Adaptation of *Azolla Mycrophyla* to Brackish Water Ecosystem

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**ABSTRACT:** *Azolla microphylla* is a tropical and subtropical fern with a length of 1.5-2.5 cm. Some genera can grow in brackish waters, but these biota are more commonly referred to as freshwater plants. Utilization of this macrophyte as fish and livestock feed (cattle, goats, ducks, ducks and chickens) have been introduced in some part of the world. Therefore, it is necessary to know the best way to adapt the fern into brackish water ecosystem and examine the potential for growth in such environment. In this study, two adaptation methods were performed, namely instant adaptation (IA) method and the step by step adaptation (SSA) method. A number of 25-liter plastic basin were filled with 20 l of freshwater or sea water, salt marsh sediment (500 g) and fermented chicken manure (500 g) and used as experimental unit. In the IA approach, the water salinity was adjusted from the beginning at 0, 5, 10, 15 and 20 ppt, planted with fern and observed for 21 days. While in the SSA method, the fern was firstly grown on an experimental unit of 0 ppt for 7 days. Then the water salinity was increased of 1 ppt every 7 days, until day 49. All experimental units were observed daily, including; survival rate, absolute growth, total growth, doubling time, and histological observations of leaves, rhizomes and macrophyte roots. The study showed that in IA approach, *A. microphylla* grew normally only in salinity 0 ppt (freshwater). In SSA method, the fern grew well and the population increased until day 49 at salinity 6 ppt. From the study can be suggested that in case of growing *A. mycrphylla* in brackish water ecosystem, the organism can be cultivated at salinity below 6 ppt by using step by step method of adaptation.

**Keywords:** Fern, Azolla, salinity, growth, nitrogen fixation, and adaptation.

1. Introduction

Coastal areas, where brackish water is present, are mostly populated areas. Provision of food sources for livestock and fish in coastal communities in the form of green protein is one alternative problem solving for protein limitation in the area. The use of feed produced by the plant in the form of pellets and concentrates on the one hand has been proven to provide good growth of fish and livestock. However, high factory feed prices, remote area position, limitation of local consumer purchasing ability, poor transportation facilities, limited capital owned by farmers (Hidayat, 2012) makes this business unprofitable.
Macrophyte *Azolla microphylla* is a relatively small water-sized fern (Christenhusz, 2014), has a length of 1.5-2.5 cm. This plant grows in the tropics and subtropics (Tjitrosoepomo, 2014). Some genera can grow in brackish waters, but the biota are more commonly referred to as freshwater plants (Kitoh and Shiomi, 1991; Shiomi and Kitoh, 2001). They have lateral root where with a sharp shave like a hair or feathers on water. Small leaf shape with a length of about 1-2 mm with the position of the leaves that overlap each other. Taxonomists classify Azollas into 7-9 species. They are *Azolla caroliniana* Willd, *A. circinata* Oltz & Hall, *A. cristata* Kaulf, *A. filiculoides* Lam, *A. imbricata* Nakai, *A. japonica* Franch. & Sav, *A. Mexicana*, *A. microphylla* Kaulf, *A. nilotica* A. pinnata and *A. rubra* R.Br (Christenhusz, 2014; Sadeghi et al., 2013). Furthermore, Utilization of *Azolla* as a fish feed and other livestock has begun to be introduced in the Indonesia. In Java some internet sites have known and sell these commodities online for those needs (Madigan et al, 2009). Some of the advantages *Azolla* as fish feed include the following. 1) High protein content (23-30% dry weight, Kusumanto, 2014). 2) Very fast growth (3-7 days, Smith et al, 2010). 3) Relatively easy to cultivate (Chapman, 2010). and 4) Easy to digest and distributed to fish and other livestock.

In Indonesia, *Azolla* cultivation is currently still in limited area. The cultivation techniques applied are still conventional and home industry scale and mainly for their own needs. This biota is only stocked in columns of stagnant water in the soil (pond, tub and so on) leading to minimum growth so the benefits provided certainly limited. Provision of fish and livestock (cattle, goats, ducks, ducks and so forth) feed for coastal communities in the form of green protein is one of the alternative solutions to the economic problems of coastal communities. Macrophyte *Azolla microphylla* along with its symbionts *Anabaena azollae* and nitrifying bacteria can be expected to answer both problems. Various studies have shown the negative effect of salinity on growth of different Azolla species. There are still no in depth studies about variation in s

### 2. Materials and Methods

The research was conducted in March - August 2017 at Marine Biotechnology Laboratory, Faculty of Fisheries and Fisheries, University of Riau. The samples (seawater and sediment) were taken from coastal waters in Desa Kayu Ara, Siak Regency, Riau Province.

A number of 25-liter plastic basin were filled with 20 l of freshwater or sea water, salt marsh sediment (500 g) and fermented chicken manure (500 g) and used as experimental unit to grow *A. microphylla*. In this study, two adaptation methods were performed, namely instant adaptation (IA) method and the step by step adaptation (SSA) method. In the IA approach, the water salinity was adjusted from the beginning at 0, 5, 10, 15 and 20 ppt, planted with fern and observed for 21 days. While in the SSA method, the fern was firstly grown on an experimental unit of 0 ppt for 7 days. Then the water salinity was increased of 1 ppt every 7 days, until day 49. All experimental units were observed daily, including; survival rate, absolute growth, total growth, doubling time, and histological observations of leaves, rhizomes and the macrophyte roots.

### 3. Results and Discussion

The study showed that in IA aproach, *A. microphylla* grew normally only in salinity 0 ppt (freshwater). In 5 ppt experimental unit, the organisms survived until day 14. Similarly, at 10 ppt, the macrophytes grew and branched up to day 7. While the treatment of salinity 15 and 20 ppt *A. microphylla* survived up to 5 and 3 days only, respectively. The biomass growth and absolute weight, growth of specific biomass, growth doubling time, root growth only occurred at salinity 0, 5 and 10 ppt. More detailed growth data are presented in Table 1. Similar result was indicated also by absolute biomass weight, in which the macrophytes only grew normally at salinity 0. At brackish water with 5 ppt salinity, biomass biota weight only increased up to the 9th day (Table 2).
The macrophytes *A. microphylla* exhibited negative response to salinity can be understood, since the fern are actually a brackish water biota. Some researcher considered that Azolla is extremely sensitive to NaCl (Rai and Rai, 2000; Fernández-Zamudio et al., 2010). Masood et al. (2006) stated that salinity inhibits growth of *A. pinnata* and *A. filiculoides* leading to a significant decrease when NaCl concentration increased. *A. filiculoides* was more sensitive to high salt concentration than *A. pinnata*. Salt concentrations above 10mM NaCl inhibited growth of *A. filiculoides*, but growth of *A. pinnata* was only stopped at 40mM NaCl. Some genera *Azolla* can grow in brackish waters, but these biota are more commonly referred to as freshwater plants. Azolla has been used for centuries in China, Vietnam and the Philippines as a source of N for fresh water wetland rice (Chapman, 2010).

**Table 1.** The growth and number of population (individu) and the biomass conditions of *A. microphylla* in brackish water at different salinity.

| Salinity (ppt) | Day   | 0      | 5      | 10     | 15     | 20     |
|----------------|-------|--------|--------|--------|--------|--------|
|                | 1     | 20.00; NGF | 20.00; NGF | 20.00; NGF | 20.00; NGF | 20.00; NGF |
|                | 2     | 23.66 NGF | 22.66 NGF | 21.33; NGF | 20.66; NGF | 20.00; NGF |
|                | 3     | 32.33; NGF | 25.00; NGF | 22.66; NGF | 21.00; RW | 20.00; TW |
|                | 4     | 44.66; NGF | 28.66; NGF | 25.33; NGF | 21.00; TW | TW     |
|                | 5     | 44.66; NGF | 35.33; GF | 25.33; RW | TW     | TW     |
|                | 6     | 64.00; NGF | 42.00; GF | 25.33; TW | TW     | TW     |
|                | 7     | 83.33; NGF | 45.00; GF | TW     | TW     | TW     |

NGF = normal, green and fresh; GF = green and fresh; RW = rather wilted; TW = totally wilted

Sadeghi et al. (2012) found that salinity can play an intermediately role on the growth of *A. filiculoides* in the Anzali wetland. Some other researcher (Fernández-Zamudio et al. 2010) studied germination characteristics and spore germination success of *A. filiculoides*. Germination rate showed a significant decrease when salinity increased. Arora and Singh (2003) conducted a study on six different Azolla species (*filiculoides, mexicana, microphylla, pinnata, rubra* and *caroliniana*). They noted that salinity drastically decreased biomass production in all 6 species. *A. microphylla* showed high tolerance to salinity than other species. Khosravi et al. (2005) stated that increasing salt concentration in water (in terms of NaCl) can reduce the removal of heavy metals by Azolla. In other words, high salinity level can inhibit Azolla growth (Arora and Singh 2003; Fernández-Zamudio et al., 2010) leading to a decrease in removal of heavy metals by the species.

**Table 2.** Absolute growth of biomass (gram) *A. microphylla* in brackish water at different salinity.

| Salinity (ppt) | Day   | 0       | 5       | 10      | 15      | 20      |
|----------------|-------|---------|---------|---------|---------|---------|
|                | 1     | 0.1400 g | 0.1600 g | 0.1533 g | 0.1267 g | 0.1400 g |
|                | 2     | 0.1333 g | 0.1667 g | 0.1567 g | 0.1467 g | 0.1400 g |
|                | 3     | 0.1400 g | 0.1733 g | 0.1600 g | 0.1467 g | 0.1400 g |
|                | 4     | 0.1733 g | 0.1900 g | 0.1600 g | 0.1467 g | NW      |
|                | 5     | 0.1733 g | 0.1600 g | 0.1667 g | NW      | NW      |
|                | 6     | 0.1933 g | 0.1967 g | NW      | NW      | NW      |
|                | 7     | 0.3200 g | 0.2033 g | NW      | NW      | NW      |

NW : Not Weighted
An encouraging result was noted in SSA method. It seems that within 7 days period, *A. microphylla* had enough time adjust their body structure and function to higher salinity. Growth doubling time became lower with the higher salinity, however the fern grew well and the population increased until day 49 reaching salinity at salinity 6 ppt. More detailed growth data are presented in Table 3. It is tempting to speculate that the organism will grow well in shallow land pond or plastic made pond in coastal area, an alternative to provide green protein to fish and other lifestocks in the area.

**Table 3.** The growth and number of population and the biomass conditions of *A. microphylla* in brackish water at different salinity.

| Day and Salinity | Sample 1 | Sample 2 | Sample 3 |
|------------------|----------|----------|----------|
|                  | Biomass  | Weight   | Biomass  | Weight   | Biomass  | Weight   |
| 0 d (0 ppt)      | 60       | 0.96     | 60       | 1        | 60       | 0.88     |
| 7 d (1 ppt)      | 304      | 3.36     | 312      | 4.42     | 336      | 3.2      |
| 14 d (2 ppt)     | 531      | 7.22     | 856      | 19.78    | 623      | 8.93     |
| 21 d (3 ppt)     | 1176     | 21.04    | 1974     | 56.2     | 1340     | 24.68    |
| 28 d (4 ppt)     | 3960     | 70.56    | 6646     | 186.83   | 4505     | 84.56    |
| 21 d (5 ppt)     | 6743     | 136.67   | 8478     | 256.68   | 7650     | 112.87   |
| 28 d (6 ppt)     | 8244     | 184.65   | 9803     | 326.45   | 8904     | 187.65   |

4. Conclusions and Suggestions

The study showed that in instant adaptation (IA) approach, *A. microphylla* grew normally only in salinity 0 ppt (freshwater). In 5 ppt experimental unit, the organisms survived until day 14. Similarly, at 10 ppt, the macrophytes grew and branched up to day 7. While the treatment of salinity 15 and 20 ppt *A. microphylla* survived up to 5 and 3 days only, respectively. While in step by step adaptation (SSA) method, the fern grew well and the population increased until day 49 at salinity 6 ppt.

From this study can be suggested that in case of growing *A. mycrphylla* in brackish water ecosystem, the organism can be cultivated at salinity below 6 ppt by using step by step method of adaptation.

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