A review of the known distribution of Halophila spinulosa in Indonesia with herbarium from Laikang in South Sulawesi

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Abstract. Seagrasses are true plants living in the marine realm. The Coral Triangle is also the centre of seagrass diversity with 14 species recorded in Indonesian waters. There is a growing awareness of the ecological and socio-economic importance of seagrass meadows as well as the growing threats to these valuable coastal ecosystems. However, there is a lack of accessible data on seagrass distribution at ecosystem and species scales. The most speciose seagrass genus is Halophila, with at least 7 species (Halophila beccarii, H. decipiens, H. major, H. minor, H. ovalis, H. sulawesii and H. spinulosa) known to occur in Indonesia. Reported from Malaysia to eastern Australia, Halophila spinulosa is a data deficient tropical seagrass. We review the known distribution of H. spinulosa based on a search of Indonesian and international literature. We also present an annotated herbarium of H. spinulosa samples collected from Laikang in South Sulawesi, Indonesia. Samples were collected in September 2020 at low tide at depths of around 0.75 to 1.25 m on a silty sand substrate along with samples of other seagrass species present. Herbarium specimens were cleaned of epiphytes and sand. The samples were laid out carefully on A4 paper, arranged so that all parts were clearly visible, covered with gauze, and placed on folded newspaper. The prepared samples were then sandwiched between piles of newspaper and placed in a wooden press. The herbarium specimens will be preserved in the Marine Biology Laboratory collection, Marine Science Department, Universitas Hasanuddin. We consider the distribution data for H. spinulosa are far from complete; this review and herbarium should inform and spur further research on this species and its distribution at macro (site) and micro (within site) scales.

1. Introduction
Indonesia is recognised as a “megabiodiversity” country, comprising the greater part of the Indo-Malay or Coral Triangle marine biodiversity hotspot [1,2]. Indonesian seagrass ecosystems are important in terms of their ecosystem services and roles in the wider marine and coastal ecosystems [3,4]. Seagrasses themselves, true plants fully adapted to life in the sea [5], are also important from a biodiversity viewpoint [4,6]. There are around 72 currently recognised seagrass species [5], of which 16 species are reported from Indonesia [4]. These include 7 species of the genus Halophila, a genus currently undergoing taxonomic revision: Halophila beccarii, H. decipiens, H. major, H. minor, H. ovalis, H. sulawesii (so far only known from Spermonde Islands [7]) and H. spinulosa; while the other nine species reported are Enhalus acoroides, Thalassia hemprichii, Cymodocea rotundata, C. serrulata, Halodule pinifolia, H. uninervis, Syringodium isoetifolium, Thallasodendron ciliatum and Ruppia maritima [4].
Species of the genus *Halophila* are widely reported as important grazing species for threatened marine mega-herbivores, the dugong *Dugong dugon* and the green turtle *Chelonia mydas* [8–11]. Dugongs around Sulawesi are considered at high risk of extirpation [12]; thus species providing quality grazing for dugongs are of particular importance in the context of marine mammal conservation in this region. Within the specieose seagrass genus *Halophila*, *H. spinulosa* is one of the less well researched species, and has been classified as Data Deficient under IUCN Red List criteria [5]. There are conflicting reports regarding the importance of *H. spinulosa* for dugongs. While some studies report the genus *Halophila* and/or *H. spinulosa* in particular as a preferred species [8,13], others report that dugongs seem to have a negative preference for the genus or species, preferring other seagrass species available in their grazing habitat [10,14]. These differences are an intriguing puzzle, and may be related to individual or learned/socially transmitted preference. However, the fact remains that data and information on the distribution and ecology of *H. spinulosa* are limited.

Takalar is one district in South Sulawesi Province with extensive seagrass beds, including those in the coastal waters of Laikang Village. The purpose of this study was to describe a population of *Halophila spinulosa* in these waters including the associated seagrass species and the preparation of a reference herbarium of *H. spinulosa* from Laikang as well as to review data on *Halophila spinulosa* distribution and association with other seagrasses in Indonesia and neighbouring countries.

2. Materials and Methods

2.1. Sampling site, sample and primary data collection
The sampling site in Laikang, Takalar District, South Sulawesi Province, Indonesia is shown in Fig. 1. The geographic coordinates (S 05.59266, E 110.48483) were recorded at the landward low water line, along a transect line perpendicular to the shore which bisected the area to seaward in which *H. spinulosa* was observed. A Garmin GPSmap 62s unit was used, set to WGS 84 datum.

![Figure 1. Map of Takalar District in South Sulawesi, Indonesia showing the Laikang sampling site](image)
The first field observations were made on 24th December 2018 and a visual record was made using a digital underwater camera (Nikon Coolpix AW120). It was noted that the seagrass *H. spinulosa* occurred in an apparently quite limited area around a transect from a marker on the shore and a pole set in the sea. Further observations were made on 16th September 2020 at the same site, determined using this transect. The substrate type was noted, but a visual record was not possible because of the high turbidity due to the prevailing conditions. Wave height was around 50 cm to 1m with breakers along the shore, resulting in the suspension of the fine particles from the silty sand substrate. Extent of the area covered by *H. spinulosa* was made through direct sampling using a mask and snorkel, by feel and taking samples of the vegetation. Samples were collected at low tide at depths around 0.75-1.25 m, taking care to collect the whole plant including rhizomes and roots. Other seagrass species present were identified in the field where possible and samples of some other seagrass species present were also collected.

### 2.2. Herbarium method

Herbarium preparation begins with proper collection. This section describes the protocols used in this study and provides a guide for the preparation of a herbarium specimen. The protocols were developed and have been extensively used by the first author and are presented in illustrated form as Fig. 2.

#### A. Marine Plant Collection
- It is important to collect the entire plant
- For seagrasses, this includes leaves, rhizome, and roots in all seasons, as in this study
- Flowers and fruits should be collected when sampling during the flowering/frUITing season
- For algae, the thallus and holdfast and any other features present (e.g. floats/bladders and/or reproductive organs)
- Ideally, associated plants should be collected for ecological context
- Transport (short distance): keep cool, moist and avoid direct sunlight
- Spread the specimen out nicely and photograph it while it is still fresh, using a plain background

#### B. Preparation
- Clean off epiphytes and sand with a brush and clean water
- Wash repeatedly
- Lay out the specimen on a sheet of white herbarium paper (blotting paper is ideal if available)
- Place on a wad of newspaper
- Cover with a layer of gauze

#### C. Pressing
- Place on the base of a wooden press
- Sandwich the herbarium paper/specimen in newspaper
- Tie the frame firmly to press the herbarium and then place in sunlight to dry
- For the first 2 days, the damp newspaper should be changed daily with a new dry one
- The tied frame can then be left until the plant material is well desiccated and firmly attached to the herbarium paper

**Figure 2.** Method used in preparing the herbarium specimens of *Halophila spinulosa* and other seagrasses collected from the Laikang site
2.3. Secondary data collection and analysis
Secondary data were collected through a desk study of scientific literature and reports available online. There is little doubt that more data are available in unpublished and inaccessible formats; however, a search for such data was not part of this study. The data obtained were tabulated and analysed descriptively together with the primary data.

3. Results and Discussion

3.1. Halophila spinulosa in Laikang
The site at which Halophila spinulosa was observed faces the open sea and can be considered exposed to water movement, especially wave action. The silty sand nature of the substrate means that even quite small waves can result in a significant decrease in underwater visibility due to suspended fine sediment particles. Despite this limitation, field observations by the first author in 2018 yielded documentary evidence of the presence of H. spinulosa as well as the seagrass and macroalgal community with which this species was associated (Fig. 3). Species seen on this occasion included stands of Enhalus acoroides, forming a relatively sparse upper canopy, with an understory of smaller species, including some quite dense patches of H. spinulosa. These included Syringodium isoetifolium and Halophila ovalis, as well as another Halophila species with more elongated leaves. This last species (possibly H. decipiens) is currently being identified. In addition to the morphological characters of a sample collected and preserved, we plan to use DNA sequence data, such as that used to verify the occurrence of H. major in Indonesia [15] or the biogeography of Enhalus acoroides [16].

Figure 3. Halophila spinulosa and associated vegetation at the Laikang site

Based on the September 2020 observations, the H. spinulosa patch began around 10 m from the low tide line, with around 10-15 m of other small seagrasses exposed on the substrate; the shoreward
edge of the patch was therefore around 20 m from the shoreward edge of the seagrass meadow. *H. spinulosa* was found for around 50 m to seawards of this line, and extended around 20-30 m parallel to the shore in each direction from the transect line noted in 2018. This indicates that the patch is limited in extent but appears to be stable in time. There evidence from 2007 for several apparently small dispersed patches of *H. spinulosa* approximately 1-2 km to the east along the Takalar coast and around the Punondo headland, at the entrance to Laikang Bay [17,18]. This patchy pattern of occurrence together with the limited sampling of seagrass meadows in this area makes it seem likely that other *H spinulosa* patches will be discovered in the future, both nearby and in other areas around Sulawesi and other Indonesian islands, and calls for an increase in fine-scale sampling effort.

3.2. *Herbarium process and results*

The specimens collected were prepared according to the method described above. Herbarium curation is vital to make the preserved specimens available over the long term to those interested in or needing such material. This study is intended as part of an effort to establish a well-maintained and accessible reference collection for marine plants within the Marine Biology Laboratory collection of the Marine Science Department, Faculty of Marine Science and Fisheries at Universitas Hasanuddin. A selection of the herbarium specimens are shown in Figure 4. The marine herbarium collection will serve as a resource for education and outreach as well as a reference collection for research.

![Figure 4. Herbarium of *Halophila spinulosa* from Laikang](image)

3.3. *Distribution and association of H. spinulosa with other seagrasses*

The distribution of *Halophila spinulosa* is quite extensive but appears to be patchy at site scale (as noted above) and at wider geographical scales. Many papers reporting on seagrass surveys in
Indonesia list *H. spinulosa* in the introduction, as one of the species (typically 12-14 are listed) occurring in Indonesia; however, very few report *H. spinulosa* among the species found at the sites surveyed. An extensive survey of the published literature only found five reports confirming the identification of *H. spinulosa* at specific sites in Indonesia (Table 1), far fewer than the number of reports from surrounding countries, of which a selection is also shown in Table 1.

| No | Location | Associated seagrasses | References/notes |
|----|----------|-----------------------|-----------------|
| 1  | Laikang  | *Enhalus acoroides*, *Halophila ovalis*, *(Halophila sp. possibly *H. decipiens*), *Syringodium isoetifolium* | This study |
| 2  | Putondo  | *Cymodocea serrulata*, *C. rotundata*, *E. acoroides*, *Thalassia hemprichii*, *Halodule uninervis*, *Halophila ovata*, *H. ovalis*, *Syringodium isoetifolium* | [17] (1 spot) [18] (occasional occurrences) |
| 3  | Lombok Timur | Not clear, several | [19] (1 of 3 sites) |
| 4  | Riau Islands | Not clear, several | [20] |
| 5  | Wakatobi | Not clear, several | [21] |
| 6  | Singapore | *E. acoroides*, *H. ovalis*, *H. minor*, *H. decipiens*, *S. isoetifolium*, *Halodule pinifolia*, *H. uninervis*, *Cymodocea rotundata*, *C. serrulata* | [22] |
| 7  | Hervey Bay, Queensland, Australia | *H. ovalis*, *Halodule uninervis*, *Halodule pinifolia*, *Zostera capricorni* | [8] |
| 8  | Orman Reef area of Torres Strait | *T. hemprichii*, *Cymodocea sp.*, *S. isoetifolium*, *H. ovalis*, *H. uninervis*, *E. acoroides and Thallasodendron ciliatum* | [10,11] |
| 9  | Green Island, Australia | *Halophila capricorni*, *Halophila decipiens* | [23] |
| 10 | Moreton Bay, Australia | *H. ovalis*, *H. decipiens*, *Halodule uninervis*. *Zostera capricorni*, *Cymodocea serrulata* | [11,24] |
| 11 | Groote Eylandt, northern Australia | *Cymodocea serrulata*, *Syringodium isoetifolium*, *Thalassia hemprichii*, *Halophila ovalis*, *Halodule uninervis* | [25] |
| 12 | Johore Malaysia (3 sites) | *Halodule uninervis*, *Halophila ovalis* | [26] |

The known *H. spinulosa* sites in Indonesia extend from Riau in the Sunda Shelf region to the west [20] to Lombok and Wakatobi in the Wallacea region of eastern Indonesia [19,21]. These sites are all in the southern hemisphere, as are the reports from other countries such as Singapore and Malaysia, with the majority of reports from Australia. The occurrence of *H. spinulosa* in the Torres Strait region, where Papua New Guinea faces Australia [10,11], raises the possibility that the Indonesian distribution of this seagrass could extend further east beyond Lombok and Wakatobi across the islands in the Banda and Timor seas, towards Papua.

Surveys further north in Indonesia (e.g. [27,28]) do not list *H. spinulosa*, although most (with the notable exception of [28]) list at least one species of the genus *Halophila*. Indeed, there appear to be no records of *H. spinulosa* from Manado and North Sulawesi Province more generally, despite the fact that seagrass observation effort can be assumed to have been relatively high compared to many regions of Indonesia due to the presence of Universitas Sam Ratulangi (with a strong record in marine science) as well as the frequent visits by scientists from many countries to that area, both before and
after the colonial era. Although it is possible that this apparent southern hemisphere distribution may be an artefact of insufficient sampling (survey) effort, it seems likely that the core distribution of *H. spinulosa* is in Australia, with a patchy distribution of possibly isolated populations, typically limited in extent (but potentially dense) further north in Southeast Asia, close to the equator.

In terms of associated seagrass species, despite the relatively frequent reporting of the large-leaved seagrass *Enhalus acoroides*, it would seem that *H. spinulosa* is typically associated with other smaller species (Table 1). In Australia these include seagrasses of the sub-tropical to temperate genus *Zostera* [8,11,14]. The distribution of *H. spinulosa* as far south as Moreton Bay [11,24] indicates that this may be a sub-tropical to tropical rather than a truly tropical/equatorial species, possibly preferring waters somewhat cooler than are common in the equatorial region. Such a preference could account for the lack of sightings during seagrass surveys along the coasts of the equatorial Central Sulawesi Province involving the second author (2001-2015) as well as further north.

Such a preference could also explain the presence of *H. spinulosa* in Takalar, in an area known for upwellings and relatively lower seawater temperatures compared to many surrounding areas, including the Spermonde islands. It is worth noting that the species was not found at 19 of the 20 sites in South Sulawesi surveyed by [17], and was not recorded in a study on the stomach contents of a dead dugong stranded in the Spermonde Archipelago [29], or in any other reports from extensive seagrass survey efforts in the Spermonde (e.g. [30–32]). This lack of records indicates that *H. spinulosa* may not be found in these islands. This is despite the geographical position of the Spermonde Archipelago between confirmed sites in Singapore [22], Riau [20] and peninsular Malaysia [26] to the west and in Takalar (this study, [17,18]) to the east. Further sampling effort specifically targeting seagrass meadows in suspected areas (e.g. between known distribution points) and in habitat suspected to be suitable (e.g. near upwellings) might provide a more complete picture of *H. spinulosa* distribution and abundance. With respect to the mechanisms by which the observed distribution occurred, multidisciplinary biogeographical research such as that advocated by [33] may be the answer. For example, phylogenetic studies using molecular biology (DNA-based) methods within the context of plate tectonics and oceanographic conditions past and present.

4. Conclusion

We consider the distribution data for *H. spinulosa* to be far from complete. It is our intention that this review and herbarium should inform and spur further research on this species and its distribution at macro (site) and micro (within site) scales. The herbarium specimens will be preserved in the Marine Biology Laboratory collection, Marine Science Department, Universitas Hasanuddin as a resource for education and research. We hope that the methods and concepts presented in this paper will spur researchers and citizen scientists, including students and nature lovers of all ages, to observe, record and preserve the marine flora of the Coral Triangle, and specifically the seagrass meadows of Indonesia. In particular we recommend a systematic search for “off-line” records and curating them in such a way as to make them available and avoid their loss. Data on plant communities will help to guard against the “shifting baselines” syndrome, while herbarium specimens can serve as reference material for classical and molecular taxonomy.

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