

# Seasonal biomass and alginate stock assessment of three abundant genera of brown macroalgae

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## Seasonal biomass and alginate stock assessment of three abundant genera of brown macroalgae using multispectral high resolution satellite remote sensing: A case study at Ekas Bay (Lombok, Indonesia)



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## ABSTRACT

The potential of Indonesian bays as alginate producers was assessed by determining the stock of wild brown algae and exploring their biomass as alginophytes at the scale of entire bay, using a combination of field observations, remote sensing high resolution data and GIS tools. Ekas Bay in Lombok Island presented a stock of brown macroalgae which varied with season and species: for *Padina* the biomass reached  $97.85 \pm 12.63$  and  $79.54 \pm 2.53$  tons in May/June and November respectively; for Sargassaceae species, it reached  $669.70 \pm 109.64$  and  $147.70 \pm 77.97$  tons in May/June and November respectively. The best alginate yields occurred during the May/June period: *Padina* could produce  $9.10 \pm 0.06$  tons DW of alginates. Interestingly, *Sargassum/Turbinaria* together allow  $207.61 \pm 0.42$  tons DW of alginates. This study suggests that wild Sargassaceae represent an interesting stock in terms of biomass, alginate yield and M/G ratio.

## 1. Introduction

Alginate is the major polysaccharide that structure brown algal cell walls; it consisted of mannuronate (M) and guluronate (G) acids with different ratios of M/G depending on the type of species, age, and location (Draget et al., 2005; Zubia et al., 2008; reviewed by Stiger-Pouvreau et al., 2016). Alginate makes a valuable resource and the global market has reached > 20,000 tons/year (Mc Hugh, 2003; Bixler and Porse, 2011; Rebours et al., 2014; Hafting et al., 2015; Buschmann et al., 2017). Alginate is mostly used as thickening, gelling and stabilizing agents in food, cosmeceutical and pharmaceutical industries (Milani and Maleki, 2012). *Macrocystis pyrifera* (Linnaeus) C. Agardh, *Ascophyllum nodosum* (Linnaeus) Le Jolis, and *Saccharina/Laminaria* spp. are the main sources of alginate from temperate regions, whereas in tropical regions, the genera *Sargassum*, *Turbinaria*, and *Padina* are mainly used as major alginate sources (Critchley and Ohno, 1998; Zemke-White and Ohno, 1999), including from China, Philippines, India and Vietnam. Indonesia is also producing alginate but not as successfully as others phyco colloids produced by red macroalgae, agar

and carrageenan, which are greatly produced all around the world by agarophytes (*Gelidium*, *Gracilaria*, ...) and carrageenophytes (*Kappaphycus/Eucheuma*, *Chondrus*, ...) in temperate and tropical areas. In fact, alginate production in Indonesia has decreased over past decades for the benefice of the production of phyco colloids from red seaweeds, making this country the leading producer of carrageenan (Cai et al., 2013; Hurtado et al., 2015). However, Indonesia production of carrageenan remains fragile, especially with the emergence of illnesses, like the ice-ice disease which decreases yield and quality of carrageenan (Solis et al., 2010).

To maintain its worldwide leadership in the production of phyco colloids, Indonesia began a diversification program concerning phyco colloids sources and turn towards the exploitation of brown seaweeds to increase alginate productions (MMAF, 2015). It is believed that alginophytes production in Indonesia remain low due to a lack of information about their types and components (Mushollaeni, 2011). To generate and to develop alginate production from brown seaweeds, it is important to quantify the alginate yield and quality together with the quantity of algae available in Indonesia. Alginate content and quality in

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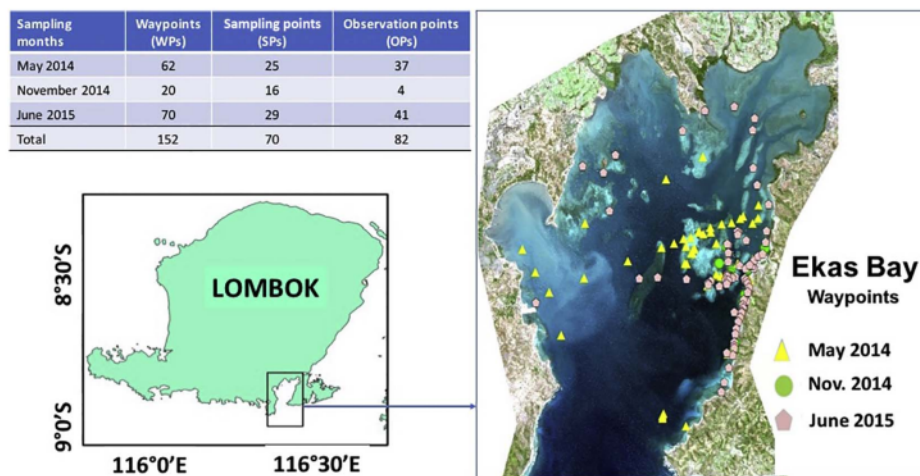


Fig. 1. Localization of the Ekas Bay in the South of Lombok Island (Indonesia) (A) and Map of the study area with waypoints (WP) representing the sampling design carried out at three field periods during a one year and a half monitoring (B). Each geometrical form represents an observation of a population of brown algae. The table presents a summary of all waypoints in Ekas Bay, dividing in sampling points (with quadrats) and observation points.

brown algae highly depend on species, environmental conditions, season of harvest, and also on the method of extraction used (Mirshafiey and Rehm, 2009). The genera *Sargassum*, *Turbinaria*, and *Padina* had been considered to have a great potential for the Indonesia alginate production (Rasyid, 2003), but they have not yet been optimally exploited (Subaryono, 2011). It is then a priority to explore the potential offered by Indonesia coastlines in term of brown macroalgae distribution, biomass and production of high yields of alginate suitable for market's demand. However, the presence and availability of the three genera *Sargassum*, *Turbinaria*, and *Padina* in natural populations remains poorly known. These macroalgae can also experience seasonal blooming, as it was highlighted in several regions all around the world (Stiger and Payri, 1999; Ateweberhan et al., 2009). Therefore, seasonal monitoring of natural populations would be crucial for an accurate estimation of brown algae stocks to eventually foster development of an alginophyte industry in Indonesia.

In this context, remote sensing and mapping can be helpful. They have been used in several geographic and spatial contexts for seaweed exploitation development and management, including for farms site selection, planning and zonation mapping, environmental impact assessment, inventory and monitoring, including in Indonesia (Rossi et al., 2010; Radiarta et al., 2011; Oppelt, 2012; Setyawidati et al., 2017a, 2017b). Hu et al. (2015) also demonstrated the feasibility of sensor satellite capture for the monitoring of spatio-temporal distribution of drifting pelagic macroalgae. Of interest here, multispectral sensors were already used in mapping benthic macroalgae and to estimate their biomass (Andréfouët et al., 2003, 2004, 2017; Mattio et al., 2008a, 2008b; Noiraksar et al., 2014; Hoang et al., 2015; Zubia et al., 2015; Tin et al., 2016; Setyawidati et al., 2017a, 2017b).

The present study focused on the stock assessment of brown algae using satellite remote sensing, to explore their biomass and give to Indonesian managers the potential of an area in term of dry material and yield of alginate production. For this demonstration, we choose a bay on the island of Lombok and monitored natural populations of brown algae during two different seasons, i.e. dry and wet seasons. In parallel, we extracted alginates from each species of brown algae to give information about the seasonality of potential alginate yields at the scale of the bay.

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## 2. Materials and methods

### 2.1. Study area and environmental data

Ekas Bay is situated in the southeast corner of Lombok Island, Indonesia (8°53'00"S, and 116°27'00"E). It is directly facing the Indian Ocean. It is the largest bay in Lombok covering > 50 km<sup>2</sup> and with an intertidal zone reaching > 20 km<sup>2</sup> (Fig. 1). Ekas Bay is characterized by two different zones distinguished according to seawater depth and substrates. One zone covers the intertidal shoreline coastal areas, i.e. the zone which is above water at low tide and under water at high tide (0 up to 5 m water depth), and the other zone covers the always submerged deeper lagoon. Environmental conditions of Ekas Bay, i.e. water temperature, salinity, velocity, wind together with tide range, were monitored at each sampling period.

### 2.2. Field observations and sampling for the phenological monitoring of populations

Field observations and sampling were carried out in May and November 2014 and in June 2015 to follow broadly the monsoon regime pattern. May 2014 and June 2015 represented the southeast monsoon which is a fresh and dry season known for macroalgal bloom in Indonesia. Conversely, November 2014 represented the northwest monsoon which is a hot and wet season generally not favorable for the growth of brown algae. These three time-periods allow monitoring variations across three successive seasons.

Field observations consisted in recording presence of algae and density (visual assessment only) for a variety of locations within the intertidal zone of the bay chosen according to the interviews of local inhabitants who pointed out to usual locations of algae presence (Fig. 1). The location of algae in the Ekas Bay were then localized and their actual locations were recorded (waypoint, hereafter WP) using a GPS Garmin GPSMAP 78 s data logger (Fig. 1). The field survey was conducted during the lowest tide when seaweeds were exposed. At each WP with sufficient macroalgae abundance, biological variables were determined in each population (see further sections). Each specimen of

brown macroalgae was determined at the species level using morphological criteria (Stiger-Pouvreau, pers. observ.), determination keys and local flora guidebooks (Rohfritsch et al., 2007; Mattio et al., 2008a, 2008b, 2009; Mattio and Payri, 2011; Atmadja and Prud'homme van Reine, 2012; Guiry and Guiry, 2016), but also using NMR analysis as already described for the genus *Turbinaria* (Le Lann et al., 2008, 2014), *Cystoseira* (Jégou et al., 2010) and *Sargassum* (Tanniou et al., 2015).

The number of WPs varied according to the presence of abundant brown macroalgae all along the study period. A total of 62, 20 and 70 WPs were recorded on May 2014, November 2014 and June 2015 respectively, representing a total of 152 points (Fig. 1). Among these 152 WPs, only 70 were used for the phenological monitoring because of the presence of enough thalli for the measurement of biological variables. We refer to them as sampling points (SPs). They were spread as 25, 16 and 29 SPs on May 2014, November 2014 and June 2015 respectively (see Fig. 1 with a summary of each type of points monitored during the field survey). The remaining 82 points presented not enough algae to be sampled; then we just checked the presence/absence of brown macroalgae and noted the type of substratum for each alga. These 82 points were called observation points (OPs). All the 152 WPs were also used for the mapping of brown algae within Ekas Bay (see next section).

The phenological monitoring consisted in the sampling of triplicates of 0.25 m<sup>2</sup> quadrats at each of the 70 SPs, leading to sample a total of  $n = 210$  quadrats. Quadrats were haphazardly placed in the structured populations of algae following the methodology described in several studies of population biology of marine macrophytes (Rogers et al., 1994; Mumby et al., 1997; Stiger and Payri, 1999; Plouguerné et al., 2006; Le Lann et al., 2012). Within each quadrat were recorded for the most abundant species: the percentage cover, the density (number of thalli per m<sup>2</sup>), the length of thalli together with the total biomass in fresh (FW) and dry (DW) weights.

### 2.3. Field observations for mapping algal biomass

For the mapping of algae populations, we considered only the genus level with *Padina*, *Sargassum* and pooled *Turbinaria* observations. The mean percentage cover of the three brown genera was used to determine the potential total area colonized by the three genera of brown seaweeds at Ekas Bay.

At each waypoint (70 SPs + 82 OPs), the substrate considered as a favorable habitat for living brown macroalgae was also recorded and photographed. Five categories of substrates associated to the brown macroalgal habitat were identified: 1) Sand (S); 2) Muddy Sand (MS); 3) Heterogeneous on soft (HS); 4) Hard bottom (HB) and 5) Living coral (LC).

Heterogeneous on soft corresponds to any combination of sand, rubble coral, and submerged vegetation that covers 10–100% of any substrate, while hard bottom represents hard substrate composed of exposed bedrock, hard or organic banks (Setyawidati, 2017).

### 2.4. Satellite data and analysis

A Worldview-2 (WV-2) satellite image acquired on 13 August 2014 (dry season) was used for the mapping. The WorldView-2 sensor provides multispectral imagery with eight bands centered at 427, 478, 546, 608, 659, 724, 833 and 947 nm thus providing higher spectral resolution than other common multispectral imagers. Image preprocessing consisted in pan sharpening operation to guide field data collection, including the choice of ground-control points to test the accuracy of the map (Green et al., 2000). Areas with inaccurate results were edited based on in-situ observations to produce a final map corrected for errors.

The image was acquired at a time different than all field surveys, hence the applied strategy was to map the dominant substrates observed in situ and assign to each substrate an average cover based on all the three study periods. A supervised Maximum Likelihood

Classification performed with the ER Mapper software was performed for the 5 identified substrates of the intertidal zone. Training data were the 82 WPs not used for phenology. The 70 points used for phenology were used for accuracy assessment, to test if the correct substrates were identified by the mapping process.

To create surface grid for cover and non-cover macroalgal potential areas, SPs macroalgal percentage cover from field observations (Fig. 1) were interpolated within polygons for the 5 substrates categories, and for each species and seasons. The Kriging method provided within the Spatial Analyst ESRI toolbox was used (Childs, 2004). This procedure permitted then to cartography, the populations of brown macroalgae in relation with their preferred substrate at the different seasons.

### 2.5. Alginate extraction and measurement of M/G ratio of alginate

Three genera of macroalgae, i.e. *Padina*, *Sargassum*, *Turbinaria*, and four species were selected from our pre-sampling period due to their abundance. After the measurement of biological variables, thalli of about 5 cm to 50 cm, from the holdfast to the top of the individual, were sampled within SPs quadrats, cut and put in plastic bags. All the collected thalli were washed with sterile seawater to remove epiphytes or impurities, and wringed. Later, they were weighted, sun-dried, blended into fine powder, then stored in an aerated bag and kept in shaded and ventilated site prior to the extraction.

#### 2.5.1. Extraction of alginate

All chemicals used for the alginate extraction process were obtained from Sigma-Aldrich (Germany). Alginate extraction with alkaline protocol was a laboratory adaptation of the industrial process. It was conducted in several steps as follows, using triplicates of extraction: 10 g of algal powder were rinsed and immersed in a 150 mL HCl solution (0.01 N) and 2% of CaCl<sub>2</sub>, subsequently at room temperature and at 70 °C for two times each. The liquid supernatants as soluble fractions were centrifuged, filtered and collected for evaporation process (Vol/30). The dialysis (48 h in – 20 °C) was one subsequent process prior to freeze drying and yield analysis. Alginate yield was expressed for each species and each genus of brown macroalgae as a percentage of dry weight (% DW).

#### 2.5.2. Estimation of the M/G ratio of alginate

The estimation of mannuronic acid/guluronic acid ratio (M/G ratio) of alginate extracted from the three brown macroalgae collected at Ekas Bay was obtained with Fourier transformed Infrared spectroscopy (FTIR), as described in previous works (Torres et al., 2007; Lean et al., 2008; Zubia et al., 2008 and references cited; Fenoradosoa et al., 2010). Alginate powder obtained from entire thalli of brown species collected at Ekas Bay was recorded using a Thermo Scientific Nicolet iS 5 iD 7 ATR accessory (a monolith diamond ATR type) in the 4000–400 cm<sup>-1</sup> region. Spectra from FTIR were then analyzed for the M/G ratio. The ratio of absorption band intensities appearing at 1290 and 1320 cm<sup>-1</sup>, 808 and 787 cm<sup>-1</sup> and 1030 and 1080 cm<sup>-1</sup> in the IR spectra (as used in Lean et al., 2008 and Setyawidati, 2017), gives a fairly good estimation of the M/G ratio of alginate extracts based on the intensity band from those absorptions. We then estimated the M/G ratios using the three different methods described in Setyawidati (2017) to qualify each alginophyte species in regard of their alginate and to estimate the property of alginate to create gel (by the estimation of M/G ratio).

### 2.6. Assessment of macroalgal biomass and alginate production at the scale of the bay

Quadrat field data provided for each brown seaweed species the average of dry weight (referred as DW<sub>T,S</sub>) in gram per meter square (g m<sup>-2</sup>) and the percentage cover referred as %C<sub>T,S</sub>, as they are estimated for different sampling time *T* and for each type of substratum *S*. The map provided the total area for each substrate (A<sub>s</sub>). The estimated

**Table 1**

Temporal variation of biological variables determined in triplicates of quadrats (Means ± SE) and monitored along three periods of observation on populations of dominant brown macroalgal species in Ekas Bay (Lombok, Indonesia): thallus density, mean thallus length and mean estimate of the thallus biomasses in dry (DW) and fresh (FW) weights per m<sup>2</sup>. During the monitoring of brown macroalgae, 3 quadrats were sampled per population for each species and at each sampling period (May 2014, November 2014 and June 2015).

Taxa	Season and month of observation	Percentage cover (%)	Length of thalli (cm)	Density (Ind/m <sup>2</sup> )	DW (g/m <sup>2</sup> )	FW (g/m <sup>2</sup> )
<i>Sargassum aquifolium</i> (Turner) C. Agardh	Dry/May 2014	79.9 ± 6.5	48.0 ± 4.8	45 ± 1	65.3 ± 4.7	797.0 ± 191.5
	Wet/Nov 2014	60.4 ± 3.7	15.2 ± 2.3	28 ± 2	17.8 ± 0.8	142.0 ± 24.3
	Dry/June 2015	80.8 ± 4.3	44.0 ± 6.3	32 ± 1	34.0 ± 2.3	266.7 ± 76.0
<i>Padina boryana</i> Thivy	Dry/May 2014	60 ± 9.8	7.1 ± 0.8	38 ± 6	13.5 ± 2.0	83.0 ± 16.0
	Wet/Nov 2014	60 ± 4.7	7.1 ± 0.4	36 ± 1	12.6 ± 0.4	38.4 ± 14.3
	Dry/June 2015	38.6 ± 6.7	7.4 ± 0.8	43 ± 2	17.5 ± 1.2	98.4 ± 8.7
<i>Turbinaria decurrens</i> Bory	Dry/May 2014	59.6 ± 10.1	16.7 ± 0.7	55 ± 3	28.4 ± 1.7	245.2 ± 28.0
<i>T. conoides</i> (J. Agardh) Kützting	Wet/Nov 2014	Carpet of individuals	3.2 ± 0.5	87 ± 4	15.9 ± 1.0	18.3 ± 8.7
<i>T. decurrens</i> Bory	Dry/June 2015	49.8 ± 8.2	15.4 ± 1.0	56 ± 2	33.4 ± 1.0	158.2 ± 41.9

biomass for each species or genus (*B*, in kg) at each sampling time *T* was computed by summing across all substrates the result of the equation:

$$B_{T,S} = A_S \% C_{T,S} * DW_{T,S}$$

DW can represent the dry weight of algae or the dry weight of alginates. Results are presented as mean value ± standard deviation.

**3. Results**

**3.1. Environmental conditions**

Sea surface temperature (SST) reached 30.0 ± 0.72 °C in early dry season (May/June) and 26.0 ± 1.15 °C in early rain season (November 2014). This condition was reversely correlated with salinity. Salinity was 32.0 ± 0.41 psu and 34.0 ± 0.59 psu in May/June 2014 and November 2014 respectively. Wind came from the east with 12–15 m/s of velocity in May 2014 and in June 2015. Meanwhile, in November 2014, Northwest wind was dominant with a velocity at 15–20 m/s. In Ekas Bay, tide is mixed semi-diurnal and the maximum amplitude was measured at 314 cm HAT (Highest Astronomical Tides). The intertidal and shallow water area showed important hydrodynamic condition, with the maximum current velocity at the inner Ekas Bay.

**3.2. Phenology of brown macroalgae**

Finally, four different brown macroalgal species were identified, which were well-identified and abundant all along the monitoring period. These were *Padina boryana* Thivy, *S. aquifolium* (Turner) C. Agardh, *Turbinaria conoides* (J. Agardh) Kützting and *T. decurrens* Bory, as listed on Table 1. Most SPs were in fact monospecific in coverage, and we were then able to monitor the percentage cover for each species across the study period, with *T. decurrens* present in May/June and *T. conoides* present in November (Table 1).

Our field surveys shown an alternation of *Turbinaria* species, i.e. *T. decurrens* dominated in dry season (May/June) and *T. conoides* in wet season (November), while *Sargassum* and *Padina* species were present all along the monitoring period (Table 1, Fig. 2). The Table 1 showed that *Sargassum aquifolium* was also present all over the monitoring period. May 2014 and June 2015, representing the dry season coincided with the season for the blooming of *Sargassum aquifolium* at Ekas Bay. The density within the population reached 45 ± 1 and 32 ± 1 individuals per m<sup>2</sup> in respectively May 2014 and June 2015, while in November 2014, the density was minimal with 28 ± 2 individuals per m<sup>2</sup> (Table 1). Concerning the length of individuals, an increase was noted in May 2014 and June 2015 with individuals reaching a mean size of 48 ± 4.8 cm and 44 ± 6.3 cm respectively, (Table 1) while in November 2014, the length of individuals was smaller (15.2 ± 2.3 cm). The alternation in *Turbinaria*

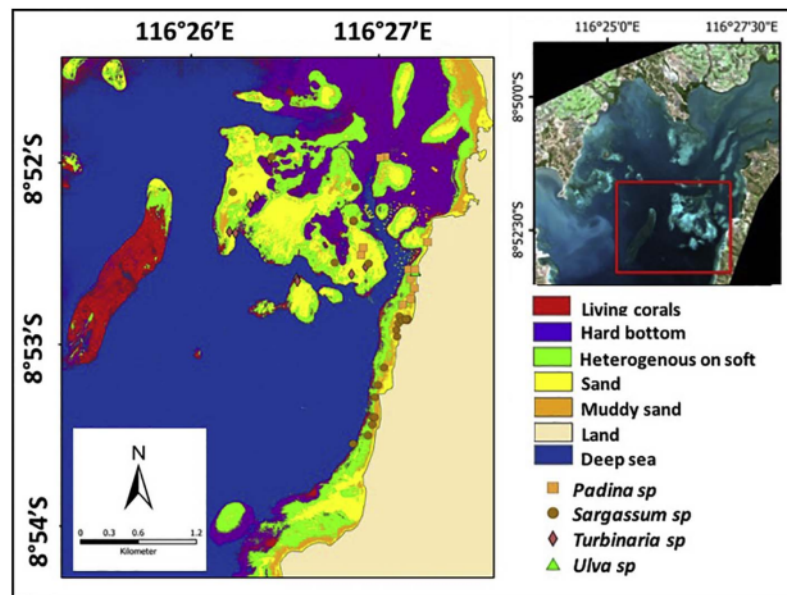


Fig. 2. Thematic map of substrates distribution in the intertidal zone of the study area obtained using satellite imagery and field sampling, together with the points of sampling of brown macroalgae (Ekas Bay, Lombok, Indonesia).

species was visible by a seasonality of measured variables. *T. decurrens* was dominant in May 2014 ( $55 \pm 3$  individuals per  $m^2$ ) and June 2015 ( $56 \pm 2$  individuals per  $m^2$ ) while *T. conoides* was present only in November 2014 with a density of  $87 \pm 4$  individuals per  $m^2$  (Table 1). The length of individuals of *T. decurrens* ( $16.7 \pm 0.7$  cm and  $15.4 \pm 1.0$  cm in respectively May 2014 and June 2015) was higher than *T. conoides* ( $3.2 \pm 0.5$  cm). Individuals of *Padina boryana* reached a size that did not vary between season and years, with  $7.1 \pm 0.8$  cm in May 2014 and  $7.4 \pm 0.8$  cm in June 2015 (Table 1). Density reached a maximal value in June 2015 with  $43 \pm 2$  individuals per  $m^2$  (Table 1).

### 3.3. Habitat mapping

The field observation data, from 152 WPs, indicated that the brown algae were concentrated at the Eastern part of Ekas Bay (Figs. 1, 2). Among the 70 SPs (210 quadrats) sampled and thought to be potential as habitat for brown algae, 25 points were on 'heterogeneous on soft' substrate, 16 were on sand, 14 on 'muddy sand', 9 on 'hard bottom' and 6 on 'living corals' (Fig. 2, Table 2). Living corals were mainly present in the west part of studying area, while hard bottom dominated the east part of the Bay (Fig. 2). Sandy substratum, often surrounded by 'heterogeneous on soft' substratum, was present in the middle of the sampling area and all along the coast (Fig. 2).

**Table 2**

Error matrices for major substrate structure in satellite image. The major diagonal (in grey) presents correct classifications from waypoints during sampling.

Ground truth data Satellite image classification data	Sand	Muddy sand	Coral reef	Heterogeneous on soft	Hard bottom	Total	User's Accuracy (%)
Map Sand data	10	2	–	2	2	16	62.5
Muddy sand	2	10	–	–	–	14	71.4
Living coral	–	–	4	–	–	4	100
Heterogeneous on soft	–	–	–	21	–	21	84
Hard bottom	–	–	–	2	7	9	77.8
Total	16	14	4	23	9	66	
Producer's accuracy (%)	62.5	71.4	100	84	77.8		
Overall accuracy (P <sub>c</sub> )	79.59%						
Kappa coefficient	0.72						

**Table 3**

Estimation of the potential area of abundant brown macroalgae present on the intertidal zone at Ekas Bay (Indonesia). Following results from Fig. 2 (sampling points of brown macroalgae), distinction is made between area colonized by *Padina* (\*) and by the couple *Sargassum/Turbinaria* (\*\*).

Substrate	Intertidal zone		Potential habitat area		Total area (%)
	Nb of pixels	Total area (km <sup>2</sup> )	Nb of pixels	Total area (km <sup>2</sup> )	
Sand (S)	1,483,603	5.93	228,150	2.912*	15.4
Hard bottom (HB)	1,858,002	7.43	1,204,450	1.817**	64.8
Muddy sand (MS)	2,284,122	9.14	1,793,500	3.401*	78.5
Heterogeneous on soft (HS)	1,428,729	5.72	850,250	5.174**	59.5
Living corals (LC)	550,177	2.20	330,304	1.321**	60.0
Total Area		30.42		14.625	
Area colonized by <i>Padina</i>				6.313	
Area colonized by the couple <i>Sargassum/Turbinaria</i>				8.312	

The overall accuracy quantified with the 70 SP points, was good, at 78.59% (kappa = 0.72). The total number of mapped pixels, representing the intertidal area, was 7,604,633 pixels or 30.42 km<sup>2</sup> (Table 3). The map of the potential areas covered by the studied brown macroalgae during all seasons is shown Fig. 3 (light grey). The total area mapped as potential habitat for macroalgae was estimated at 14.625 km<sup>2</sup> with depth < 3 m and dominated by a 'heterogeneous on soft' substrate and clear water (turbidity < 80–90%).

### 3.4. Coverage, biomass and alginate estimation

Field observation data (SPs) showed that the maximum coverage at Ekas Bay was  $80.8 \pm 4.3\%$ , observed for *Sargassum aquifolium* in June 2015 (Table 1). Overall, *S. aquifolium* and other brown species presented high coverage during dry season (June 2015). Meanwhile, minimum coverage was observed at the same time for *Padina boryana* ( $38.6 \pm 6.7\%$ ). *Turbinaria decurrens* dominated in May 2014 and June 2015 with respectively  $59.6 \pm 10.1$  and  $49.8 \pm 8.2$  (Table 1) while *T. conoides* dominated in November 2014 with small thalli recovering the substratum as a continuous carpet.

Biomass of the three brown genera *Padina*, *Sargassum* and *Turbinaria*, were determined for both Fresh weight (FW) and Dry weight (DW). The coefficient of correlation between Fresh weight (FW) and Dry weight (DW) of brown algae (r) was calculated at 0.9253. The average ratio between wet and dry weights for the three brown seaweeds was 8:3:1. Moisture content of the three brown algae did not vary; the average was about  $87.3 \pm 0.05$ .

The alginate yields per species for the three genera are shown in Table 4. The extraction of alginates from *T. conoides* was not possible as not enough dry matter was available from the collected thalli (dwarfism of individuals in November 2014, Table 1). The alginate yield was maximal for *S. aquifolium* ( $39.01 \pm 0.03\%$  DW) and for *T. decurrens* ( $38.70 \pm 0.02\%$  DW), both collected during the dry season (Table 4). Conversely, *P. boryana* presented a low alginate yield comprised between 6 and 9% DW, with the maximal yield found during the wet season ( $9.30 \pm 0.01\%$ , Table 4).

The property of alginate was demonstrated by the estimation of the M/G ratio of all extracted alginates (Table 4). Using three different manners to determine this ratio, alginates from the three species were able to form gel as values of ratio oscillated between 0.689 (*P. boryana*, dry season) and 1.031 (*T. decurrens*, dry season). One should note the great intraspecific variability of ratio depending of the used method. Using the Mackie method, which is the most used in the literature, *Sargassum* and *Turbinaria* species from Ekas Bay produced an alginate with a M/G ratio slightly inferior to 1, then with a slightly dominance of G units.

At the scale of the bay, we estimated the stock in *Padina boryana* within a range of 85.23 tons DW to 110.48 tons DW, obtained respectively in May 2014 and June 2015, and at 79.54 tons in November 2014 (Table 5), with this biomass distributing along 6.31 km<sup>2</sup>. Larger DW biomasses values were determined for the couple *Sargassum/Turbinaria* species with an estimated 779.33 tons and 560.06 tons DW for May 2014 and June 2015 respectively, while the biomass decreased for November 2014 at 147.70 tons DW, due to *Turbinaria decurrens* absence at this sampling date and *T. conoides* represented by dwarf individuals impossible to collect as they constituted a carpet on the substratum. These brown seaweeds were distributed within an area of 8.312 km<sup>2</sup>.

The alginate stock for *Padina boryana* was estimated at 9.10 and 4.97 tons in the dry and wet seasons respectively. Larger alginate stocks were calculated for the couple *Sargassum/Turbinaria*, with 207.61 and 57.60 tons in dry and wet season respectively (Table 5).

## 4. Discussion

Our study confirmed a great exploitation potential for brown seaweeds in Ekas Bay (Indonesia) by estimating the potential area of these

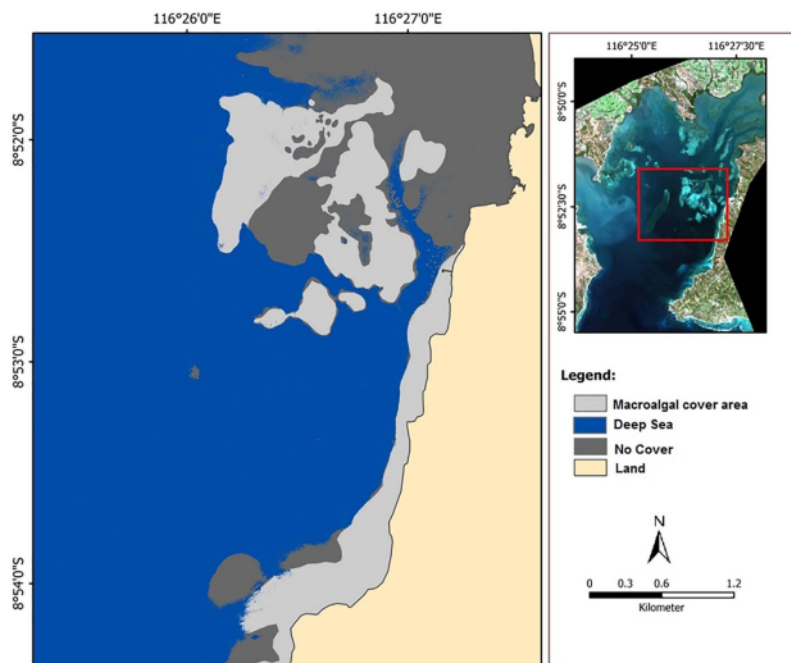


Fig. 3. Thematic map showing the potential area covered by brown macroalgae (grey color on the map) in the Ekas Bay (Lombok, Indonesia).

brown seaweeds (14.625 km<sup>2</sup>), the biomass and alginate stock at two seasons and for two taxonomic entities, namely *Padina boryana* and the couple *Sargassum/Turbinaria*.

Brown algae were mostly found at the Eastern part of Ekas Bay near the shoreline. The Western part of the Bay was dominated by high turbidity water and high suspended matter which was not likely favorable to fleshy macroalgae. These poor conditions are related to the presence of an estuary more suitable for seagrass and mangrove at the north of Ekas Bay. In term of biomass interesting to harvest, our study highlighted a relative stable biomass of *Padina boryana* for the two periods of sampling while the biomass was more variable for the couple *Sargassum/Turbinaria* with high biomasses for May/June and a decrease in November. Indeed, during our monitoring period, *Padina boryana* and *Sargassum aquifolium* were present all year-round while *Turbinaria* species were seasonal, with *T. conoides* present in November and *T. decurrens* observed in May/June. *T. conoides* was represented in November 2014 as a carpet of small individuals covering the substratum. The seasonality or dwarfism of *Sargassaceae* species was already highlighted in other tropical areas (Stiger and Payri, 1999, 2005; Ateweberhan et al., 2009; Le Lann et al., 2012; Hoang et al., 2016) with highest biomass values occurred during cooler months. Similar results on biomass and percentage cover were also obtained by a similar study

as here, conducted for another Indonesian site, Malasoro Bay in South Sulawesi.

The production in brown algae within the Ekas Bay is interesting to compare with other sites. An estimation of the biomass in brown algae living in the South West lagoon of New Caledonia, using space borne high resolution Landsat (30-m resolution) and Quickbird (2.4-m resolution), was done by Mattio et al. (2008a, 2008b). In this area, the biomass of *Sargassum* beds was estimated at 2900 kg DW on 9 km<sup>2</sup>, thus lower than the biomass of brown algae found in our present study. Moreover, prospective industrial use of drifting algae was also conducted in the South Pacific, including estimations of biomass, biomass renewal and agronomical trials by Zubia et al. (2015) in Moorea Island. In this study, the drifting biomass in rafts was estimated at 13062 ± 1998 kg dry mass based on aerial photographs. Compared to these areas, i.e. a large coral reef lagoon in New Caledonia and a narrow lagoon in French Polynesia, Ekas Bay also represents an interesting area which could be used for the harvesting of brown seaweeds, as the potential in brown algae is high. The area is enough diversified in substrata to permit the settlement of a large cover of brown seaweeds, like *Padina boryana* in muddy sand and sandy areas, and like *Sargassum* and *Turbinaria* species in the others substrate types (see Fig. 2).

We were able to estimate the alginate stock of brown seaweeds at

Table 4

33 Yields related to dry weight (DW) and M/G ratio of alginate extracted from dominant species of brown macroalgae in Ekas Bay (Lombok, Indonesia). For each species, the best alginate yield is highlighted (in bold). ND: not determined as not enough biomass. SD: Standard deviation. n corresponds to the number of replicates of 10 g of algal matter used for the extraction of alginates by species. M: mannuronic acid; G: guluronic acid. M/G ratio was determined using three different methods as described in Setyawidati (2017) corresponding at three different areas on FT-IR spectra (see Materials and methods for the different spectral areas).

Species	Season	Sampling date	n	Alginate yield (% DW ± SD)	M/G ratio		
					Filippov & Kohn	Mackie	Sakugawa
<i>S. aquifolium</i>	Dry	May 2014, June 2015	6	<b>39.01 ± 0.03</b>	1.004	0.999	0.833
	Wet	November 2014	3	24.26 ± 0.08	1.002	0.992	0.731
<i>P. boryana</i>	Dry	May 2014, June 2015	6	<b>6.25 ± 0.04</b>	1.013	1.016	0.689
	Wet	November 2014	3	<b>9.30 ± 0.01</b>	1.007	1.023	0.901
<i>T. decurrens</i>	Dry	May 2014, June 2015	6	<b>38.70 ± 0.02</b>	1.031	0.977	0.789
<i>T. conoides</i>	Wet	November 2014	3	ND	–	–	–

**Table 5**  
 Biomasses fresh weight (FW) and dry weight (DW), and alginate stock determined for dominant brown macroalgae genus (*Padina*, *Sargassum* and *Turbinaria*) at the scale of Ekas Bay (Lombok, Indonesia). The alginate yield for the couple *Sargassum*/*Turbinaria* was calculated from *S. aquifolium* and *T. decurrens* (see text). The best results are written in bold.

Model based on	Dry season (mean ± SD)	Wet season (mean ± SD)
Biomass (tons FW in the Bay)		
<i>Padina</i>	572.72 ± 48.74	242.10 ± 90.09
<i>Sargassum</i> / <i>Turbinaria</i>	<b>6096.81 ± 1175.28</b>	<b>1180.55 ± 201.57</b>
Biomass (tons DW in the Bay)		
<i>Padina</i>	97.85 ± 12.63	79.54 ± 2.53
<i>Sargassum</i> / <i>Turbinaria</i>	<b>669.70 ± 109.64</b>	<b>147.70<sup>a</sup> ± 77.97</b>
Stock Alginate (tons DW in the Bay)		
<i>Padina</i>	9.10 ± 0.06	4.97 ± 0.25
<i>Sargassum</i> / <i>Turbinaria</i>	<b>207.61 ± 0.42</b>	<b>57.60 ± 0.66</b>

<sup>a</sup> Only *Sargassum aquifolium* was taken in consideration as no harvestable *Turbinaria* species occurred in the area in November 2014.

the scale of Ekas Bay and found an alginate yield of 9.10 tons in May/June and 4.97 tons in November for *Padina boryana* together with an alginate yield of 207.61 tons in May/June and 57.60 tons in November for Sargassaceae species, which represented valuable yields to begin a harvest of brown algae for alginate production within the Bay. The alginate yield of *Sargassum aquifolium* and *Turbinaria decurrens* from Ekas Bay are high and comparable to yields published by Zubia et al. (2008) for two Sargassacean species, *Sargassum pacificum* and *Turbinaria ornata*, that the authors also positively compared to published M/G ratio. Both Indonesian species presented yield between 24 and 39% DW in alginates, which categorize them as interesting alginophytes for further industrial uses. *S. aquifolium* presented an alginate yield near the one determined in *S. cristaeifolium* C. Agardh, *S. duplicatum* C. Bory, *S. ilicifolium* (Turner) C. Agardh, *S. siliquosum* J. Agardh, *S. vulgare* C. Agardh and *S. wightii* Greville ex J. Agardh (Zubia et al., 2008) while the Indonesian *T. decurrens* presented yield similar to the ones determined in others species of *Turbinaria*: *T. conoides*, *T. murrayana* E.S. Barton and *T. ornata* (Turner) J. Agardh listed in the review of Zubia et al. (2008). Concerning their M/G ratios, all were near 1 and also slightly inferior to 1, highlighting a tiny dominance of G blocks in alginate. In their work, Zubia et al. (2008) compared alginophyte species in term of M/G ratio and demonstrated a high variability of this ratio depending of the considered species. In term of alginate, both Indonesian Sargassaceae genus, *Sargassum* and *Turbinaria*, represent an interesting biomass which could be used as sources of alginates with thickening and probably gelling properties.

Another study on a biomass estimation of potential phycocolloid macroalgae using high resolution image, was published by Setyawidati et al. (2017b). It estimated the biomass together with the stock in carrageenans from the red macroalga *Kappaphycus alvarezii*, a species that is mass-cultivated in Indonesia. Setyawidati et al. (2017b) worked in a cultivation area, divided into 108 parcels representing a surface of about 32.2 ha, and they estimated the stock at 368 tons for the studied area. At bay scale, the stock was estimated at 3575 tons. The extracts of carrageenan and the carrageenan yields obtained in this study fulfilled the specification recommended for industry (followed FT-IR and NMR analysis). The cultivation of *K. alvarezii* (Doty) Doty ex P.C. Silva could also be carried out throughout the year, especially at the time of optimal environmental condition in April–September (Setyawidati et al., 2017a, 2017b). However, this potential has to be considered fragile, due to the appearance of numerous illnesses which weakens this production mode (cultivation).

According to our preliminary results, brown algae in Ekas Bay are of interest for industrial uses especially as a source of raw materials for the preparation of alginates. Our study indicates that *Sargassum* would be a better source compared to the two other genera, i.e. *Padina* and *Turbinaria*. Furthermore, the collection of *Padina* species could be problematic because of a large quantity of sediment, sand and detritus, associated with the algae which would require washing the biomass before its use as alginophyte. Moreover, *Turbinaria* species were seasonally observed with *T. conoides*, visible only as carpet during the rain-to-dry season (November) which then eliminate this species as potential harvestable species. *T. decurrens* is an interesting species but present only during the dry-to-rain season (May/June).

This study highlights the possibility to develop the harvest of wild stock of brown algae, which could represent a novel activity for local people but a management scheme will have to be put in place to preserve populations. Furthermore, if the stock natural renewal is low, one should consider brown algae farming in Ekas Bay as well. The harvest of brown algae in Ekas Bay should take place preferably during from April to September, then during the dry-to-rain season. Exploitation could focus on both Sargassaceae species, *Sargassum aquifolium* and *Turbinaria decurrens*, and farmers could choose to collect *T. decurrens* in May/June and *S. aquifolium* in November as a rotational scheme to avoid harvesting entirely the Sargassaceae fields. Before to choose these periods, one should also monitor the quality of alginates produced by each species.

Future research effort should be targeted at the characterization and properties of alginate extraction of *Sargassum aquifolium* and *Turbinaria decurrens*. Others molecules could be extracted from the dry matter, such as phenolic compounds as already shown by Le Lann et al. (2012) or Stiger et al. (2004). These high added-value molecules represent activities of interest in many industrial sectors (Zubia et al., 2009; Tanniou et al., 2013; Stiger-Pouvreau et al., 2014; Le Lann et al., 2016 as examples of works on Sargassaceae species). The search for new active molecules could benefit others applications for the large biomass of brown seaweeds occurring with the Ekas Bay.

To conclude, the brown algae from Ekas Bay have a great potential as sources of alginate and could be a source of diversification in Indonesia offering the opportunity for this country to remain a major player in the phycocolloid industry. Anonyme (2009) also mentioned that several Indonesian provinces possess sites with similar environment characteristics (substrate and bottom type) such as Maluku (372,408 ha), South Sulawesi (224,172 ha), South East Sulawesi (206,052 ha), Central Sulawesi (178,296 ha), West Papua (130,081 ha), East Nusa Tenggara (104,320 ha), Riau Islands (93,316 ha) and many other Indonesia provinces that have wider coral reef areas than Ekas Bay. These areas require further exploration to fully assess the potential of brown algae growth and alginate production.

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