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Fantastic Flatworms and Where to Find Them: Insights into Intertidal Polyclad Flatworm Distribution in Southeastern Australian Boulder Beaches

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Abstract: There is a rapid and extensive decline of our marine biodiversity due to human impacts. However, our ability to understand the extent of these effects is hindered by our lack of knowledge of the occurrence and ecology of some species groups. One such group of understudied organisms are marine flatworms of the order Polycladida, a conspicuous component of southeastern Australia's marine ecosystems that has received little attention over the years. Intertidal boulder beaches support a diverse range of polyclad flatworms in other countries, but the role of these environments in maintaining biodiversity is not well understood. In this study, we identified hotspots of flatworm occurrence by assessing the diversity and overall abundance of flatworms at boulder beaches along the southeast Australian coast. Bottle and Glass, Sydney Harbour, was found to be the most diverse site for flatworms. We also identified a higher occurrence of flatworms under large boulders and less exposed beaches and noted an increased presence of flatworms at higher latitudes. Probable influences on these patterns such as the requirement for shelter and protection are discussed. This study contributes to our knowledge of Australia's coastal biodiversity and can be used to assist in the management and conservation of our marine environments.

Keywords: Polycladida; ecology; biodiversity; marine ecosystems

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1. Introduction

Marine systems are facing substantial and rapid declines in biodiversity due to human impacts such as urbanisation, climate change, overharvesting and pollution [1–4]. One issue with understanding the extent and rate of decline is a basic lack of inventory of the diversity and complexity of marine communities and systems [5]. This is particularly pertinent for Australian marine systems where large gaps exist in our baseline knowledge of some species groups and their distributions. Such gaps hinder our ability to understand ecosystem functioning, and the magnitude of biodiversity loss in response to perturbations [6].

For rocky intertidal regions, the type of substratum can substantially influence the presence and success of the diversity of inhabiting organisms, particularly if the substratum is dynamic. Intertidal and shallow subtidal rocky areas of unstable rock substrata comprising pebbles, cobbles and boulders (referred to as 'boulder beaches' here) are dynamic in that they create disturbance events when the substrata move in response to high energy events [7,8]. These boulder beaches typically support a broad range of biota, including rare species [9–12]. While the ecological processes of animals inhabiting intertidal boulder beaches in southeastern Australia have been assessed for some species [13–15], the role of boulder beaches in maintaining biodiversity is not well understood in this area due to the lack of inventory of organisms, especially less abundant species. This is particularly concerning for this area of southeastern Australia, which is a known biodiversity hotspot for numerous other groups of marine intertidal organisms [16–19]. Many of these organisms are cryptic in habit, such as those occurring in crevices or under rocks, and/or with external features and colouration patterns that mimic the substrate they inhabit, making species identification difficult [20]. Such paucity in our understanding of community structure and functioning of these habitats in southeast Australia hinders development of ecologically driven resource management plans in such habitats vulnerable to anthropogenic perturbations [21].

One such group of understudied organisms are marine flatworms of the Order Polycladida. Polyclads have the potential to substantially impact their communities by predating on a range of invertebrates such as crustaceans, corals and molluscs, including some commercially valuable species, such as bivalves [22–25]. Recent studies have shown the rich biodiversity of polyclad flatworms in boulder beaches across southeastern Australian coasts [26,27]; however, their abundance and geographical distribution are only eclectic and poorly understood.

Records from overseas show that polyclad flatworms usually reside on the underside of rocks on boulder beaches [28–32]. In southeastern Australia, boulder beaches occur along the coast and in estuaries, presenting different levels of wave exposure [33]. Due to the abundance of these habitats and the lack of data on polyclads in temperate southeastern Australia, an assessment of the suitability of boulder beaches as habitat for polyclad flatworms was done. This is the first assessment of polyclad flatworm diversity and abundance in temperate Australian waters.

Here, we document the diversity and abundance of polyclad flatworms (hereafter called 'flatworms') in intertidal beaches along over 1367 km of the southeastern coastline of Australia and covering a range of beaches with different wave exposures. Specifically, the research asked the following questions: (1) Do flatworms inhabit the underside of boulders on these intertidal beaches? (2) Are there areas with higher species diversity (i.e., biodiversity hotspots) along the coastline than others? (3) Does the size of the boulder influence the abundance or diversity of flatworms? (4) Does beach exposure influence the relationship between rock size and the diversity and abundance of flatworms?

2. Materials and Methods

The demography of intertidal polyclad flatworms in boulder beaches along 532.45 km of the southeastern coastline of Australia, from 29°49′01.6″ S, 153°17′34.4″ E to 38°30′24.6″ S, 145°07′33.8″ E, was assessed (Figure 1). Boulder beaches, defined as those with a mean rock diameter of >256 mm [34], are distinct sedimentary coastal features with unique morphological characteristics [33,35], typically occurring in higher wave-energy environments [36]. Appropriate boulder beaches were selected by assessing maps and from discussions with other researchers and the local communities. Sites suitable for flatworms were then chosen based on the requirement of the presence of gravel to boulders (between 8.1 mm and 100 cm, respectively) and accessibility by foot at low tide (Figure 1).

Rocks on beaches suitable for flatworms were classified using a Wentworth scale according to an expanded version of Oak (1984) [33], as outlined in Table 1. Beach exposure was calculated using Baardseth's wave exposure index which involved counting the number of 9° sectors that contained a fetch of greater than 7.5 km [37,38]. In this index, beaches are classified from 0–9, where 0 is least exposed and 9 is most exposed. According to this classification system, Bottle and Glass, Chowder Bay and Shelly Beach were classified as index 0, Phillip Island and San Remo were classified as index 3, Port Macquarie was classified as index 4, Diggers Camp and Foster as index 8 and Boat Harbour was classified as index 9.

Figure 1. Locations surveyed during this study (**left**) and examples of boulder beaches: Boat Harbour, Gerroa, NSW (**top right**), Inverloch, VIC (**bottom right**). Photos: ESRI ArcGIS (**left**), Jorge Rodríguez (**right**).

Table 1. Overview of sampled rock size categories from boulder beaches in southeastern Australia.

Boulder Size Range (cm)	Wentworth Category
0.10–0.40	Coarse sand
0.41–0.80	Fine gravel
0.81–1.60	Coarse gravel
1.61–3.20	Medium gravel
3.21–6.40	Cobble
6.41–12.80	Coarse cobble
12.81–25.60	Small boulder
25.61–51.20	Medium boulder
51.21–102.40	Large boulder

Sampling each beach involved two hours of continuous searching in which rocks of varying sizes were lifted and the underside inspected for flatworms. The longest length of the rock was then measured as a proxy for boulder size (Table 1). Following inspection and measurement, the rocks were returned to their original upright position. Beaches were methodically sampled to ensure that rocks of all sizes were sampled, and only sampled once. Sampling started 1.5–2 h prior to a daytime low tide, depending on the weather conditions and tidal heights (Table 2). To characterise the range and abundance of boulder sizes on each beach (Figure 2), three radial 2 m plots were also assessed at a subset of the beaches (Boat Harbour, Bottle and Glass, Chowder Bay, Port Macquarie, San Remo and Shelly Beach, Eden), where the longest length of all bounders within the circumference of each plot was measured. Radial plots were chosen for their representativeness of the boulder beach habitat that had been searched. Finally, a range of other substrata at each beach were also searched for flatworms to ensure the flatworms collected were representative of the species occurring at that location.

Table 2. List of sites and sampling times.

State	Locality	Latitude	Longitude	Date	Sampling Time	Baardseth's Wave Exposure Index
New South Wales	Diggers Camp Beach, Diggers Camp	29°49′01.6″ S	153°17′34.4″ E	8 December 2019	12:00 p.m.–14:00 p.m.	8
	Shelly Beach, Port Macquarie	31°27′27.7″ S	152°56′04.4″ E	7 January 2020	12:30 p.m.–14:30 p.m.	4
	Pebbly Beach, Forster	32°10′46.0″ S	152°31′10.6″ E	6 December 2019	09:00 a.m.–11:00 a.m.	8
	Chowder Bay, Sydney Harbour	33°50′19.8″ S	151°15′16.2″ E	20 February 2020	13:30 p.m.–15:30 p.m.	0
	Bottle and Glass, Sydney Harbour	33°50′54.0″ S	151°16′13.1″ E	25 October 2019; 21 February 2020	12:00 p.m.–14:00 p.m.; 14:30 p.m.–16:30 p.m.	0
	Boat Harbour, Gerroa	34°45′02.0″ S	150°49′56.5″ E	7 July 2018	07:30 a.m.–8:30 a.m. *	9
	Shelly Beach, Eden	37°04′22.0″ S	149°54′45.6″ E	10 July 2018	11:20 a.m.–12:20 p.m. *	0
Victoria	San Remo	38°31′11.9″ S	145°22′02.2″ E	13 July 2018	07:30 a.m.–8:30 a.m. *	3
	Cats Bay, Phillip Island	38°30′24.6″ S	145°07′33.8″ E	12 July 2018	16:00 p.m.–17:00 p.m. *	3

* Sites sampled simultaneously by two teams during a one hour interval.

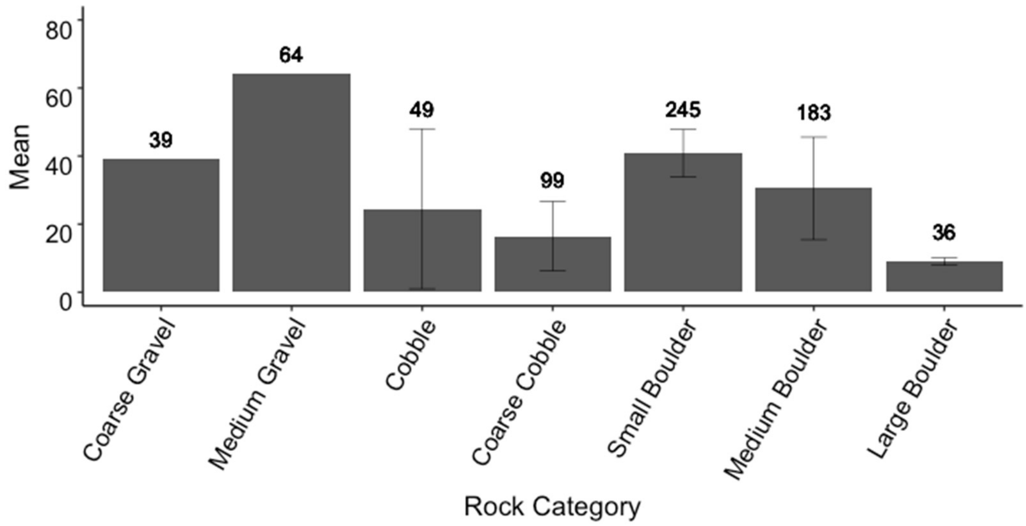

Figure 2. Mean (±SE) number of rocks measured in the representative radial plots in each of the boulder categories across the subset of sampled sites. Numbers above each bar refer to the frequency of rocks counted in each category. Refer to Table 1 for definitions of rock sizes. *Note: There is no SE for coarse and medium gravel as these rock sizes were only counted in the radial plots at Port Macquarie*.

Once a flatworm was seen, it was removed from the substratum with a fine paintbrush and kept in a separate container filled with seawater in a portable cooler for live transport to a fieldwork laboratory. This was necessary to ensure correct identification to species [26]. In the laboratory, animals were fixed with either 10% buffered formalin or Bouin's liquid after a small tissue sample was taken for sequencing, then stored in 70% ethanol. Once back at the University laboratory, specimens were dehydrated in an ethanol series from the original 70% to 90% and finally 100%, cleared in benzyl benzoate, embedded in paraffin wax using a Leica EG1150 H Paraffin Embedding Station, and sagittally sectioned in serial using an American Optical Spencer Rotary Microtome 820 at a thickness between 7 and 10 µm, depending on the size of the individual. Sections were stained with AZAN (trichrome staining method), mounted on glass slides in DPX (Dibutylphthalate Polystyrene Xylene) and observed and photographed under an Olympus BX53 compound microscope for species identification. For details regarding sequencing, see [26].

Statistical Analyses

All statistical analyses were completed in R [39]. Bottle and Glass was sampled at two timepoints (25 October 2019; 21 February 2020). To ascertain if data could be pooled for this site, we assessed differences in abundance between the two sampling timepoints using a generalised linear model (glm) [40]. Because flatworm abundance is count data, the model was fitted with a Poisson distribution. Flatworm abundance was included as the response variable and the sampling timepoint and boulder size included as the explanatory variables with an interaction term between them. There was no significant interaction (p = 0.961), nor was there any significance between the two timepoints (p = 0.722) or boulder size (p = 0.401), thus flatworm counts for the two sampling points at Bottle and Glass were pooled for any further abundance analysis.

To detect any beaches with increased biodiversity, three measures of alpha diversity were obtained: Shannon's H′, species richness and species evenness (Pielou's Eveness/J). Diversity measures for Shannon's H and richness were obtained using *diversity* and *specnumber* functions in the vegan package [41]. Pielou's evenness was obtained by dividing Shannon's H by its log. To explore relationships between alpha diversity (Shannon's H′) and environmental variables we ran simple linear models that included Shannon's H′ as the response variable and mean rock size and beach exposure as explanatory variables. We included an interaction term between rock size and exposure. Where the interaction term was not significant, the main effects in the model were analysed.

To identify any hotspots of occurrence in overall abundance of flatworms, a glm with Poisson distribution was constructed using the lme4 package [40]. Flatworm abundance was included as the response variable with site as the explanatory variable. Pairwise comparisons between sites were run using the *emmeans* function in the emmeans package [42] and the fitted model checked for overdispersion and excess zeros. To establish whether there was a relationship between flatworm abundance and boulder size, a glm with a Poisson distribution was fitted with the number of flatworms as the response variable and boulder size as the explanatory variable. To establish if there was a particular category of boulder that flatworms were more likely to be found on, a second glm was fitted with flatworm number as the response variable and rock size category (according to Table 1) as the explanatory variable. Pairwise comparisons between rock size categories were run using the emmeans function in the emmeans package. The models were checked for overdispersion and excess zeros.

To test whether beach exposure level influenced the distribution of flatworms on rock sizes, a glm with Poisson distribution was constructed with flatworm abundance as the response variable and the main effects of rock size and exposure, with an interaction between rock size and exposure. The model was not over-dispersed, nor did the fitted model have excess zeros compared to the number of zeros in the data. Significance of interactions were obtained from an analysis of deviance table obtained using a Chi-squared test. To interpret the coefficients of the rock size and exposure, we obtained slopes of rock size by exposure and pairwise differences between slopes using the *emtrends* function in the emmeans package. We used the *emmip* function to create the interaction plots of estimated marginal means based on the fitted model described above [42].

3. Results

3.1. Species of Flatworms Identified

We found that flatworms found on temperate boulder beaches inhabit the underside of boulders in all cases. No flatworms occurred on the top or sides of any of the rock size categories that we assessed in this study. A range of species from several families were identified as inhabiting intertidal boulder beaches in southeastern Australia (Table 3).

Species occurrence varied among species, both within and between families. For example, *Echinoplana celerrima* Haswell, 1907, occurred in all sites sampled except San Remo, whereas the closely related *Ceratoplana falconerae* Rodriguez et al., 2021, was extremely rare in our sampling and occurred at only one site. *Notoplana australis* (Schmarda, 1859) rarely occurred at sites but was common at the sites where it did occur (Table 3).

Table 3. Species and number of individuals found in intertidal boulder beaches in southeastern Australia.

Family	**Species**	**Locality**								
		Diggers Camp	**Port Macquarie**	**Forster**	**Chowder Bay**	**Bottle and Glass**	**Boat Harbour**	**Shelly Eden**	**San Remo**	**Phillip Island**
Acotylea										
Gnesiocerotidae	*Echinoplana celerrima* Haswell, 1907	6	10	7	4	9	4	4		4
	Ceratoplana falconerae Rodriguez et al., 2021									1
Notocomplanidae	*Notocomplana distincta* (Prudhoe, 1982)									1
Notoplanidae	*Notoplana australis* (Schmarda, 1859)							17		3
	Notoplana felis (Rodriguez et al., 2021)									1
	Notoplana longiducta Hyman, 1959				1	7				
Pseudostylochidae	*Tripylocelis typica* Haswell, 1907		2	2				2		
Planoceridae	*Planocera edmondsi* Prudhoe, 1982								1	
	Planocera sp.					1				
Stylochidae	*Leptostylochus victoriensis* Beveridge, 2017						2			
	Stylochus sp.				2	4				
Cotylea										
Cestoplanidae	*Cestoplana rubrocincta* (Grube, 1840)					2		2		
Euryleptididae	*Eurylepta* sp.					1				
Prosthiostomidae	*Enchiridium* sp.	1								
Pseudocerotidae	*Pseudoceros* sp.					1				

3.2. Hotspots of Abundance and Biodiversity

Polyclad flatworms occurred in all boulder beaches assessed. Bottle and Glass hosted the highest abundance of flatworms, with significantly higher numbers compared with Boat Harbour (z = 3.803, p = 0.004), Chowder Bay (z = 3.076, p = 0.054), Phillip Island (z = 3.977, p = 0.002), Port Macquarie (z = 3.103, p = 0.049) and San Remo (z = 3.954, p = 0.002). Shelly Beach, Eden, also had a significantly higher abundance of flatworms compared with San Remo (z = 0.342, p = 0.018) (Figure 3).

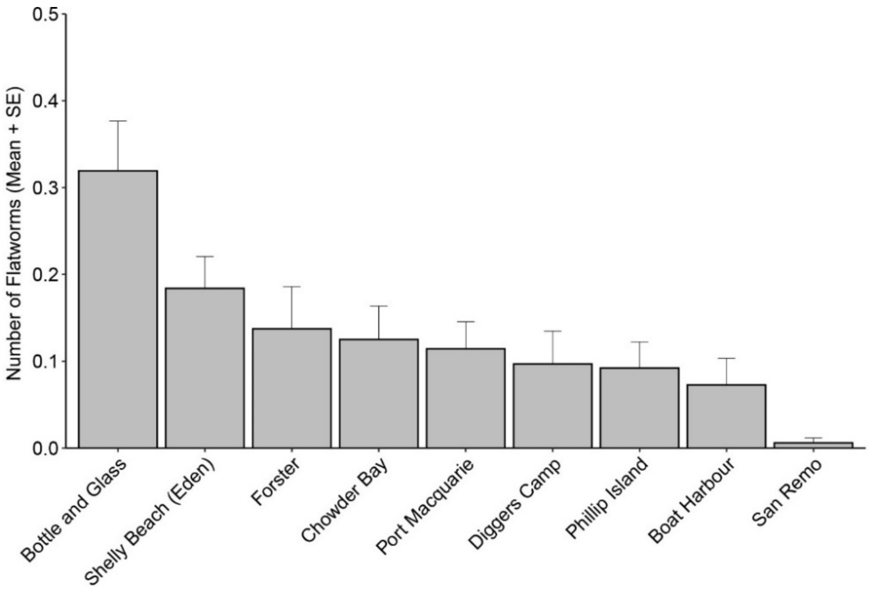

Figure 3. Differences in the average (+SE) abundance of flatworms found at each site sampled along the southeastern coast of Australia.

Patterns of flatworm diversity were similar to those of abundance. Bottle and Glass, Chowder Bay and Phillip Island all scored high across all diversity measures, while Boat Harbour, Diggers Camp, Forster, Macquarie Port, San Remo and Shelly Beach (Eden) presented lower diversity values (Figure 4).

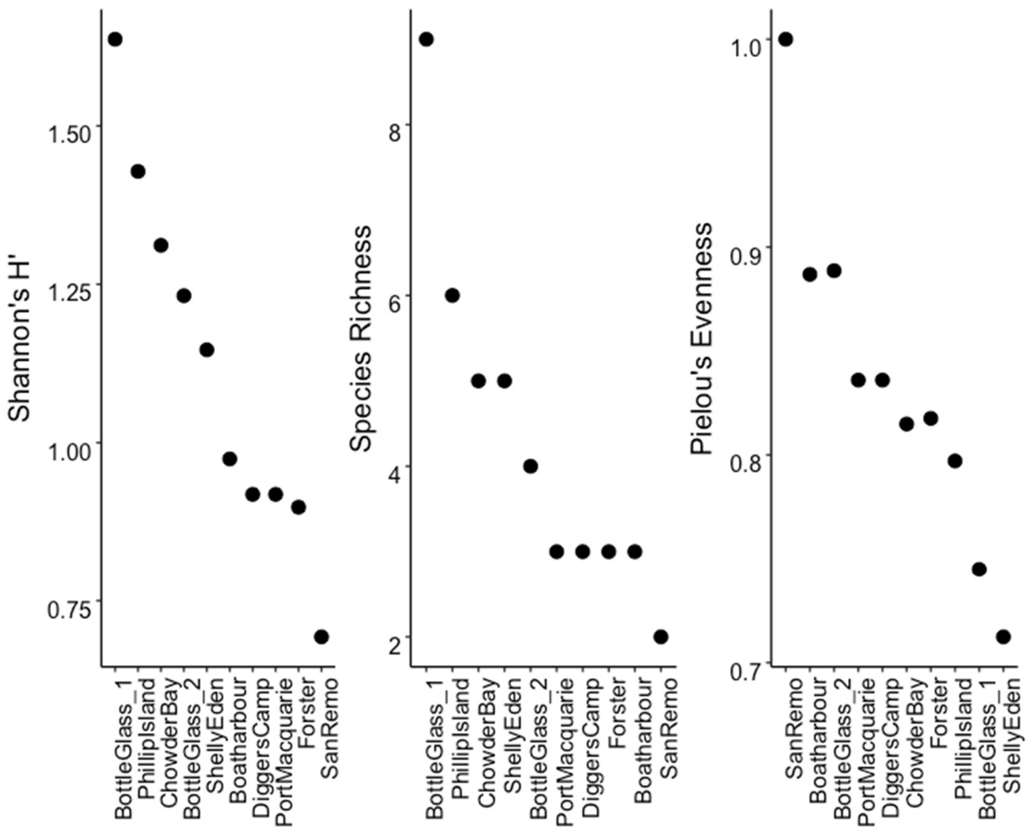

Figure 4. Different diversity indices for flatworms at each site. Bottle and Glass was sampled on two occasions and as such each sampling event has been separated in the figure.

3.3. Influence of Boulder Size and Beach Exposure on Flatworm Diversity

There was no significant interaction between mean rock size and exposure ($F = 3.664$, $p = 0.214$) on the diversity of flatworms. Examination of the main effects also showed no effect of mean rock size ($F = 0.048$, $p = 0.838$) or exposure ($F = 0.551$, $p = 0.711$) on flatworm diversity.

3.4. Relationship between Rock Size and Flatworm Abundance

We found that increasing boulder size had a significant effect on flatworm abundance ($z = 5.543$, $p < 0.001$). Across all sites, the large boulder category had significantly higher abundance of flatworms when compared with the medium boulder ($z = 3.435$, $p = 0.011$), small boulder ($z = 5.869$, $p < 0.001$) and coarse cobble ($z = 4.141$, $p < 0.001$) categories. There was no significant difference in the abundance of flatworms found between the coarse cobble, small boulders or medium boulders (Figure 5).

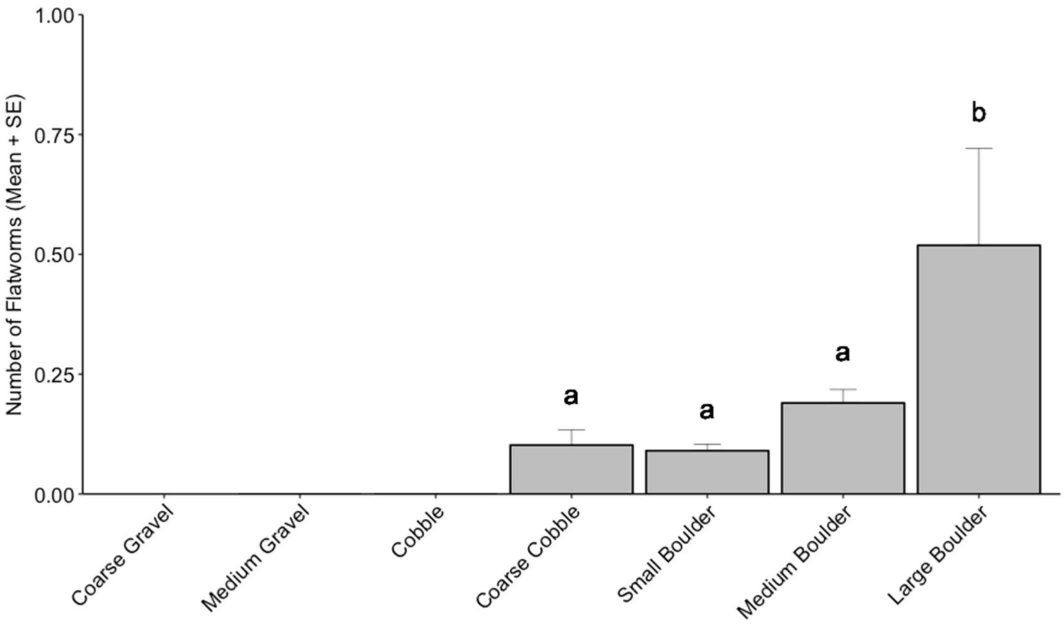

Figure 5. Average (+SE) number of flatworms under each rock size pooled across all boulder beaches. Differences in lower case letters indicate significant difference in flatworm abundance.

Within a beach, flatworm distribution did not mirror the relative distribution of rock sizes. For example, flatworms from Port Macquarie occurred under rock of medium boulder to coarse cobble size classes but not under the broad range of smaller rock sizes. No flatworms occurred under cobble, despite the size class occurring in four of the beaches and being the predominant rock category at Phillip Island and Shelly Beach, Eden.

3.5. Influence of Beach Exposure on Preferred Boulder Size for Flatworms

There was a significant interaction between rock size and exposure ($F = 6.470$, $p = 0.0015$) on the abundance of flatworms. The mean numbers of flatworms increased with boulder size on exposure level 3 when compared with exposure levels 8 and 9. There was no other significant interaction between exposure levels and boulder sizes (Figure 6).

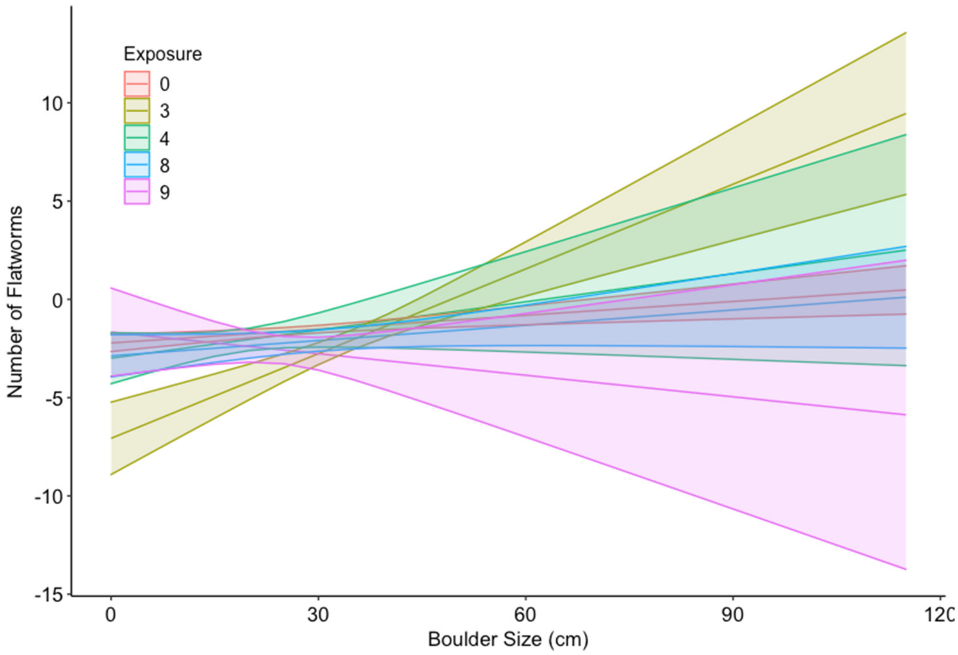

Figure 6. Interaction plot comparing the estimated marginal means of flatworms across boulder sizes and different levels of exposure (0 being a more protected beach and 9 being more exposed). Non-parallel lines indicate an interaction between factors. Ribbons represent 95% confidence interval limits.

4. Discussion

This is the first study to document the occurrence of polyclad flatworm species on intertidal boulder beaches in southeastern Australia, and the first to provide an ecological context for the distribution of polyclad flatworms in such habitats in the southern hemisphere. Fifteen species of flatworms from 10 families were observed on the boulder beaches, which spanned 9 degrees of latitude and 1367 km of coastline. Some species regularly occurred in the sampling, whereas the occurrence of others was rare. A higher number of flatworms occurred under rock sizes of medium boulders to coarse cobble, even though a diversity of other rock size classes occurred on the beaches sampled. We also observed that more flatworms occurred under larger rock size classes at more protected beaches.

Bottle and Glass from Sydney Harbour contained the highest abundance and the most flatworm diversity of all beaches sampled, and thus appears to be a hotspot for polyclad flatworms. Chowder Bay, less than 2 km away from Bottle and Glass in Sydney Harbour, also scored a high diversity measure; however, so did Phillip Island, which is over 1000 km away via the coastline. Sydney Harbour is a known global hotspot for marine and estuarine diversity, with a relative greater number of species and habitats represented than most of the harbours and estuaries in Australia and worldwide [43]. Sydney Harbour is a hotspot for other benthic invertebrate species including molluscs, crustaceans, polychaetes and echinoderms [16]; it is thus not surprising that a higher diversity and abundance of polyclad flatworms is found in these locations. While geographically distant from Sydney Harbour, Phillip Island resides on the edge of Bass Strait, a body of water also known for its unique biodiversity [2]. All three sites are more likely to be affected by anthropogenic perturbations than other sites sampled due to their proximity to major cities, making this diversity an interesting phenomenon leading to speculation as to whether polyclad flatworms in more disturbed areas have an advantage over other species in under-rock assemblages. Unfortunately, we were unable to directly assess this concept in the present study.

The concept of natural disturbance impacts on polyclad flatworms was examined by screening our research for impacts associated with wave exposure and rock size. Flatworms occurred under rock diameters of 6.4 cm or more at all beaches. Larger boulder sizes supported more individuals than smaller rocks, despite the greater abundance of smaller rock sizes compared to larger boulders. Since polyclad species found in the intertidal are usually small (5 to 30 mm body length) and fragile, their more common occurrence in larger boulders and less exposed beaches could be attributed to a need for shelter from high wave action. Larger boulders could also provide better protection against potential predators such as some fish and crustacean species [44,45]. Furthermore, many flatworms feed on sessile organisms [44,45], and it is possible that larger boulders may have greater abundance of their preferred prey. Future studies should investigate if distribution of flatworms is also related to the availability of sessile fauna on boulders. Most polyclad species may also be photophobic and actively hide from the light during the day, which would explain the higher occurrence of flatworms under larger boulders where it is presumably darker. Similar nocturnal behaviours have been reported from other intertidal and shallow subtidal marine invertebrates such as chitons [46], gastropods, sea urchins and sea cucumbers [47,48] that appear cryptic during the day and emerge at night-time to feed.

Our observations do not support predictions of the Intermediate Disturbance Hypothesis (IDH), which states that species diversity should be highest at intermediate levels of disturbance (i.e., intermediate boulder sizes) [49]. While the IDH traditionally refers to sessile organisms, polyclad flatworms are unlikely to move beyond the underside of their rocks in areas of high wave action (Rodriguez, personal observation) and can thus be considered semi-sessile in these situations. According to the IDH, highly disturbed areas, analogous to smaller rock sizes that would be more tossed about on exposed beaches, should support less diversity because organisms do not have the opportunity to successfully colonise in the harsh environment. Similarly, less disturbed areas, analogous to the underside of rocks that do not move, should harbour less diversity due to competitive exclusion by dominant species [50]. However, neither was the case in this study. Polyclad flatworms form only one part of the under-rock species assemblage at these beaches, and it is likely that more complex interactions at play may impact or mask effects of the IDH. More research on the movement of flatworms in relation to wave energy, and the composition of the under-rock communities are needed to tease apart such patterns.

Another obvious hypothesis to explain the absence of pattern associated with the IDH is the low sampling effort in this study. With the exception of Bottle and Glass, which was sampled twice, all sampling consisted of a single survey per site. It is highly possible that our snapshot of diversity was not at an appropriate temporal scale to measure such ecological patterns. We are confident that our biodiversity snapshot is rigorous. Patterns of biodiversity on other intertidal invertebrates have been successfully done along the Australian coastline using similar single standardised time searches as ours [51], and with experienced researchers as with our team. We are not confident that the diversity of flatworms is static over time; however, and that our diversity estimates are comprehensive. It is far more likely that our flatworm diversity estimates grossly underestimate the diversity of flatworms at each beach. It is well known that intertidal species vary in species occurrence and abundance over seasons and years [52]. We therefore propose a future study that assesses the diversity and abundance of flatworms on these boulder beaches at least seasonally over several years to glean an understanding of processes that may affect polyclad flatworm demography.

A trend of increasing abundance of flatworms with increasing latitude was observed in these data (see supplementary Figure S1). However, given that lower latitudes were sampled in summer time and higher latitudes sampled in winter, it is not possible to substantiate this trend until a more comprehensive study over multiple seasons is undertaken. The trends observed at different latitudes may be driven by the different seasons that sampling was undertaken. The eastern coast of Australia is susceptible to East Coast Lows, a dangerous weather system which can bring gales and heavy rain. While these low-pressure systems can occur at any time of the year, they are much more common in Autumn and Winter [53]. Given that storm intensity can influence intertidal communities [54,55], it is possible that flatworms are also affected by winter storms. While higher average population richness at higher latitudes has been observed in other studies [56], our results are biased by the increased presence of 17 *N. australis* in Shelly Beach (Eden). Further research needs to be done to ascertain if there is a trend in flatworm abundance and those of other under-boulder communities at higher latitudes or if there is a seasonal influence.

Echinoplana celerrima was the most common polyclad species found in intertidal boulder beaches on the southeastern Australia coast. This species occurred at all boulder beaches except for San Remo, while other polyclad species occurred in only one to three of each of the nine studied boulder beaches. *Echinoplana celerrima* presents the common acotylean body plan, with a small size (10 to 25 mm long, 5 to 10 mm wide), light brown colouration, eyes arranged in two elongate groups, ruffled pharynx located in the middle of the body and genital systems found in the posterior body third [26]. None of these characteristics indicate at first sight why this particular species is so successful in southeastern Australia boulder beaches compared to other taxa such as *Notocomplana longiducta* Hyman, 1959 or *Notocomplana distincta (*Prudhoe, 1982*)* which present similar anatomical traits and habits. Polyclad flatworms are generally highly selective in prey choice; however, some species exhibit different dietary preferences related to the abundance of suitable prey in a particular locality [57] and others have been reported to feed on a wide variety of invertebrates [58]. On shores where the preferred mussel prey is abundant, the Mediterranean flatworm *Stylochus mediterraneus* Galleni, 1976 feeds almost exclusively on these, while in locations where this primary prey species is rare and the oysters are widespread, flatworms feed on the latter ([57] and references within). It is thus possible that *E. celerrima* is an opportunistic predator and able to feed on a range of prey present, or switch between preferred prey species, compared to the other studied species. Without knowledge of prey preference and feeding habits for *E. celerrima* in relation to the other under-boulder species on the rocks they inhabit, it is difficult to understand any mechanisms underlying the relative importance of ecological processes such as feeding and competition on this group of species.

The only other location where *E. celerrima* has been documented to occur in addition to southeastern Australia is the Mediterranean and Black Sea. It is unclear whether the species occurs in areas between these two regions due to the lack of research targeted at flatworms. As this distance is so great, the most parsimonious explanation of this widespread occurrence is human-induced transportation between the two regions, possibly through ballast water, attached to the hull of a ship or carried with oysters or other animals. If *E. celerrima* is indeed an opportunistic predator with no strict preferred prey, it would explain its ability to settle away from its original habitat. Similar remarks were made for both *E. celerrima* and *Euplana gracilis (*Girard, 1850*)* [59]. Prior to being discovered in Port Phillip Bay (Victoria, Australia) by Prudhoe [59], *Euplana gracilis* was only described for the Atlantic coast of North America. Bennet and Pope [60] regarded the Victorian coast as a cold-temperate region, similar to that of the places where it was first found.

Many aspects of the biology of Australian polyclads remain unknown, hindering our ability to discern the processes driving their distribution patterns on boulder beaches. Characteristics such as the presence of a larval stage during the developing process, dietary habits, dispersion and seasonality could have major impacts on the distribution, richness and abundance of these species. Although the most common mode of development in polyclads is direct development (where the embryo develops directly into a form resembling the young adult), there are many species that develop indirectly through a planktonic phase with transient larval features [61]. Our knowledge on these matters is severely lacking; however, with the mode of development having been described for less than 8% of known polyclad species [61]. All of these characteristics are likely to have an impact on flatworm abundance and distribution, yet the lack of such knowledge hinders our ability to completely analyse these patterns.

While polyclad research has seen a resurgence in interest over the last decade, most studies are taxonomic and systematic in nature, or focus on natural products and other aspects of the flatworm's biology [62,63] and do not include data on the ecology of the investigated species. Future studies in southeastern Australian intertidal boulder beaches should focus on (1) continuing sampling of the boulder beaches to obtain an understanding of the temporal variability of flatworms, (2) assessing sessile fauna alongside flatworms to determine if there are similar distributions, (3) developing culturing techniques for flatworm larvae to close the life history loop for key species, (4) assessing the diets of key polyclad species, (5) gathering genetic data of all sampled species to study population connectivity at the intraspecific level, and (6) understanding the impacts of anthropogenic disturbances on under-boulder community diversity and abundance, including polyclad flatworms. Such information will create a strong baseline of information on polyclad flatworms and their communities, which can help inform conservation and management efforts of our coastal marine environments and contribute to our knowledge of Australia's biodiversity.

5. Conclusions

In this study, the abundance and diversity of polyclad flatworms across southeastern Australia is investigated. It is the first study to record the occurrence as well as the ecological context of flatworm species on intertidal beaches in this region. We identified 15 species of flatworms from 10 families on intertidal boulder beaches with hotspots of abundance and diversity at those sites most likely to be influenced by anthropogenic disturbance. There was higher abundance of flatworms on larger boulders at more protected beaches, which is possibly attributed to a need for shelter from high wave action, predation and daytime light. This study lacks a high degree of sampling effort over multiple time scales and future studies that assess abundance and diversity of flatworms on these beaches will obtain insights into processes driving their occurrence. Future directions for studies in southeastern Australian boulder beaches are provided so that a baseline of information on flatworms and their communities can be documented. This study is an important contribution to the knowledge of Australia's coastal marine systems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d15030393/s1, Figure S1: Mean flatworms (estimated marginal means) across differing latitudes and boulder sizes.

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