcheri* was probably relatively constant from 1996 to 2007 and a dietary shift is unlikely to have caused the decline in growth.

In addition to effects on growth, recent environmental conditions in Lake Clifton appear to have also negatively impacted on the reproductive output of *A. butcheri*. A sample of 100 *A. butcheri* collected from the lake in 1996 included ten age classes ranging from 1 to 19 years (Sarre 1999). This suggested that spawning had occurred in at least 10 of the years from 1977 to 1995. In 2007, the absence of all year classes except 1995 suggests limited or zero recruitment in 1996 and all subsequent years.

It is unlikely that many of the fish captured in 1996 were introduced (*i.e.* spawned elsewhere and translocated to Lake Clifton). In 1996, anglers removed many hundreds of large bream from the lake. Also, anecdotal reports from fishers indicate that some fishing had been occurring for several years prior to 1996. Even after this population depletion, numerous large fish were still available to be captured by Sarre (1999). These high catch rates, occurring after the effects of fishing and

natural mortality, imply a high initial abundance of each age class. This would have required substantial quantities of fish to be stocked into the lake each year. A more likely mechanism is natural recruitment. Hence, the majority of the fish collected in 1996 and in 2007 were probably spawned in Lake Clifton and were the descendants of a small number of introduced fish.

Reproduction by *A. butcheri* (as measured by sperm motility, egg fertilization, egg survival and larval development) can occur at salinities of 10–35 gL^{-1} but, within this range, is most successful at salinities of 20–35 gL^{-1} (Haddy & Pankhurst 2000). This optimal range reflects the salinities at which *A. butcheri* typically spawns in the wild (Nicholson *et al.* 2008). The potential of *A. butcheri* to reproduce successfully at salinities above seawater has not been examined. However, in the congeneric *Acanthopagrus berda*, sperm motility occurs over a wide salinity range (5–60 gL^{-1}) but peaks sharply at approximately 25–35 gL^{-1} (Molony & Sheaves 2001). In another euryhaline sparid, *Sparus sarba*, hatching of viable larvae is limited to salinities of 9–54 gL^{-1} but highest rates occur between 20–36 gL^{-1} (Mihelakakis & Kitajima 1994). The optimal salinity ranges for these species correspond to the salinities at which they typically spawn in the wild. Therefore, it is likely that reproductive success of *A. butcheri* declines rapidly as salinity increases beyond 35 gL^{-1} and is limited to salinities well below 60 gL^{-1}.

Despite substantial penalties for unlawful translocations of fish, introductions of non-endemic *A. butcheri* into inland lakes is a widespread problem in south-western Australia, with various potential ecological, social and economic impacts (*e.g.* Smith *et al.* 2009). Most recently, a population of *A. butcheri* was discovered in Lake Thetis, another inland lake containing a significant benthic microbial community (Grey *et al.* 1990; N. Casson [DEC] pers. comm.). *Acanthopagrus butcheri* in Lake Thetis were discovered during a fish kill in February 2010. The salinity of Lake Thetis is typically hypersaline and was approximately 57 gL^{-1} during the fish kill (K. Smith unpubl. data). As with Lake Clifton, this discovery generated concerns about the potential impacts to microbialites and whether management action should be taken. Improved knowledge of the upper salinity limits for reproduction, growth and survival by *A. butcheri* would assist in determining the need for management action in such cases.

In summary, the environmental conditions in Lake Clifton since 1995 appear to have been highly unfavourable for both growth and reproduction of *A. butcheri*. High salinity is likely to be the primary factor contributing to slow growth and recruitment failure. Given that salinity is predicted to remain high, and possibly increase, it appears unlikely that any surviving *A. butcheri* in the lake will successfully breed again.

The proposed conservation strategy for Lake Clifton aims to address a range of identified threats to thrombolites, including the eradication of the introduced *A. butcheri* population (Threatened Species Scientific Committee 2010b). However, management action in response to this particular threat may no longer be required. The abundance of *A. butcheri* in the lake is probably very low and the population is likely to become extinct if current environmental trends continue.

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Benthic algae and seagrasses of the Walpole and Nornalup Inlets Marine Park, Western Australia

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Abstract

A survey of the marine plants of the Walpole and Nornalup Inlets Marine Park has recorded 49 species of marine benthic algae and seagrasses, including 15 green algae, 11 brown algae, 18 red algae, 4 seagrasses, and one cyanobacterium, representing a substantial increase on the 14 previously recorded species. Most species are relatively common elements of the south-western Australian marine flora, but several are of taxonomic or biogeographic and ecological interest. Included in this group are: a new species of the green algal genus *Codium*, the first records of previously unknown reproductive phases in the red algae *Mazoyerella australis* and *Spermothamnion cymosum*, and a new distribution record for *Ossiella pacifica*, a species hitherto known only from warmer waters of the Pacific Ocean and not recorded for mainland Australia. The species diversity in the inlets decreases markedly with increasing distance from the ocean, reflecting a reducing marine and increasing estuarine influence.

Keywords: algae, benthic, *Codium*, *Mazoyerella*, Nornalup, *Ossiella*, range extensions, *Spermothamnion*, Walpole

Introduction

The estuaries of Western Australia's (WA) south provide important highly productive and sheltered aquatic habitats on an otherwise typically high energy stretch of coast (Brearley 2005). While the physical conditions of estuaries vary widely due to the mixing of marine and fresh waters, the wave-dominated estuaries of southern WA vary also with regard to their underlying geomorphology, rainfall characteristics and frequency and duration of times at which they remain open to the ocean (Hodgkin & Hesp 1998; Chuwen *et al.* 2009). While systems in relatively high rainfall areas tend to be permanently or seasonally open to the ocean, those in the drier east of WA's south coast have longer periods of closure.

Estuaries typically accumulate terrestrially-derived sediments and nutrients in their lower basins, and for this reason they may support particularly high densities of some organisms such as invertebrates and fishes (Edgar 2001). However, as relatively few species can persistently tolerate the highly variable environmental conditions associated with, for example, seasonal freshwater flows and periods of bar closure, the number of true estuarine species is relatively low. It is more common for mobile organisms to opportunistically enter estuaries when conditions are suitable and/or exploit

their productivity and sheltered waters during particular stages of their life-cycle (Potter & Hyndes 1999). By accumulating sediments and nutrients from surrounding catchments, estuaries are also particularly susceptible to pollution and eutrophication, and many of WA's estuarine systems are significantly degraded from such impacts (Brearley 2005). In particular, massive growth of algae and seagrasses has occurred in some estuaries as a consequence of eutrophication.

The Walpole and Nornalup Inlets are among the few estuaries in the south west of WA that have not been significantly modified by development and/or catchment degradation. The inlets are a very popular recreation area for both locals and the broader WA community, and the high ecological and social conservation values of the inlet system led to it being gazetted as a marine park in 2009 (DEC 2009). The Walpole and Nornalup Inlets Marine Park comprises two discrete basin inlets and the tidal reaches of the Frankland, Deep and Walpole Rivers. The Nornalup Inlet is connected to the sea by a relatively narrow entrance channel that remains permanently open to the ocean due to the relatively high rainfall of this area, the highest in WA of *ca.* 1300 mm, and the protection of the channel entrance from wave action by a rocky headland (Hodgkin & Clark 1999). For this reason essentially marine conditions are maintained in most of the system throughout the drier months, and aspects of the biota are relatively diverse compared to most other estuaries in this region (Brearley 2005). In contrast to Nornalup Inlet, Walpole Inlet is much smaller and

shallower. Exchange between the two water bodies is via a narrow deep channel. During winter, the surface water of Nornalup Inlet can be quite fresh, although the bottom waters generally remain at about 30 ppt. Water in the Walpole Inlet, however, is generally of a lower salinity. The sediments of the inlets comprise fine mud in the deeper basins and clean, well-sorted sands in the entrance channel and shallows, which are particularly extensive around the Nornalup Inlet (Hodgkin & Clark 1999; DEC 2009). Notably, the inlets support little fringing marsh habitat and have relatively small areas of rocky shoreline or sub-tidal rocky substratum.

Creation of the marine park has provided impetus for updated and new scientific knowledge of the system to assist management and provide a basis for long-term monitoring of conservation values. The aim of this study was to conduct a methodical survey of marine plants in the entrance channel and Walpole and Nornalup inlet basins, with a particular emphasis on the relatively limited areas of hard substratum.

Previous studies

Prior to the current study, records of marine plants from the Walpole and Nornalup Inlets were exceedingly scant, and Brearley (2005) suggested that there are "not many types of aquatic plants" growing in the system. The only specific accounts include a short summary provided by Hodgkin & Clark (1999), wherein they described surveys of the aquatic vegetation undertaken by Marion Cambridge (October, 1976) and Jane Chambers (January, 1987); on both occasions the vegetation was found to be "sparse". Ten species were recorded, including the seagrasses *Ruppia megacarpa* and *Heterozostera tasmanica* (probably *Zostera polychlamys*, see below), the brown alga *Cystoseira trinodis*, the red alga *Monosporus australis* (= *Mazoyerella australis*), and the green algae *Chaetomorpha billardierei*, *C. linum*, *C. aerea*, *Enteromorpha intestinalis* (= *Ulva intestinalis*) and *Acetabularia caliculis*. Later, Brearley (2005) also provided an account of the marine plants, this in part derived from unpublished notes by E. Hodgkin (Brearley, pers. comm., Nov. 2010), listing *Halophila ovalis*, *Halophila decipiens*, *Cladophora* sp. and *Polysiphonia* sp. in addition to the species recorded by Hodgkin & Clark (1999). For the most part these earlier records cannot be verified, as only a few specimens from the surveys are lodged in the Western Australian Herbarium, and only one of those a named voucher (*Chaetomorpha linum*). These specimens include a plant of *Cystoseira trinodis* (with epiphytic *Polysiphonia infestans*, *Ulva paradoxa*, *Cladophora* sp. and the diatom *Melosira*), and the aforementioned *Chaetomorpha linum* voucher, which has been re-examined and found to be an admixture of *Chaetomorpha indica* and *C. billardierei* Most species previously reported from the inlets, however, were recollected during the present survey.

Anecdotal evidence indicates that summer seagrass growth (probably *Ruppia megacarpa*) occurred to such an extent in the Walpole Inlet during the 1950s and 1960s that it clogged boat propellers (WAPC 2009). As this growth no longer occurs, it was most likely caused by nutrients entering the inlet from local agricultural sources that have since been controlled or removed (DEC 2009).

Methods

Sampling sites

Specimens were collected by JMH and MJR (assisted by Anna John and Darren Stevens) during April 2010; primarily from six shallow rocky substratum sites in the Walpole and Nornalup Inlets and by a single collection of *Halophila decipiens* (and associated epiphytes) made by benthic grab from a seventh site (Fig. 1).

Site 1 (S35.03129, E116.73309) was at Skippy Rock, a granite formation on the western shore close to where the entrance channel meets the sea (Fig. 2A). This location was visited twice (20 and 21 April), the second occasion for an incidental collection at the adjacent jetty.

Site 2 (S35.01460, E116.70937) was on rocky shoreline in Weedy Bay on the western shore of the Nornalup Inlet (Fig. 2B).

Site 3 (S34.99208, E116.74197) was on the northern side of the Nornalup Inlet; on small sub-tidal reef structures not far east of the Coalmine Beach boat ramp.

Site 4 (S34.98680, E116.72431) was in the Walpole Inlet on rocky shore on the southern side of this basin, one of the few areas of hard substratum.

Site 5 (S35.00731, E116.71220) was on the rocky shoreline of Newdegate Island, a granitic outcrop on the west side of the Nornalup Inlet, adjacent to the Deep River delta.

Site 6 (S34.99496, E116.73148) was close to Site 3, on the northern side of the Nornalup Inlet, on the rocky eastern shore of the Knoll.

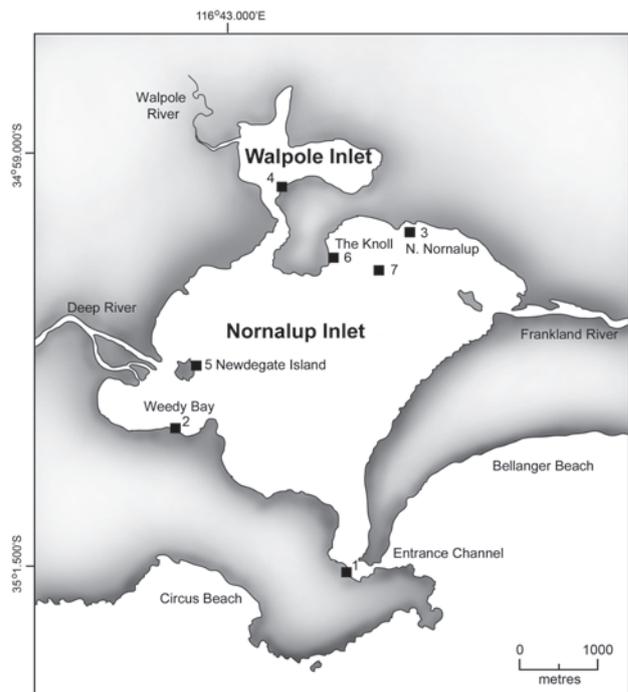Site 7 (S34.99648, E116.73780) comprised sand and was located on the north-eastern side of the Nornalup Inlet basin.

Figure 1. Map of the Walpole and Nornalup Inlets, showing the location of collection sites.

Specimen collection/preservation

Specimens were collected by snorkeling and were photographed in-situ (see Figs 2A–H, 3A–H) by JMH using a Canon G9 camera in a housing. Voucher specimens were pressed onto herbarium sheets on the day of collection, with portions of larger plants and small filamentous species preserved in 5% formalin/seawater for later detailed examination. Additional portions were dried in silica-gel for DNA sequence analyses, although the results of these will not be reported here. In the laboratory, plant sections or whole-mounts were stained in a solution of aniline blue and mounted in 50% Karo/water. Microscope preparations were examined and photographed using either a Nikon SMZ800 stereo microscope or a Nikon Eclipse 80i compound microscope, in both cases with a Nikon DS Fi1 digital camera. All herbarium sheets and microscopic preparations have been lodged in the Western Australian Herbarium (PERTH). The distribution of species within the inlets (Table 1) is based on presence as observed during the survey, and on subsequent laboratory examination of smaller species and epiphytes.

Environmental conditions

Environmental conditions across the Walpole and Nornalup inlets were measured at 19 sites (15 in the Nornalup and 4 in the Walpole) encompassing both the shallow peripheral sand flats and the deeper waters. At each site, a single measurement of temperature (°C), salinity (ppt), dissolved oxygen (mgL^{-1}) and pH were taken using a calibrated Hydrolab DS5 Surveyor water probe. In addition, secci depth was measured at each site. For sites >1m deep, measurements were made both at the surface and approximately 30 cm above the bottom, and the readings averaged. All measurements were made between the hours of 8am–2pm on two consecutive days at the time of the algal collection.

Arrangement of the taxonomic account

Taxonomic results are grouped into Phaeophyceae (= brown algae), Chlorophyta (= green algae), Rhodophyta (= red algae), Seagrasses, and Cyanobacteria (blue-green algae), with constituent species listed alphabetically. For each species, the name, authority, and date and page of publication are given (the place of publication listed in the references), with author names abbreviated according to Brummitt & Powell (1992). The PERTH voucher is cited, followed by a comments section.

Results

Environmental conditions

This study took place in autumn prior to the onset of significant river discharge and when environmental conditions in the inlet system were relatively stable and essentially marine. Only relatively small variations in physical parameters were apparent at the time of the survey. The mean water temperature was 20.6 °C, with the maximum (22.5 °C) and minimum (19.8 °C) occurring on the shallow peripheral sand flats and deeper waters of the Nornalup Inlet, respectively. Salinity was approximately 38 ppt throughout the inlet basins. The

mean dissolved oxygen was 7.3 mgL^{-1}, and this ranged from 5.9 mgL^{-1} in the Walpole Inlet to 8.4 mgL^{-1} in the Nornalup Inlet. Although water clarity was notably high during the survey, underwater visibility in the Walpole Inlet was less than at other sites due to the widespread prevalence of fine muddy sediments in this basin.

Taxonomic account

The following account details the species of seagrass, brown, green, and red macroalgae, and conspicuous cyanobacteria recorded from the Walpole and Nornalup Inlets. In addition to these taxa, numerous species of epiphytic diatoms and several microscopic cyanobacteria were present in the collections, but as these groups were not the primary focus of this study they will not be treated in detail. The former group included *Grammatophora oceanica* Ehrenb., *Striatella unipunctata* (Lyngb.) C.Agardh, *Biddulphia biddulphiana* (Sm.) Boyer, *Melosira moniliformis* (O.F.Müll.) C.Agardh, and species of *Licmophora*, the latter *Calothrix* sp. and *Lyngbya* sp.

Phaeophyceae

Colpomenia sinuosa (Mert. ex Roth) Derbès & Solier 1851: 95.
Specimen: Entrance channel, Nornalup Inlet (Site 1), epilithic at 1.5 m depth, 20 Apr. 2010, *J.M.Huisman* (PERTH 08227624).
Only a single plant was collected and the species appears to be rare in the inlets. *Colpomenia sinuosa* is widely distributed and is found on all Australian coasts (Womersley 1987: 297).

Cystoseira trinodis (Forssk.) C.Agardh 1820: 67–68.
Specimen: East Coalmine, Nornalup Inlet (Site 3), 20 Apr. 2010, *J.M.Huisman* (PERTH 08243670).
This species formed dense beds near Newdegate Island (Fig. 2C) and was also common at Coalmine. *Cystoseira trinodis* is widespread in tropical and subtropical regions of the Indian Ocean and extends along southern Australia to Victor Harbour, S.A. (Womersley 1987: 358). It appears to be the only large brown alga capable of surviving in the upper reaches of south-western Australian estuaries (*e.g.*, Waychinicup Estuary, see Phillips & Lavery 1997). *Cystoseira trinodis* was previously recorded for Nornalup/Walpole by Hodgkin & Clark (1999) and Brearley (2005: 366).

Dictyota fastigiata Sond. 1845: 50
Specimen: Entrance channel, Nornalup Inlet (Site 1), epilithic at 1.5 m depth, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243468).
Dictyota fastigiata was formerly included in *Dilophus* (see Womersley 1987), but that genus was subsumed into *Dictyota* following DNA sequence studies (see De Clerck 2003). Plants have dark brown, dichotomously branched, flattened axes that structurally have a medulla several cells thick and a cortex of a single cell layer.

Dictyota furcellata (C.Agardh) Grev. 1830: xliii.
Specimen: Entrance channel, Nornalup Inlet (Site 1), epilithic at 1.5 m depth, 20 Apr. 2010, *J.M.Huisman* (PERTH 08227616).

Table 1

Algae species collected from six rocky sites in the Walpole and Nornalup Inlets Marine Park during April 2010. Sites are arranged from the most southern (Site 1) to northern (Site 4), reflecting increasing distance from the open coast.

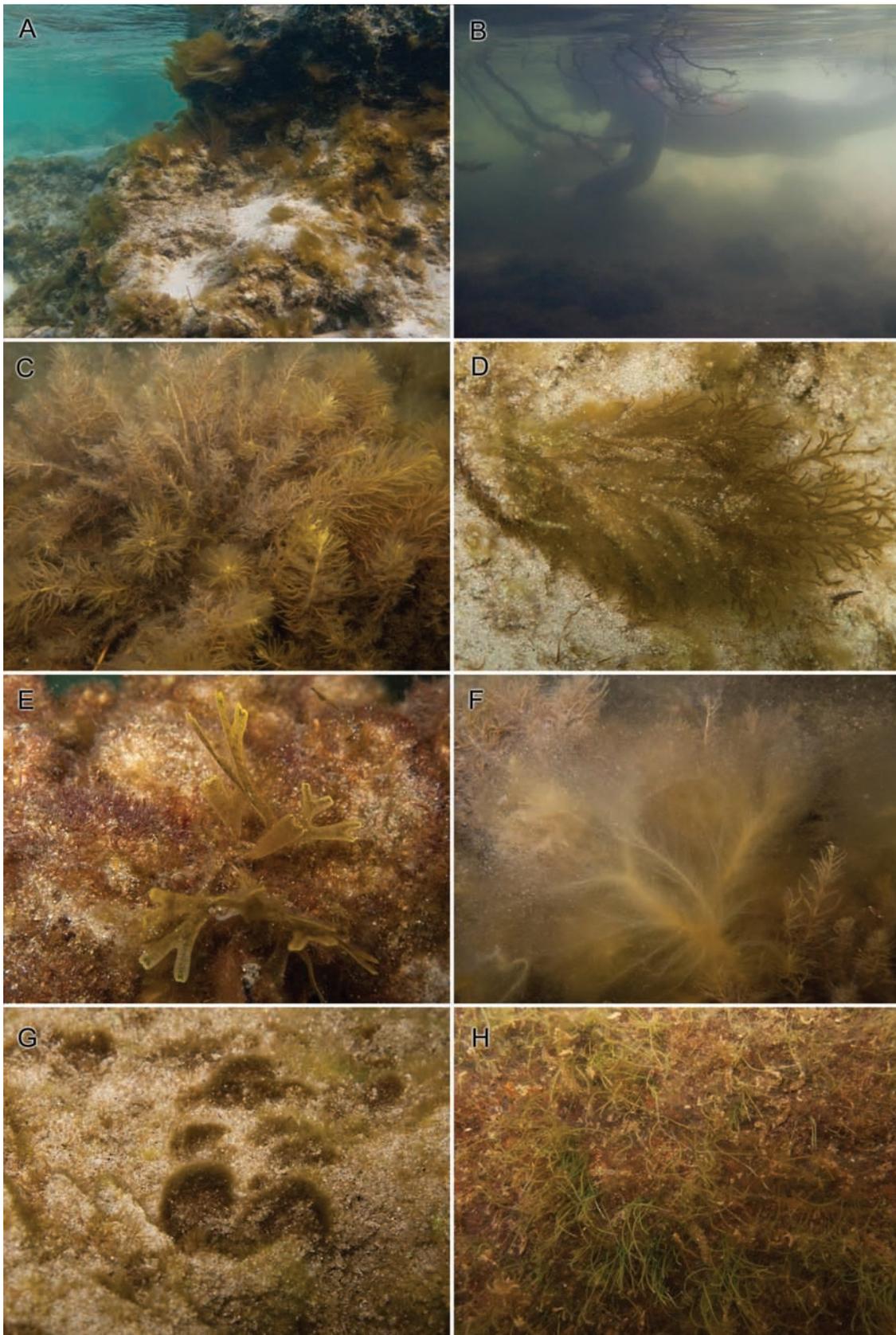| Species	Site 1 Entrance Channel	Site 2 Weedy Bay	Site 5 Newdegate Island	Site 6 The Knoll	Site 3 N. Nornalup Inlet	Site 4 Walpole Inlet
CHLOROPHYTA						
Acetabularia caliculus					X	
Chaetomorpha aerea				X		
Chaetomorpha billardierei					X	
Chaetomorpha ligustica	X					
Chaetomorpha indica	X		X			
Cladophora dalmatica	X				X	
Cladophora feredayi			X			X
Cladophora laetevirens	X					
Cladophora lehmanniana	X	X			X	
Codium sp.		X	X	X	X	X
Rhizoclonium implexum	X	X		X		
Struvea plumosa			X	X		
Ulva paradoxa	X	X			X	
Uronema marina	X	X	X	X	X	
PHAEOPHYCEAE						
Colpomenia sinuosa	X					
Cystoseira trinodis		X	X	X	X	X
Dictyota fastigiata	X					
Dictyota furcellata	X	X		X	X	
Dictyota dichotoma	X					
Feldmannia irregularis	X					
Sphacelaria biradiata	X					
Sphacelaria fusca		X	X			
Sphacelaria rigidula	X		X	X	X	X
Sphacelaria tribuloides	X					
RHODOPHYTA						
Acrochaetium microscopicum	X					
Aglaothamnion tenuissimum						X
Centroceras clavulatum	X	X		X		
Ceramium cliftonianum	X					
Ceramium sp.	X		X			
Chondria angustissima	X	X				
Colaconema savianum	X			X	X	X
Erythrotrichia carnea	X			X	X	X
Gelidium crinale	X	X	X			
Gracilaria ramulosa	X		X			
Hincksia mitchelliae	X	X	X	X	X	
Mazoyerella australis	X	X	X	X	X	X
Ossiella pacifica	X					
Polysiphonia infestans	X	X		X		
Polysiphonia subtilissima						X
Polysiphonia teges	X	X	X	X	X	X
Spermothamnion cymosum	X			X		
Spyridia filamentosa	X					
Stylonema alsidii	X	X		X		
SEAGRASS						
Zostera polychlamys	X	X				
CYANOBACTERIUM						
Spirulina subsalsa			X			
Total # species	**34**	**17**	**15**	**17**	**14**	**10**

Figure 2. A. Reef near channel entrance, Nornalup Inlet (Site 1). The filamentous brown alga is *Hincksia mitchelliae*. B. Weedy Bay (Site 2), with higher turbidity typical of most inlet sites. C. *Cystoseira trinodis*, a large brown alga common at several sites. D. *Dictyota furcellata*, another common brown alga. E. *Dictyota dichotoma* (Site 1). F. *Hincksia mitchelliae*, epiphytic on *Cystoseira trinodis* at Newdegate I. (Site 5). G. *Sphacelaria rigidula*, forming bristly tufts at the channel entrance (Site 1). H. *Chaetomorpha aerea*, a filamentous green alga attached to rock at the Knoll (Site 6).

Dictyota furcellata was moderately common near the Nornalup entrance and also near Coalmine and Newgate Island (Fig. 2D).

Dictyota dichotoma (Hudson) J.V.Lamour. 1809: 42.
Specimen: Entrance channel, Nornalup Inlet (Site 1), epilithic at 1.5 m depth, 20 Apr. 2010, *J.M.Huisman* (PERTH 08214433).
The present specimen is not fertile but agrees with *Dictyota dichotoma* as described by Womersley (1987). De Clerck (2003: 166) suggests that many reports of this supposedly widespread species might be incorrect, and that *D. dichotoma* could be restricted to the north-east Atlantic and the Mediterranean. The identification of this and other southern Australian records of *D. dichotoma* are therefore tentative (Fig. 2E).

Feldmannia irregularis (Kütz.) Hamel 1939: XVII.
Specimen: Entrance channel, Nornalup Inlet (Site 1), 20 Apr. 2010, *J.M.Huisman* (PERTH 08243409).
This species forms light brown, filamentous tufts and is almost indistinguishable from *Hincksia mitchelliae* without microscopic examination. In *Feldmannia*, the meristematic zones (regions of active cell division, recognisable as series of shorter cells) tend to be proximal to long, unbranched, hair-like filaments. Fertile material is readily distinguished by the plurangia distinctly tapering to a point, whereas those of *Hincksia mitchelliae* are cylindrical with rounded apices (compare Figs 4A and 4B).

Hincksia mitchelliae (Harv.) P.C.Silva in Silva, Meñez & Moe 1987: 73, 130.
Specimen: Newgate I., Nornalup Inlet (Site 5), epiphytic on *Cystoseira trinodis*, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243727).
Hincksia mitchelliae is another filamentous brown alga (Figs 2A, 2F) and was very common at most sites in Nornalup, where it was both epilithic and epiphyte on a variety of larger algae and seagrasses. Several specimens in the collection were reproductive, with cylindrical plurangia characteristic of this species (Fig. 4B).

Sphacelaria biradiata Askenasy 1894: 15.
Specimen: Entrance channel, Nornalup Inlet (Site 1), mixed with *Sphacelaria rigidula*, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243956).
Four species of *Sphacelaria* were collected during the survey. Each of these forms dark brown, bristly tufts and are similar in general appearance (see Fig. 2G of *S. rigidula*). They can be distinguished by their microscopic structure and the form of their propagules (vegetative reproductive structures); those of *S. biradiata* have two basally constricted, elongate arms, plus an apical elongate hair, those of *S. fusca* generally have three elongate arms, whereas propagules in *S. rigidula* have two arms and in *S. tribuloides* they are triangular without elongate arms (Womersley 1987: 168).

Sphacelaria fusca (Huds.) Gray 1821: 333.
Specimen: Newgate I., Nornalup Inlet (Site 5), on old oyster shells, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243611).
Sphacelaria fusca was found only on old oyster shells near Newgate Island and was the least common of the four species of *Sphacelaria* recorded.

Sphacelaria rigidula Kütz. 1843: 292.
Specimen: Newgate I., Nornalup Inlet (Site 5), on old oyster shells, 21 Apr. 2010, *J.M.Huisman* (PERTH 08214441).
This species (Fig. 2G) was more widespread but was also found on oyster shells near Newgate Island, the same habitat as *S. fusca*. *Sphacelaria rigidula* has narrower branches and propagules typically with 2 arms (Fig. 4C).

Sphacelaria tribuloides Menegh. 1840: [2]
Specimen: Entrance channel, Nornalup Inlet (Site 1), on rock, 20 Apr. 2010, *J.M.Huisman* (PERTH 08227594).
Sphacelaria tribuloides was only found at the Entrance channel site (Site 1), where it formed epilithic tufts. The specimens bore the triangular propagules (Fig. 4D) characteristic of this species.

Chlorophyta

Acetabularia caliculus J.V. Lamour. in Quoy & Gaimard 1824: 621.
Specimen: East Coalmine, Nornalup Inlet (Site 3), on old bivalve shells, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243689).
Acetabularia caliculus is a common species that is often found growing in clusters on old bivalve shells on sandy substrata, which was also the habitat of the present specimens.

Chaetomorpha aerea (Dillwyn) Kütz. 1849: 379.
Specimens: The Knoll, Nornalup Inlet (Site 6), epilithic at 1 m depth, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243379).
Chaetomorpha is a simply constructed genus that forms unbranched filaments composed of cylindrical cells. Species distinctions are based on habit (whether attached or free-floating) and differences in cells diameter and proportions (length/breadth ratio = L/B) (Womersley, 1984), although often published accounts vary in the size ranges included in single species. These morphological features, especially cell dimensions, are now regarded as poor indicators of phylogenetic relationships in the green algae (Leliaert *et al.* 2007) and it is clear that *Chaetomorpha* is in need of revision (Leliaert & Boedeker 2007). For the interim, however, it is prudent to at least recognise the different morphologies. *Chaetomorpha aerea* was one of four species of the genus recorded and was the only one found attached. Plants grew on rock in the shallow subtidal (Fig. 2H) and were mostly inundated with silt, such that they were only visible after cleaning. Cell diameters were 250–300 μm, with an L/B of 1–2 (–3). Their basal cells, at 600–1200 μm in length, can be slightly longer than those recorded by Womersley (1984: 172), but were comparable to dimensions recorded for this species from elsewhere (*e.g.*, Alves *et al.* 2009), and otherwise the specimens are comparable. The lowermost cells also had thicker walls than reported by Womersley (1984; to 30 μm as opposed to 2–4 μm), which relates these specimens to the unattached *C. valida*. *Chaetomorpha aerea* was also recorded by Brearley (2005: 366).

Chaetomorpha billardierei Kütz., 1847: 166.
Specimen: East Coalmine, Nornalup Inlet (Site 3), entangled on *Cystoseira trinodis*, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243336).
Plants of this species were unattached, with cells 290–400

μm in diameter and L/B 1–2, the largest of the four *Chaetomorpha* species recorded here. The cells are of a similar diameter to those reported for *C. linum* (Womersley 1984), but are generally longer than broad (L/B 1–2). *Chaetomorpha billardierei* was also recorded by Hodgkin & Clark (1999), epiphytic on *Ruppia* and *Zostera* (as *Heterozostera*), and Brearley (2005: 366). The spelling of the species epithet is variously given as "billardierii" (Womersley 1984: 176; Guiry & Guiry 2010) or "billardieri" (Index Nominum Algarum 2010) but as the name commemorates French botanist J.J. de Labillardière it should be corrected to "billardierei" (International Code of Botanical Nomenclature 2006: Art. 67, Ex. 15).

Chaetomorpha indica (Kütz.) Kütz. 1849: 376.
Specimen: Entrance channel, Nornalup Inlet (Site 1), unattached, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243964).
This species formed entangled skeins and was common near the Nornalup entrance (Fig. 3A). The filaments were 70–100 μm in diameter, which is at the lower end of the range reported by Womersley (1984), but the L/B ratios are comparable. Also recorded for Walpole Inlet by Womersley (1984: 180).

Chaetomorpha ligustica (Kütz.) Kütz., 1849: 376.
Specimen: Entrance channel, Nornalup Inlet (Site 1), unattached, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243972).
Plants of *C. ligustica* were mixed with *C. indica* and have a similar appearance, but with slightly smaller filament diameters and cells that are proportionally shorter (L/B 1–2). Womersley's (1984) treatment would place these specimens in *Chaetomorpha capillaris*, a species now regarded as a taxonomic synonym of *Chaetomorpha ligustica* (see Guiry & Guiry 2010).

Cladophora dalmatica Kütz., 1843: 268.
Specimen: East Coalmine, Nornalup Inlet (Site 3), growing on *Cystoseira trinodis* amongst *Polysiphonia*, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243522).
This is one of four species of *Cladophora* collected. The specimens agree with the description provided by Hoek & Womersley (1984: 202). *Cladophora dalmatica* is sometimes treated as a taxonomic synonym of *C. vagabunda* (L.) C.Hoek, but is retained here due to its distinctly narrower apical cells (15–20 μm, versus 25–70 μm reported for *C. vagabunda* by Hoek & Womersley 1984: 203). *Cladophora dalmatica*, *C. laetevirens*, and *C. lehmanniana* are included in Section Glomeratae, differing primarily in their cell diameters.

Cladophora feredayi Harv. 1858: pl. 47.
Specimen: Newdegate I., Nornalup Inlet (Site 5), epilithic, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243476).

Cladophora laetevirens (Dillwyn) Kütz. 1843: 267.
Specimen: Entrance channel, Nornalup Inlet (Site 1), 21 Apr. 2010, *J.M.Huisman* (PERTH 08243557).

Cladophora lehmanniana (Lindenb.) Kütz. 1843: 268.
Specimen: East Coalmine, Nornalup Inlet (Site 3), epilithic, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243395).
The branching pattern of *Cladophora lehmanniana* is similar to that of *C. dalmatica*, but plants are considerably larger, with apical cells ca 100 μm in diameter.

Codium sp.
Specimens: Walpole Inlet (Site 4), 21 April 2010, *J.M.Huisman* (PERTH 08243492)
This species was common at Coalmine and Newdegate Island, and was the only large alga present in the Walpole Inlet (Fig. 3B), where it was attached to rock and typically covered in silt, such that it was visible only after cleaning. Plants are structurally similar to *Codium harveyi* P.C.Silva (see Womersley 1984: 236) but have flattened lower branches, some subtle differences in utricle morphology, and unique DNA sequences, suggesting they represent an undescribed species (Hart & Huisman 2010). A formal description will be published elsewhere.

Struvea plumosa Sond. 1845: 50.
Specimen: The Knoll, Nornalup Inlet (Site 6), epilithic, 21 Apr. 2010, *J.M.Huisman* (PERTH 08227640).
When fully developed, *Struvea plumosa* is one of the most distinctive and attractive of Western Australia's marine algae, as it forms a paddle-shaped frond composed of anastomosing filaments forming a coarse net (see Huisman 2000). The present record, however, is based on less developed plants that are present as dense clusters of unbranched stipes (Fig. 3C). These are often indistinguishable from stipes of *Apjohnia laetevirens* Harv. (Womersley 1984: 182). Fortunately, one stipe in the collection showed some distal branching similar to that of fully developed *Struvea plumosa*, allowing a positive identification.

Rhizoclonium implexum (Dillwyn) Kütz. 1845: 206.
Specimen: Entrance channel, Nornalup Inlet (Site 1), unattached, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243530).
This species occurs as unattached, unbranched filaments, similar in appearance to species of *Chaetomorpha* but with considerably narrower cells that are only 20–22 μm in diameter.

Ulva paradoxa C.Agardh 1817: xxii.
Specimen: Entrance channel, Nornalup Inlet (Site 1), 20 Apr. 2010, *J.M.Huisman* (PERTH 08243646) (Fig. 3A).
This species was common at most locations. *Ulva paradoxa* was previously included in the genus *Enteromorpha*, but that genus has been subsumed into *Ulva* (see Hayden *et al*. 2003). *Ulva paradoxa* is not typical of the 'sea lettuce' form of *Ulva*, with flat green sheets, or, superficially, the tubular form of the species previously included in *Entermorpha*. Plants are very narrowly tubular, linear, with common uniseriate lateral branches (Fig. 4E). As is distinctive of this species, the cells are arranged in longitudinal and often transverse rows, with chloroplasts mostly with 3–4 pyrenoids.

Uronema marinum Womersley 1984: 131.
Specimen: East Coalmine, Nornalup Inlet (Site 3), epiphytic on *Cladophora*, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243999).
This is a small, unbranched filamentous epiphyte, typically less than 250 μm tall, which is generally only recorded during microscopic examination. It was very common in the inlets and was observed on larger algae collected from most sites. Leliaert *et al*. (2009: 493) queried the placement of this species in *Uronema*, but retained the name pending molecular investigation.
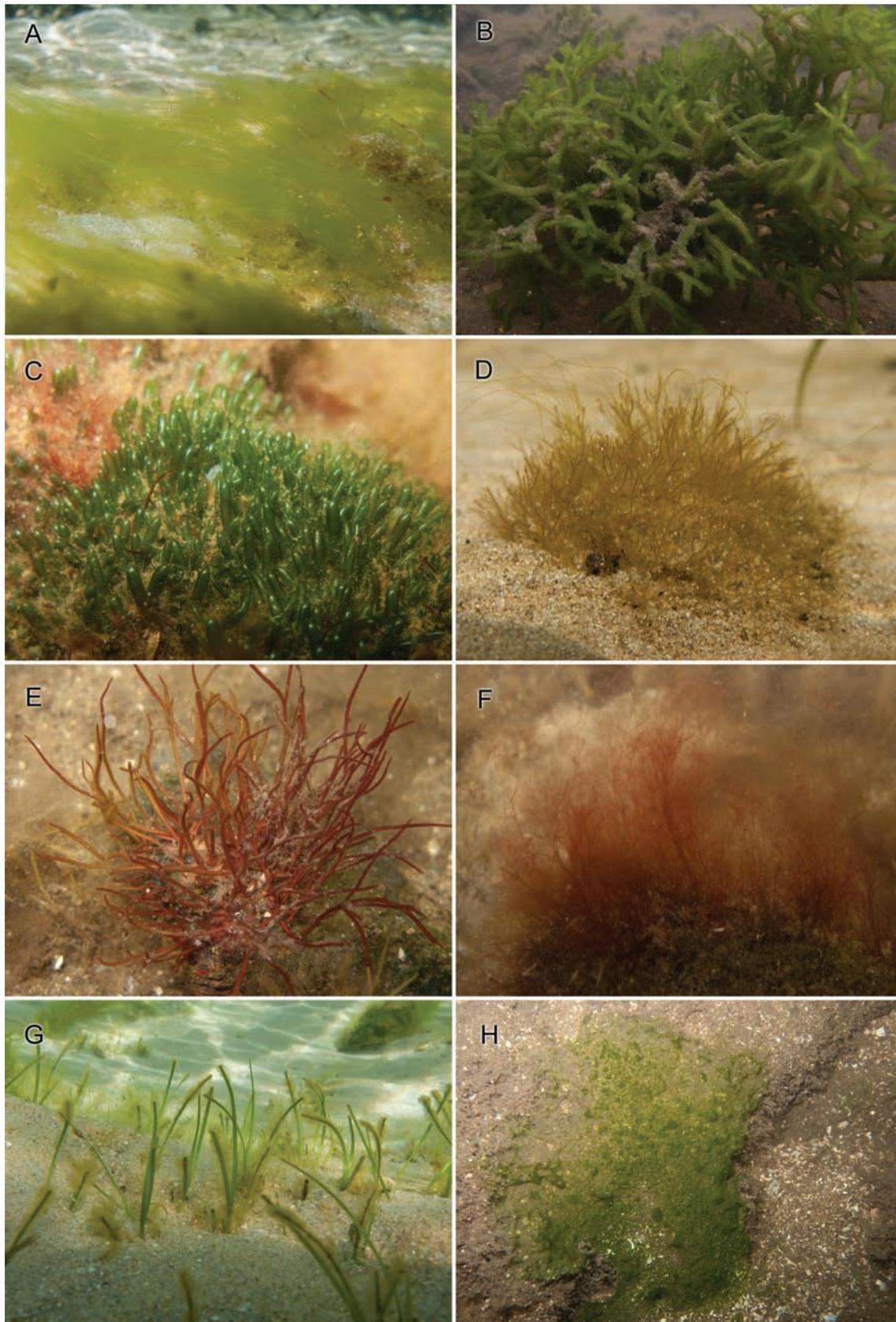
Figure 3. A. *Ulva paradoxa* and *Chaetomorpha* spp. at the channel entrance (Site 1). B. *Codium* sp., a likely new species and the only conspicuous alga in Walpole Inlet (Site 4). C. Massed stipes of *Struvea plumosa* at Newdegate I. (Site 5). D. *Centroceras clavulatum*, a filamentous red alga at the channel entrance (Site 1). E. *Gracilaria ramulosa* at Newdegate I. (Site 5). F. *Mazoyerella australis*, one of the most common red algae at inlet sites (Site 5). G. The seagrass *Zostera polychlamys* on sand at the channel entrance (Site 1). H. The cyanobacterium *Spirulina subsalsa* formed colonies on the sand surface at Newdegate I. (Site 5)
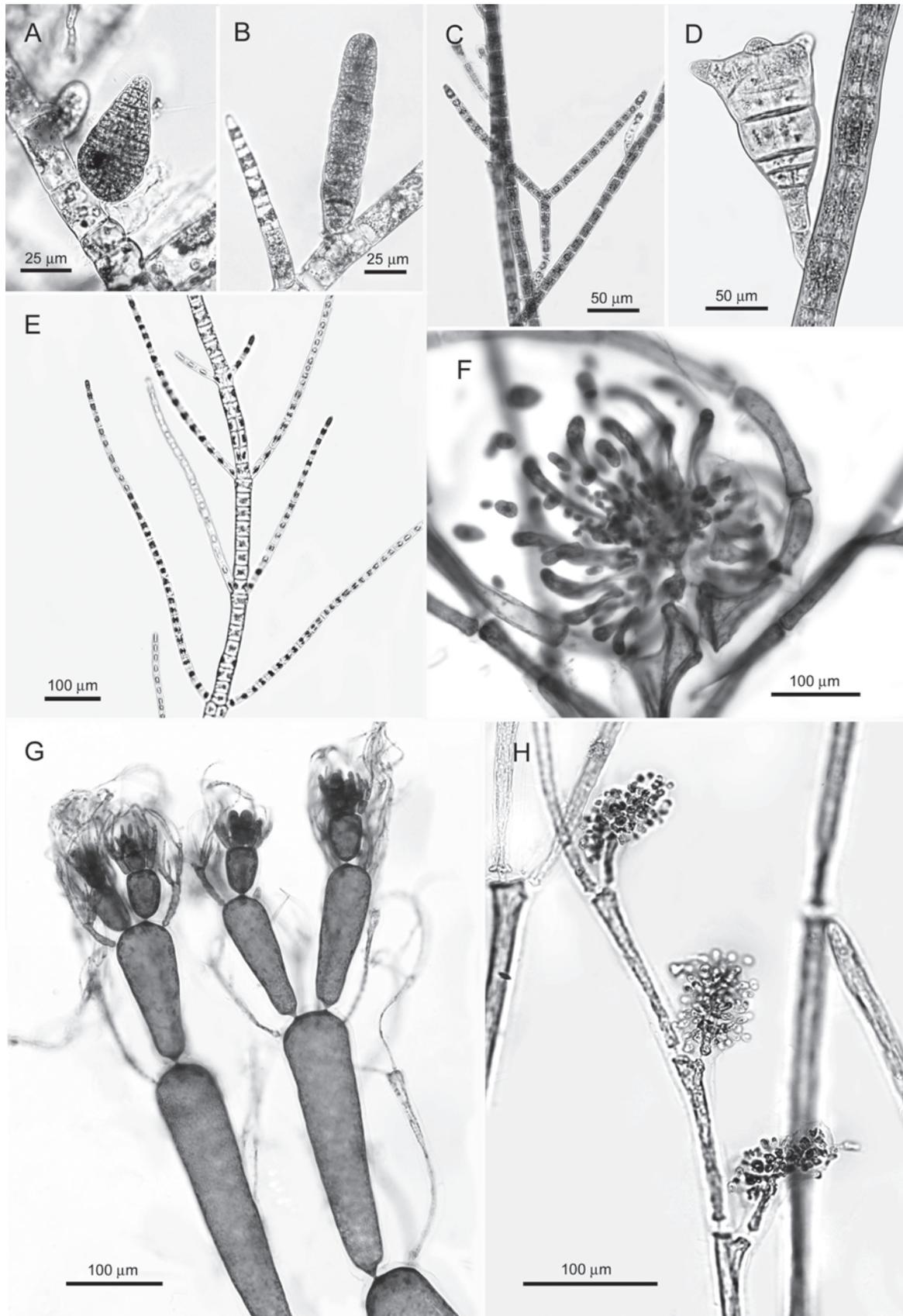
Figure 4. A. *Feldmannia irregularis*. Detail of conical plurangium. B. *Hincksia mitchelliae*. Detail of cylindrical plurangium. C. *Sphacelaria rigidula*. Slender propagule with 2 elongate arms. D. *Sphacelaria tribuloides*. Short, triangular propagule. E. *Ulva paradoxa*. Detail showing regular production of uniseriate lateral branches. F. *Mazoyerella australis*, the hitherto unknown carposporophyte stage. G. *Ossiella pacifica*, showing filamentous structure. H. *Spermothamnion cymosum*, the hitherto unknown spermatangial organs.

Rhodophyta

Acrochaetium microscopicum (Nägeli ex Kütz.) Nägeli in Nägeli & Cramer 1858: 532, footnote**.**

Specimens: Entrance channel, Nornalup Inlet (Site 1), epiphytic on *Spermothamnion cymosum*, 20 Apr. 2010, *J.M.Huisman* (PERTH 08244014).

This species is a diminutive epiphyte on larger algae, only recorded during microscopical examination.

Aglaothamnion tenuissimum (Bonnem.) Feldm.-Maz. 1941: 3.

Specimen: Walpole Inlet (Site 4), epiphytic on *Cystoseira trinodis* 21 Apr. 2010, *J.M.Huisman* (PERTH 08243603).

Several plants were collected from the Walpole Inlet, representing all stages of the life history. This species was treated in Womersley & Wollaston (1998) as *Callithamnion byssoides* Harv. (1833: 342), but that species is regarded as a synonym of *A. tenuissimum* by Furnari *et al.* (1998). The specimens differ slightly from those described by Womersley & Wollaston (1998: 248) in having obovoid to ellipsoidal tetrasporangia, whereas those described in Womersley & Wollaston are subspherical. Maggs & Hommersand (1993), however, described ellipsoidal tetrasporangia similar to those of the Walpole specimens.

Centroceras clavulatum (C.Agardh) Mont. 1846: 140.

Specimen: Entrance channel, Nornalup Inlet (Site 1), on sand, 20 Apr. 2010, *J.M.Huisman* (PERTH 08244006) (Fig. 3D).

Centroceras clavulatum is a widespread species found on most Australian coasts. Plants are filamentous and dichotomously branched, somewhat similar to species of *Ceramium* but recognisable due to their complete cortication and the presence of whorls of spines at nodes.

Ceramium cliftonianum J.Agardh 1876: 93.

Specimen: Entrance channel, Nornalup Inlet (Site 1), on jetty pilings, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243387).

Plants essentially agree with the description given by Womersley (1998: 415), although the frequency of branching is less than that of specimens depicted therein (Womersley 1998: figs 189H, I).

Ceramium sp.

Specimen: Entrance channel, Nornalup Inlet (Site 1), 21 Apr. 2010, *J.M.Huisman* (PERTH 08243417).

This species has prostrate axes and slender, sparsely branched upright axes with straight apices, 4 periaxial cells per node and only acropetal development of cortical cells. This morphology relates it to species such as *Ceramium affine* Setch. & N.L.Gardner, but accurate identification requires reproductive specimens, which were unfortunately not collected during the present survey. Whatever the eventual determination, this species will represent a new record for southern Australia, as the vegetative structure differs from all species recorded for the region by Womersley (1978; 1998).

Chondria angustissima Gordon-Mills & Womersley 1987: 531.

Specimen: Entrance channel, Nornalup Inlet (Site 1), on jetty pilings mixed with *Ceramium cliftonianum*, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243913).

Both cystocarpic and male specimens were present in the collection, both agreeing with descriptions of this species by Gordon-Mills & Womersley (1987: 531) and Womersley (2003: 426). This species is confined to sheltered estuarine conditions (Womersley 2003: 427) and was previously recorded from the Leschenault Inlet in south-west Western Australia (Womersley 2003: 427; AD A56818).

Colaconema savianum (Menegh.) R.Nielsen 1994: 715.

Specimen: Entrance channel, Nornalup Inlet (Site 1), epiphytic on leaves of *Zostera polychlamys*, 1 m depth, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243506).

Colaconema savianum is a small (less than 2 mm tall), branched, filamentous epiphyte, distinguished by its straight filaments bearing monosporangia (single-celled vegetative reproductive structures).

Erythrotrichia carnea (Dillwyn) J.Agardh 1883: 15.

Specimen: Entrance channel, Nornalup Inlet (Site 1), epiphytic on *Chondria angustissima*, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243921).

Erythrotrichia carnea is another small, filamentous epiphyte, distinguishable from other taxa sharing this habitat in being unbranched (*Acrochaetium*, *Colaconema* and *Stylonema* are branched)

Gelidium crinale (Turner) Gaillon 1828: 362.

Specimen: Weedy Bay, Nornalup Inlet (Site 2), epilithic, 20 Apr. 2010, *J.M.Huisman* (PERTH 08214468).

Plants of *G. crinale* are turf-like and wiry, with prostrate and sparsely branched erect axes that are terete or flattened. It is a common species in south-western Australia and elsewhere.

Gracilaria ramulosa J.Agardh 1876: 417.

Specimen: Entrance channel, Nornalup Inlet (Site 1), 20 Apr. 2010, *J.M.Huisman* (PERTH 08243360).

This species and *Gelidium crinale* were the only cartilaginous red algae encountered during the survey. Plants (Fig. 3E) grew to a height of approximately 12 cm and can be recognised by their terete branches and multiaxial construction (see Womersley 1996: 18). *Gelidium crinale* is a much smaller species and is structurally uniaxial.

Mazoyerella australis (Harv.) Huisman & Womersley 1998: 302.

Specimen: Weedy Bay, Nornalup Inlet, (Site 2), epiphytic on various larger algae, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243719).

Mazoyerella australis is a filamentous red alga (Fig. 3F) that was one of the more common species in the inlets, being recorded at most sites (see Table 1). Collections from The Knoll also provided the first known record of field-collected reproductive specimens. Until recently, this species was included in *Monosporus*, a genus in which the only known reproductive structures are single-celled propagules (the genus name is based on the incorrect interpretation of these structures as monosporangia, see Huisman & Kraft 1982). Subsequently, Huisman & Gordon-Mills (1994) described the presence of sexual reproductive structures in material

grown in culture, demonstrating (at least) the potential for a sexual life history in addition to vegetative propagation by propagules. The structure of these reproductive organs then provided the basis for Huisman & Womersley's (1998: 302) transfer of the species to *Mazoyerella*, although this was somewhat tentative as fertilization, and subsequent post-fertilization events, were not observed in Huisman & Gordon-Mills' (1994) culture studies. As post-fertilization and the development of the carposporophyte generation are important in generic delineation, the discovery of these stages in the Nornalup collections (Fig. 4F) will allow confirmation (or not) of the taxonomic placement of *M. australis*. These results will be incorporated into a DNA sequence study and will be reported elsewhere.

Ossiella pacifica A.Millar & I.A.Abbott 1997: 89.
Specimen: Entrance channel, Nornalup Inlet (Site 1), mixed with *Polysiphonia infestans*, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243514) (Fig. 4G).
This species was originally described in 1997 for specimens from Norfolk Island and the Hawaiian Islands (Millar & Abbott 1997), and was subsequently recorded from Micronesia (McDermid *et al.* 2002; Lobban & Tsuda 2003; Guiry & Guiry 2010). Given this known distribution in warmer waters of the Pacific Ocean, the occurrence of *O. pacifica* in Nornalup Inlet was certainly surprising. The specimen is not reproductive, but displays several distinctive vegetative features unique to this genus, including the simultaneous production of determinate whorl branches from the distal ends of axial cells (Millar & Abbott 1997: 94). Millar (pers. comm. July, 2010) examined images of this specimen and confirmed its identity. As this represents a new record for Western Australia (and mainland Australia), a full description of the specimen is given below.
Thallus filamentous, primarily upright, 8 mm tall, attached to the substratum by unicellular rhizoids arising from lower cells, uncorticated, dichotomously branched near apices, but unbranched for most of its length. Cells elongate, near base cylindrical, 120–145 μm diam., 680–1070 μm long, towards apices becoming shorter and clavate, 60–80 μm diam., 300–350 μm long, at apices obovoid to ellipsoid, 35–40 μm diam., only slightly longer than broad. Apical cells simultaneously cutting off distally a whorl of 4 villous pigmented caducous determinate whorl branches, these dichotomously branched, markedly thinner than the axial filament, basal cells 7–10 μm diam., 120–170 μm long, distal cells more slender and hair-like. Reproductive structures not observed.

Polysiphonia infestans Harv. 1855: 539.
Specimens: W of boat shed, Nornalup Inlet, epiphytic on *Cystoseira trinodis* on rocks on sand flat, 30–50 cm depth, 23 Oct. 1976, *M.L.Cambridge* (PERTH 07023146); Entrance channel, Nornalup Inlet (Site 1), epiphytic on *Dictyota furcellata*, 20 Apr. 2010, *J.M.Huisman* (PERTH 08227608).
PERTH 07023146 is a dried specimen that did not rehydrate well, and the determination is therefore tentative. The specimen has 4 pericentral cells, tetrasporangia in gently spiral series, and the dimensions of *P. infestans*, but the origin of the lateral branches (important for species identification) could not be ascertained. Specimens from the present survey were

male and essentially agree with Womersley's description (2003: 188–189), including the lateral branches replacing trichoblasts. However, the segments were often proportionally longer and the spermatangial branches did not have a prominent sterile apical cell, which are usually present according to Womersley (2003: 189). *Polysiphonia infestans* is a common species in the region and was originally described from material from Princess Royal Harbour, Albany (Womersley 2003: 184).

Polysiphonia subtilissima Mont. 1840: 199.
Specimen: Walpole Inlet (Site 4), epiphytic on immersed branches of terrestrial vegetation, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243549).
This species typically occurs in sheltered locations (Womersley 2003: 180). The specimens have a limited prostrate system attached by rhizoids in open connection to the bearing cell, upright axes with 4 pericental cells, and no trichoblasts or scar cells. One specimen bore immature tetrasporangia in a straight series. All of these features agree with this species as described by Womersley (1979: 470–472; 2003: 178–180).

Polysiphonia teges Womersley 1979: 494
Specimen: Newdgate I., Nornalup Inlet (Site 5), epilithic on rubble, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243565).
Prior to the present collection, this species was known only from the type locality at Frenchmans Bay, Albany, and Spencer Gulf in South Australia (Womersley 2003: 202). It is a distinctive species characterized by prostrate axes with rhizoids in open connection with the bearing pericentral cell, and axial cells each with 6 pericentral cells. Trichoblasts were absent or rudimentary in the present collection, but many apices were denuded.

Spermothamnion cymosum (Harv.) De Toni 1903: 1226.
Specimen: Entrance channel, Nornalup Inlet, epilithic, 20 Apr. 2010, *J.M.Huisman* (PERTH 08243980).
Spermothamnion cymosum is represented in the collections by several specimens comprising uncorticated prostrate filaments (cells 70–90 μm in diameter), bearing simple or sparsely dichotomously branched upright axes. Some plants bore tetrahedral tetrasporangia and polysporangia, these 55–80 μm in diameter and arising terminally on lateral branches. Both the habit and tetrasporangia agree with the description and figures given by Gordon (1972: 117–119, fig. 38E) and Womersley (1998: 225). The species is rarely collected and the only reproductive structures previously known are the aforementioned tetrasporangia. Consequently, its generic assignment has been regarded as tentative, as details of female reproductive structures and carposporophyte architecture are necessary for definite placement. The present collections include the first recorded male specimens. The arrangement of spermatangia (described in full below) in distinct heads (Fig. 4H) supports the inclusion of *S. cymosum* in *Spermothmanion*, but this placement must remain unconfirmed until female gametophytes and carposporophytes are collected.
Spermatangia in heads arising in unilateral series on upright axes, sessile or with single-celled stalks (Fig. 4F), if the latter then often in pairs. Spermatangial heads 75–120 μm long, 30–50 μm broad, with 4–6 axial cells bearing radial (often obliquely cut off) spermatangial

mother cells and spermatangia, the basal cell generally proximally naked.

Spyridia filamentosa (Wulfen) Harv. 1833: 337.
Specimen: Entrance channel, Nornalup Inlet (Site 1), 20 Apr. 2010, *J.M.Huisman* (PERTH 08243581).
Spyridia filamentosa is a widespread species found on most Australian coasts and temperate and tropical seas worldwide. The present specimens agree with the descriptions given by Womersley & Cartledge (1975: 222), Huisman (2000) and Womersley (1998: 372).

Stylonema alsidii (Zanardini) K.M.Drew 1956: 72.
Specimen: Entrance channel, Nornalup Inlet, epiphytic on *Chondria angustissima*, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243948).
This species is a diminutive epiphyte that is common in most seas. Zuccarello *et al.* (2008) analyzed DNA sequences generated from geographically widespread specimens and concluded that this was a truly ubiquitous species.

Seagrasses

Halophila decipiens Ostenf. 1902: 260.
Specimen: Nornalup Inlet (Site 7), 21 Apr. 2010, *M.Rule & A.John* (PERTH 08243654).
Fruiting plants of *H. decipiens* were collected by benthic grab from a relatively deeper site in the middle of Nornalup Inlet. Leaves of these plants displayed the spiny margins and surface typical of this species, features that distinguish it from the somewhat similar looking *Halophila ovalis*. Kuo & Kirkman (1995: 336) recorded this species from several inlets along the south coast of Western Australia, including Nornalup Inlet. Their record, however, was based on a 'drift leaf' and not an *in situ* collection. This species was also recorded by Brearley (2005: 366).

Zostera polychlamys (J.Kuo) S.W.L.Jacobs & Les 2009: 423.
Specimen: Entrance channel, Nornalup Inlet (Site 1), on sand at 1.5 m depth, 20 Apr. 2010, *J.M.Huisman* (PERTH 08227632) (Fig. 3G).
The present specimens matched the description of this species (as *Heterozostera*) by Kuo (2005: 124–126), including the smooth epidermis and lack of upright stems. The inclusion of *Heterozostera* species in *Zostera* is based on the phylogenetic scheme proposed by Les *et al.* (2002) and Jacobs & Les (2009), a scheme that has not been universally accepted (see Kuo 2005) but one that appears to be the most reasonable interpretation of the available data. This species was recorded by Hodgkin & Clark (1999) as *Heterozostera tasmanica*, which at that time was more broadly conceived. *Zostera* sp. (presumably this species) was also recorded by Brearley (2005: 366).

Cyanobacteria

Spirulina subsalsa Oerst. ex Gomont 1892: 353.
Specimen: Newdegate I., Nornalup Inlet, forming mats on sand at 1.5 m depth, 21 Apr. 2010, *J.M.Huisman* (PERTH 08243638).
This species forms mats of fine green filaments on the

sandy bottom (Fig. 3H). The individual trichomes are very narrow (1–2 μm diam.) and are regularly and tightly spirally coiled. According to McGregor (2007: 43), this species is cosmopolitan and is considered to be euryhaline.

Other Records

Ruppia megacarpa R.Mason 1967: 525, "swan grass"
This seagrass species was recorded by Hodgkin & Clark (1999) and Brearley (2005) as growing in shallow water on sand flats near the Deep River delta and stunted plants in south eastern shore, and was also recorded from Nornalup Inlet by Boyce *et al.* (2001) and Svensson *et al.* (2007). Brearley (2005: 366) suggested that it was not prolific and was often covered with epiphytes such as the red alga *Polysiphonia*. Although this species was not collected during the 2010 survey, it is well known and unlikely to be misidentified, and we therefore have no reason to question its occurrence in the Walpole and Nornalup Inlets.

Halophila ovalis (R.Br.) Hook.f. 1858: 45, "paddle weed"
This species was recorded by Brearley (2005: 366), along with the congeneric *H. decipiens*, but was not collected during the 2010 survey and no vouchers are present in PERTH. It is a distinctive species, however, and we therefore have no reason to doubt the record.

Chaetomorpha linum (O.F.Müll.) Kütz. 1845: 204.
This species is the only one of the older records represented in PERTH by a named voucher [Walpole Inlet, 9 July 1971, *R.Lenanton* (PERTH 07067011)]. As described by Womersley (1984: 176), *C. linum* has filaments 200–400 μm in diameter with a length/breadth ratio (L/B) of cells 0.5–1.5. PERTH 07067011 is a mixture of 2 species of *Chaetomorpha*, neither of which appears to be *C. linum*. One, with broad cells 190–260 μm diam, L/B 1–2, agrees with *C. billardierei* Kutz. The second, with cells 75–100 μm in diameter and L/B 2.5–5, has the size of *C. capillaris* but the cell proportions of *C. indica.* This latter specimen is identical to the recent collections referred to *C. indica* based on cell proportions. Both species on sheet 07067011 were therefore also collected in the 2010 survey, but not *Chaetomorpha linum*, the presence of which is regarded as uncertain. *Chaetomorpha linum* was also recorded by Brearley (2005: 366), along with *C. aerea* (Dillwyn) Kütz., as abundant in 1973–74.

Ulva intestinalis L. 1753: 1163.
This species was previously recorded as *Enteromorpha*, but all species of that genus are now included in a more broadly conceived *Ulva*, following the studies of Hayden *et al.* (2003). *Ulva intestinalis* was recorded for the inlets by Hodgkin & Clark (1999), and Brearley (2005: 367) noted that floating mats of *U. intestinalis* and *Chaetomorpha billardierei* were present in shallow water on the east side of the inlet in 1987. This species was not collected in the present survey and no vouchers are lodged in PERTH. Of congeneric species, only *Ulva paradoxa* was recorded in the 2010 survey. Recognition of species within *Ulva* can be difficult, but *U. intestinalis* and *U. paradoxa* are both distinctive and unlikely to be confused. Thus, while we are unable to confirm the record of *U. intestinalis*, we can equally not refute it.

Discussion

Taxonomy

Forty-nine species of marine algae and seagrasses are recorded for the Walpole and Nornalup Inlets, including 15 green algae, 11 brown algae, 18 red algae, 4 seagrasses, and one cyanobacterium. In addition, several epiphytic diatoms and inconspicuous cyanobacteria are noted. This represents a substantial increase on the 14 species previously known from the inlets and is a more realistic appraisal of the diversity. Unfortunately similar studies of south-western estuaries are mostly lacking, with only Waychinicup Estuary receiving comparable detailed attention (Phillips & Lavery 1997). That study recorded 40 species of macroalgae and 5 seagrasses, a diversity seemingly comparable to that recorded herein, but significantly only 5 species were common to the two estuaries (*Chaetomorpha aerea, Cystoseira trinodis, Colpomenia sinuosa, Dictyota dichotoma,* and *Heterozostera tasmanica,* the latter probably = *Zostera polychlamys*). Other studies have focussed primarily on macrophyte populations in respect to nutrient loading (*e.g.* Lukatelich *et al.* 1987), with floristic surveys of secondary importance. Hillman *et al.* (2000) recorded 4 seagrasses and 13 macroalgae for the Leschenault Inlet, but several of these were identified only to genus, making direct comparisons difficult. Nevertheless, they recorded the dominant species as the seagrass *Halophila ovalis,* the green alga *Chaetomorpha linum,* and the brown alga *Hormophysa cuneiformis* (as *H. triquetra*), of which only *H. ovalis* is reliably recorded for the Walpole and Nornalup Inlets. Lukatelich *et al.* (1987) reported that the seagrass *Ruppia megacarpa,* a species that is relatively rare in the Walpole and Nornalup Inlets, comprised over 90% of the marine plant biomass in the Wilson Inlet. These disparities suggest that the marine flora of each estuarine system is likely to be unique, and each will require dedicated floristic surveys.

Of the 49 species recorded for the Walpole and Nornalup Inlets, most are relatively common elements of the south-western Australian marine flora, but a few warrant particular mention. One of the more common species in the inlets proper is a species of *Codium,* a genus of green algae with an essentially worldwide distribution and represented on the southern Australian coast by 16 species (Womersley 1984). The present collection does not, however, agree with any previously described species and appears to be new to science. It will be described formally elsewhere, as part of a monograph of Western Australian *Codium* currently in preparation (see Hart & Huisman 2010). *Codium* includes the invasive pest *C. fragile* subsp. *fragile,* which has been introduced to eastern and southern Australia and was recently discovered in Albany harbour (Hart & Huisman 2010). The new species, however, is clearly distinct from *C. fragile,* both morphologically and molecularly, and does not appear to pose a threat to the Walpole and Nornalup Inlets Marine Park. Collections of two species, *Mazoyerella australis* and *Spermothamnion cymosum,* included specimens bearing reproductive structures that were hitherto unrecorded. In the case of *Mazoyerella australis,* these structures will be described in detail elsewhere, as part of a DNA sequence study assessing the generic affinities of this species. Preliminary results indicate that

it is incorrectly placed in *Mazoyerella*. The collection of *Spermothamnion cymosum* included male specimens and the structure of the spermatangial heads supports the inclusion of this species in *Spermothamnion,* although the still unknown female and carposporophytic specimens are required for confirmation. Lastly, *Ossiella pacifica,* a species hitherto known from warmer waters of the Pacific Ocean, is newly recorded for mainland Australia. The reasons for this unusually disjunct distribution are unclear and will require further study.

Ecology

The marked disparity in the number of algae and seagrass species collected at sites in the entrance channel (34 taxa), Nornalup Inlet (14–17 taxa) and Walpole Inlet (10 taxa) indicates that the diversity of marine plants diminishes with increasing distance from the sea. Although it is beyond the scope of this study to clearly identify the cause of this distribution, some points relating to the hydrology of the inlet system are likely to be relevant. This survey was conducted in autumn (April) when water clarity is highest and environmental conditions were relatively stable throughout the system, but the inlet waters can change markedly with the onset of freshwater river flows during the winter (Hodgkin & Clark 1999). Winter water temperatures may drop from summer highs of >20 °C to ca. 12 °C and a pronounced halocline may form with water of <10 ppt salinity flowing above relatively saline deeper waters. Notably also, the typically tannin-stained river water can markedly lower water clarity in the inlets. Although the extent of these seasonal changes varies annually with the amount of rain received by the surrounding catchments, and the subsequent volume of riverine discharge, the change in water quality could reasonably be expected to be highest in the upper reaches, with the impact lessening further seaward where there is greater tidal exchange. These seasonally variable environmental conditions strongly influence the use of estuarine habitats by mobile organisms such as fish, which may leave the system for periods or move within it to avoid brackish water (Potter & Hyndes 1999). Such variable conditions are also most likely to limit the capacity of some marine algae and seagrasses to successfully establish within the Walpole and Nornalup inlets.

While twelve species that occurred at the entrance channel site were not recorded further inside the inlets, other species were present only within the inlet basins, including two algae species that occurred only in the Walpole Inlet (Table 1). Moreover, most of the species found in the inlet basins were reproductive, suggesting that these populations may be self-propagating and not dependent on oceanic recruitment. These findings suggest that the distributional patterns observed in this study are not simply a reflection of marine species entering the system and diminishing in diversity due to an increasing estuarine influence. It is likely that a number of the species collected during this survey are particularly tolerant of estuarine conditions, such as diminished temperature, salinity and water clarity. This may be especially so in the Walpole Inlet, which is relatively distant from the ocean and where fine silt is prevalent. In fact, some of the specimens recorded during this study were actually found to be covered by silt at the