Study on stress environmental response of riverbank plants for the purpose of applying watershed environmental management

（水辺の環境管理への適用を目的とした河岸植物のストレス環境応答に関する研究）

By

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DEDICATION

This work is dedicated my loving mother Lekkala Narayanamma

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ABSTRACT

Understanding the ecophysiology of riparian vegetation is an important factor in managing and promoting biodiversity. Additionally, because riparian vegetation along an elevation gradient is constantly prone to fluctuations in water levels, it is often subjected to environmental stress. The current study examined the effect of elevation on riparian vegetation communities along the Hii River in Japan by quantifying environmental stress using foliar hydrogen peroxide concentrations. Four abundant species occurring along the Hii River, *Phragmites australis, Phragmites karka, Juglans mandshurica*, and *Salix pierotii,* were used for the investigation. Leaf samples were collected from all four species at different elevations along the river line. The results indicated that compared to the same species living at high elevations*, P. australis,P. karka*, and *S. pierotii* at low elevations had reduced hydrogen peroxide contents, indicating low levels of stress. However, *J. mandshurica* showed the opposite trend； this species had a high concentration of hydrogen peroxide at low elevations and was thus highly stressed compared to the other species at high elevations. Moreover, *J. mandshurica* preferred highly fertile soil, especially with regard to the nitrogen content, and *J. mandshurica* had a high and low hydrogen peroxide content at low and high soil nitrogen, respectively. This clearly indicates that for *J. mandshurica*, the preferential location and stress was mainly based on soil nutrient availability. Our findings reveal that hydrogen peroxide is a very efficient and reliable index for quantifying environmental stress to help manage biodiversity.

Plants are subjected to various environmental stressors, both natural and artificial. Environmental stressors that lead to an excessive generation of reactive oxygen species (ROS) may adversely affect physiology and well-being of organisms. The amount of ROS, therefore, could potentially be used to quantify environmental stresses in plants. In this study, the effect of changes of water levels and elevation at which plants are located was examined in the

drawdown zone of a flood control dam (Miharu dam), located in Fukushima in northern Japan. The *Salix subfragilis* willows are important plant species in the reservoir as these plants are resistant to prolonged submerged conditions. *S. subfragilis* growing in the drawdown zone exhibited high mortality rates from 2008 to 2016. Leaf samples of *S. subfragilis* were collected in the drawdown zone at different elevations shortly after drawdown (June), and several months after drawdown (August and October). The samples were taken under light exposure; additionally, dark-adapted (30 min) samples were collected in order to evaluate photosystem-induced ROS generation. The mortality rate of *S. subfragilis* trees was abnormally high over the previous years, particularly at higher elevated sites. Foliar H_2O_2 concentrations were significantly increased in samples collected in August and October samples, compared to June samples. Catalase, ascorbate peroxidase, and guaiacol peroxidase activities were also increased, corresponding with the H_2O_2 content. No significant difference in H_2O_2 concentrations was found between samples from the lower edge of the drawdown zone and those collected at constantly inundated sites. The H_2O_2 concentration of light- and dark-treated samples indicated that there was no additional ROS accumulation due to photosynthesis. The results indicated that the high mortality of the trees was associated with long-term exposure to drought conditions due to the drawdown, although this was not confirmed by conventional monitoring. These findings support the use of H_2O_2 as an environmental stress indicator in vegetation management.

Egeria densa is an often-found invasive species in Japan, which has spread widely in the past two decades in rivers where no macrophytes had previously been found. As a result, these ecosystems have now become dominated by *E. densa*. The habitat preference for *E. densa* colony formation was investigated using the tissue concentrations of hydrogen peroxide (H_2O_2: a reactive oxygen species) under varying conditions in rivers and laboratory conditions. The empirical equations that can describe the macrophyte tissue H_2O_2 formation under various velocity and light conditions were produced. The H_2O_2 concentrations of dark-adapted plants are proportional to the flow velocity, and the surplus H_2O_2 concentration in the light-exposed condition corresponded to the photosystems produced H_2O_2. When the H_2O_2 concentration exceeds 16 μmol/gFW, plant tissue starts to deteriorate, and biomass declines, indicating the critical values required for long-term survival of the plant. The empirically obtained relationships between flow velocity or light intensity and the analysis of H_2O_2 concentration for different slopes and depths of channels found that the H_2O_2 value exceeds the critical H_2O_2 concentration in channels with above 1/100 at around 0.6 m depth. This agrees with the observed results where colonies were not found in channels with slopes exceeding 1/100, and biomass concentration was the largest at depths of 0.6 to 0.8 m. H_2O_2 concentration is quite applicable to understanding the macrophyte condition in various kinds of macrophyte management.

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CHAPTER I

Introduction

1.1 Riparian vegetation

Riparian zone is the interface between the land and a river or a stream. The vegetation along the riparian zone is called as riparian vegetation. It plays vital role in balancing the riparian ecosystem. The riparian zone is considered to provide best suitable habitat for the growth and reproduction of aquatic species living with in the rivers or streams(Allan et al., 1993). The healthy streams are mix of riparian plant species and aquatic species that depend upon the natural hydrologic regimes for their survival. Human alterations near the riparian zone are causing great damage to the original structure of the riparian zones which may in turn lead to the disturbance to the plant communities and associated habitats inhabiting over there (Vitousek et al., 1997) . Lakes and waterways, bounded by riparian plant communities and landforms, provides important transportation corridors. Riparian zones exhibit wide ranges of physical fluctuation appear in community characteristics that are distinctly expressed by a broad array of life history action and by chronological and analytical patterns (Vitousek et al., 1997). Therefore, riparian zones are among the biosphere's most composite ecological systems. The vulnerability of biotic virtue found in natural riparian zones reflects the deep-rooted physical assortment of drainage networks, processes shaping stream channels, and ecological characteristics of community assemblages. In effect, riparian communities are products of extreme interactions among biotic and abiotic factors. Whether they are occurring in the present or have occurred in the past (Jones et al., 1994). Farther, as we will see, the biota themselves have strong, long-term influences on the geomorphic structures and processes by which they are shaped.

The timing and extent of inundation are critical aspects in improving the species composition in the riparian zone(Jones et al., 1994). Riparian trees and shrubs are differentially fitted to the duration of flood events, most able to endure several days, or a few species can tolerate months, of flooding. Many non-native invasive weeds are killed by flooding. Thus, interactions among the physical processes of flooding, sediment deposition, channel meander, and hydroperiod across a floodplain results in a vegetation montage over time that is structurally complex(Jones et al., 1994). Groves of trees, chunk of woody shrubs, open grassy areas, and open woodlands with an understory of herbaceous perennials and native grasses are distributed and in places interlace crosswise the floodplain, and distinct habitat types created by channel meander form in the oxbow lakes and cut-off bottoms.

In recent studies it was found that the species varieties of riparian forests underwent change through time in relation to shade resilience and the flexibility of the species to disturbance. In recent years, maintaining and improving biodiversity in potential riparian areas and the surrounding environment have been areas of focus, and it has been emphasized that riparian areas are core habitats for a wide range of semiaquatic and terrestrial species(McCLAIN & Cossío, 2003). Nonetheless, dense trees in riparian zones obstruct flood flow, increasing the upstream water level and causing bank collapse during floods (Nilsson et al., 2010). As recruitment of riparian vegetation occurs when seeds or fragments colonize shorelines during floods, flood water plays a key role in this process. Seeds are primarily dispersed in various patterns, such as zoochory, anemochory, and hydrochory, and after accumulating on the ground surface of a riparian zone, seeds are transported by flood water downstream and germinate at sites with favorable condition(Merritt & Wohl, 2006). When a flood is in the receding stage, most seeds are already flushed from upstream; thus, seeds on the upstream ground surface are distributed to the downstream shoreline around the peak of a flood. In addition, the available moisture along a riparian zone is a critical factor for plant occurrence, whereby water acts as a different type of support agent by initiating germination and determining plant establishment and growth.

1.2 Importance of riparian vegetation

Riparian zones act as sources of linkage in the ecosystems within a landscape. Landscapes are composed of (often overlapping) patches of different types of vegetation, soil and available nutrients, moisture and light (Rosell et al., 2005). Disturbances in the landscape, such as fire, pest outbreaks or even the death of a single tree, create these patches, which provide different types of habitats. This diversification of environment is important, but so is relatedness between them. Riparian zones grant wildlife to travel between habitat "islands" by providing transportation corridors, and help to circulate nutrients between different ecosystems(Trombulak & Frissell, 2000). Since riparian zones are wetter than the surrounding landscape, they also often resist destruction by fire, and recover more quickly. This helps the landscape as a whole to recover.

To clarify how riparian zones, circulate nutrients, we can consider a single leaf in a forest adjacent to a stream. The leaf falls into the stream and is carried a few kilometers downstream before it becomes trapped behind a log. Here it is broken down by microorganisms and ingested by insect larvae. The insects are eaten by fish, which are preyed upon by larger fish, birds, and mammals (e.g., raccoons and bears). Mammals often take fish into the forest before eating; uneaten portions of the fish decompose and are absorbed into the soil in the forest. So, the nutrients in the leaf have been conveyed from the forest to the stream and back to the forest, some distance downstream from where this cycle began. Nutrients can also be transported upstream, for example by salmon that return from the ocean to freshwater to spawn and subsequently die; their carcasses provide important nutrients to aquatic and terrestrial ecosystems.

The vegetation that grows in riparian zones is specially adapted to wet soil conditions, and can tolerate periodic flooding(Trombulak & Frissell, 2000). Vegetation fills an important niche that connects the water's edge with dry land, and accomplishes a number of functions(Church, 2002).Trees and shrubs that border and overhang streams and lake shores moderate the temperature through shading and the cooling effect of evapotranspiration. This directly benefits fish and aquatic invertebrates and prevents excess growth of algae.

Plants growing along and near stream banks, lake shores and estuaries have root structure, collecting sediment and thus prevent banks and shorelines from being washed away(Colinvaux, 1964).Leaves, twigs, needles and whole trees that fall into water bodies provide nutrients to aquatic invertebrates, which in turn nourish fish (Carvalho et al., 2009).Large trees that have fallen into streams help to dissipate the energy of flowing water, protect stream banks and create pools and hiding places for fish. The animals that live in riparian zones also contribute to the function of these ecosystems. For example, birds and mammals help to disperse the seeds of shrubs and trees (Whelan et al., 2008). Invertebrates, such as many insects, molluscs (e.g. slugs and snails) and worms help to break down plant and animal matter, making nutrients more readily available to other organisms (Whelan et al., 2008). Salmon fulfill a unique role by connecting the ocean, freshwater, and the land, during the course of their life cycle, which begins and ends in stream corridors. Complex predator-prey relationships between animal species are also necessary to maintain a healthy balance among populations. Healthy riparian zones therefore provide habitat for a huge array of animals. Studies have shown that about 80 percent of wildlife species depend on riparian areas in whole or in part.

Historically, people depended on the wildlife, plants and drinking water that could be found abundant in these areas. Large cities have grown up around many of these sites, and even modern society depends on the products and services of riparian zones. Riparian vegetation supports a diversity of aquatic habitats, shades the river water, which reduces fluctuations in water temperature, stabilizes the stream banks. Filters sediments and nutrients from the surface and sub-surface water, which reduces pollution of the river. Riparian vegetation zone is a high productivity zone that provides food for aquatic animals. Adds agricultural value to the property by allowing selective timber harvesting, forage production and apiculture; providing a windbreak for adjoining paddocks; and providing emergency feed(Dube et al., 2016). Adds to the capital value of the land.

1.3 Description of research problem

Although, there are several plant species inhabiting around the riparian zone, it is important to find the species-specific suitable location. The plant species living along the riparian zone. In a riparian zone, floods are a source of water to the ecosystem, though water availability varies along a river gradient depending on the distance and elevation from the channel, and it changes over time. In general, water level variation strongly affects the growth patterns of vegetation. However, both hydrophilic and hydrophobic species live along riparian zones, and it is difficult to determine the most suitable location and conditions for each species. Thus, in vegetation community management, there is always a need to identify species-specific, suitable locations. Regardless, very limited information is currently available.

Plants suffer from oxidative stress when exposed to severe environments, which results in an excessive amount of reactive oxygen species (ROS) in cells. Under normal conditions, a balance exists in the activities of reactive oxygen species and antioxidants generated as a defense mechanism. However, under stressful conditions, antioxidant defenses become insufficient, resulting in oxidative stress that often leads to cell death. Among the different types of ROS, superoxide anion ($\bullet O_2^-$) and hydroxyl radicals ($\bullet OH$) are the major species generated under stress and are typically converted into relatively stable hydrogen peroxide (H_2O_2), via either superoxide dismutase (SOD) or nonenzymatic systems. Thus, the concentration of H_2O_2 has a strong potential to provide information regarding the environmental stress imposed on plants.

1.4 Objective of study

The objective of the current study is to develop an assessment method for determining the elevation preference of riparian vegetation communities by quantifying environmental stress using hydrogen peroxide concentrations. Riparian vegetation is highly affected by sediment flows during floods, particularly gravel deposition. Thus, the most typical sand-bed river in Japan, the Hii River, was selected for field observations in case study 1. Also, sometimes the changes in the water levels could be due to the artificial draw down of water levels in the dams. The change in water levels could drastically affect the vegetation physiology surrounding the reservoirs and may lead to death of the species residing over there. In order to understand a case like this there is another case study 2. It was carried out along a major flood control dam Miharu dam located in the located in Fukushima in northern Japan.

1.5 Importance of study

The environmental stress like moisture and location at which plants are located can be determined using the concentration of the reactive oxygen species. Species-specific zones of colonization or recruitment can be determined using hydrogen peroxide as an environmental stress quantification index. Additionally, because most plant species face environmental stress, the current study can also be applied as a rapid, efficient, and reliable monitoring system for vegetation management. In measuring oxidative stress, it is important to truly understand the intensity of the stress, predict probable outcomes, and take appropriate measures to protect vegetation accordingly. As there is severe threat being caused to the many ecologically, economically important species there is always a need to understand the stress caused and reliable measures need to be taken to protect these species.

1.6 Outlines and constraint

In chapter III the study focused on the major abundant species along the riparian zones in Japan such *P. australis, P. karka, S. pierotii* and *J. mandshurica.* Species-specific zones of colonization or recruitment was studied using hydrogen peroxide as an environmental stress quantification index. Additionally, because most plant species face environmental stress, the current study proposes a rapid, efficient, and reliable monitoring system for vegetation management.

In chapter IV the study was conducted near the flood control dam abundant species *S.subfragilis* were studied in different seasons just before the drawdown of the flood control dam and few months after the drawdown of the water level. The study was conducted to find out the reason behind the decline in *S.subfragilis* tress around the miharu dam from 2008 to 2016. Continuous monitoring was carried out for about two years and conclusions were drawn based on the long-term monitoring which can be applied in the real time scenario.

In chapter V the study was conducted along major Japanese rivers colonized by *E. densa* were selected from the species distribution records in Japan (MLIT, 2018). In 2016 and 2017, observations were conducted in rivers for location data of *E. densa* colonization in different seasons from Eno (Go), Saba and Hii Rivers. The reason for the dense colonization of *E. densa* was studied using the foliar hydrogen peroxide concentration.

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CHAPTER II

Literature review

2.1 Riparian vegetation structure and variations

Riparian vegetation fulfils or influences various important ecological functions in relation to aquatic habitats, including the provision of food, moderation of stream water temperature via evapotranspiration and shading, providing a buffer zone that filters sediments and controls nutrients, and stabilization of stream banks. Also, the variations in the riparian vegetation structures can devastate the vegetative cover of the particular area, which may in turn lead to the habitat damage. Human activities, such as agriculture and forestry, and natural disturbances, such as outbreaks of insects and diseases, can modify the physical environment of an ecosystem (i.e., the patterns of temperature, moisture, wind, and light) by altering structural features. Typically, forest structure is described at the stand and landscape levels. Stand structure is well defined in forestry (e.g., stocking densities, overstory coverage, and species composition). Landscape structure can be defined by the spatial arrangement (pattern) of elements of topography, vegetation, soil, or the physical environment itself.

The interaction of climate with the earth's surface has created a variety of landscapes drained by networks of streams, rivers, lakes, and wetlands. Riparian areas are found adjacent to essentially all these water bodies except where human disturbance has intervened. Although riparian areas differ considerably in their structure and function from site to site, there are patterns in the attributes of riparian areas and how they are distributed across the landscape. While a single characteristic (such as the presence of bedrock) may strongly influence the size, characteristics, and functions of a given riparian area, generally the interaction of many climatic, hydrologic, geomorphic, and biological factors shape riparian environments. For example, differences in climate dictate the seasonality of the hydrologic cycle and determine the timing and intensity of flooding. Watershed features such the slope of the land, size of the watershed, storage capacity of the soil, and supplies of groundwater and sediment interact with climate to modulate or amplify these effects. Within the riparian area itself, further sources of variation can be found in channel morphology, sediment dynamics, and floodplain structure. Ultimately, all these factors influence species composition of riparian biota.

2.2 Types of riparian vegetation

Wetland vegetation is dominated by trees, a heath/shrub layer, or a ground layer, consisting of either grasses, sedges, herbs, or a combination of these in their riparian zones or in shallow aquatic areas. Submerged aquatic vegetation is found in deeper wetland areas (Arthington et al., BJ 2003). This is particularly relevant for palustrine wetlands which are defined as being dominated by vegetation, however, all other wetland systems can and most often do include a vegetation component that plays an important role in the wetland food web and physico-chemical environment (Bunn, SE 1988).

2.3 Types of threats to riparian vegetation

The threats to riparian ecosystems that have been identified in the literature include similar threats to those identified for upland ecosystems: invasive species, herbivory (both domestic livestock and wild ungulates), wildfire and fuels treatments, ecosystem fragmentation, drought, climate change, disease and insects, legacy impacts, urban development, mineral extraction, changes in hydrology, and geomorphic change. In addition, rivers are particularly vulnerable to human water use and altered hydrologic regime (Graf 1999; Allan 2004, Dudgeon et al., 2006). Because channel processes and riparian areas are inherently tied to hydrologic and sediment regimes, activities, and conditions from throughout the watershed influence riparian conditions along a river segment or reach.

Because of complex interactive and cumulative effects, lag times, stochasticity, and site-specific characteristics, predicting deterministic/cause and effect relations is difficult. However, general 4 direction and magnitude of river change may be predicted given known changes in driving variables. In addition, generalization regarding change in systems in response to stressors is risky as different channels may respond to the same change in different ways. Common indicators of stress in ecosystems are characterized by "reduced biodiversity, altered productivity, increased prevalence of disease, reduced efficiency of nutrient cycling, increased dominance of exotic species, and smaller, short-lived opportunistic species (Naiman et al. 2005). In riparian ecosystems such indicators may be direct responses to reduced or altered flow regime that governs water availability and disturbance magnitude and frequency and changes in sediment regimes delivered from upstream hillslopes and channels.

Hydrological change Changes to natural flooding regimes through flow regulation and water extraction, pose one of the greatest threats to vegetation communities in riparian zones throughout the world (Nilsson & Svedmark 2002, Tockner & Stanford 2002). Riparian vegetation is particularly sensitive to flow alterations and changes in vegetation diversity and dynamics can occur even if mean annual flows are preserved (Auble et al. 1994). Weeds are also serious threats to the ecological integrity and productivity of many Australian vegetation communities (Grice & Brown 1999) and riparian zones are highly susceptible to weed infestation (Grice 2004). Weed infestations are often the result of disturbance or the build-up of nutrient levels caused by fertilisers or grazing animals. Primary disturbances include vegetation clearance, fire, and stock grazing. Altered flooding regimes may also enable the establishment of weed species in riparian zones (Stromberg 2001).

Grazing is the dominant land-use in Australia (Stewart 1996), and the riparian zone is often severely impacted upon by the activities of domestic stock (Jansen & Robertson 2001), especially when their access to riparian zones is not controlled. Grazing by feral and native animals can also affect the diversity and condition of riparian vegetation. Riparian zones offer a number of attractions for stock, including shelter, relatively higher quality of forage, and access to water. The primary impacts that stock have on riparian vegetation include overgrazing and trampling, both of which may lead to erosion, soil compaction, and weed infestation (Clary 1999, Shaw & Kernot 2004) which in turn causes loss of biodiversity, degradation of the natural conditions and loss of water quality.

2.4 Riparian vegetation management practices

Strips of vegetation separating a waterbody from a land use that could act as a nonpoint pollution source. Vegetated buffers (or simply buffers) are variable in width and can range in function from a vegetated filter strip to a wetland or riparian area. A vegetated buffer usually has a rough surface and typically contains a heterogeneous mix of ground cover, including herbaceous and woody species of vegetation (Stewardship Incentive Program, 1991; Swift, 1986). This mix of vegetation allows the buffer to function more like a wetland or riparian area. A vegetated filter strip can also be constructed to remove pollutants in runoff from a developed site, but a filter strip differs from a vegetated buffer in that a filter strip typically has a smooth surface and a vegetated cover made up of a homogeneous species of vegetation (Dillaha et al., 1989a).

Vegetation created areas of vegetation designed to remove sediment and other pollutants from surface water runoff by filtration, deposition, infiltration, adsorption, absorption, decomposition, and volatilization. A vegetated filter strip is an area that maintains soil aeration as opposed to a wetland that, at times, exhibits anaerobic soil conditions. Engineered systems designed to simulate natural wetlands to exploit the water purification functional value for human use and benefits. Constructed wetlands consist of former upland environments that have been modified to create poorly drained soils and wetlands flora and fauna for the primary purpose of contaminant or pollutant removal from wastewaters or runoff. Constructed wetlands are essentially wastewater treatment systems and are designed and operated as such though many systems do support other functional values.

Constructed wetlands are designed to mimic the pollutant-removal functions of natural wetlands but usually lack aquatic habitat functions and-are not intended to provide species diversity. Pollutant removal in constructed wetlands is accomplished by several mechanisms, including sediment trapping, plant uptake, bacterial decomposition, and adsorption. Properly designed constructed wetlands filter and settle suspended solids. Wetland vegetation used in constructed wetlands converts some pollutants (i.e., nitrogen, phosphorus, and metals) into plant biomass (Watson et al., 1988). Stream beds should be stabilized before revegetation works begin. If a channel is actively deepening and widening, fencing off and revegetating the riparian zone will not stabilize the channel and its banks. In this situation, stream-bed control structures should be installed, and the banks protected before revegetation begins.

Native vegetation along degraded Tasmanian waterways may regenerate without replanting if stock is excluded and weeds controlled. If revegetation is necessary, advice should be sought on the most appropriate species for the site. Using inappropriate species, such as willows, may cause environmental harm that may be difficult and expensive to remediate. The species composition and community structure of the vegetation will vary with distance from the waterway or wetland as soil conditions become drier. Riparian vegetation is critical for maintaining healthy ecosystems in small waterways and the upper reaches of large waterways.

Leafy and woody debris from the riparian vegetation of small waterways is essential for local aquatic ecosystems. It is also an important source of carbon and nutrients for ecosystems downstream, where there is less shading and less leafy and woody debris entering the waterway. If resources are scarce and the objective of management is protecting the riverine environment, preserving the riparian vegetation along small waterways should be given the same or greater priority as preserving the riparian vegetation along large waterways.

2.5 Chemical assays for riparian vegetation management

The presence of reactive oxygen species (ROS) has long been used as an indicator of environmental stress in plants, and has recently been intensely studied. Among such ROS, hydrogen peroxide (H_2O_2) is relatively stable, and can be conveniently and accurately quantified. Thus, the quantification of plant H_2O_2 could be applied as a stress indicator for riparian and aquatic vegetation management approaches while evaluating the conditions of a plant species within a habitat. Previous studies have shown that H_2O_2 concentration of the plant tissues increases in unpreferable environmental conditions (Asaeda et al., 2020), and it is highly correlated with the intensity of a single environmental factor (Asaeda et al., 2017 Parveen et al., 2017; Chalanika De Silva and Asaeda, 2018). The relationship between H_2O_2 concentration and the intensity of each environmental factor does not vary much between seasons and phenological stages of the plant (Asaeda et al.,2020). Therefore, the H_2O_2 concentration is considered an indicator of the degree of the unpreferable condition. The Chl-a concentration and the growth parameter decreased with increasing intensity of the total H_2O_2 concentration (Coleman et al., 1989; French and Moore, 2003; Boustany et al., 2010).

2.6 Riparian macro phytic environment

Macrophyte communities are fundamental for the functioning of many river ecosystems.

However, they respond to disturbances in ecosystems and are particularly sensitive to anthropogenic influences, which negatively impact their diversity and species composition. Macrophyte associations depend on various abiotic and biotic factors . Based on this, we can identify species that are reliable indicators of changes in river ecosystems and use them as a tool to assess the ecological status of rivers. The presence and diversity of macrophytes depend on water quality, water depth, flow velocity, flow rate, hydrological conditions, water level, pH, shading, and substrate characteristics. In addition, they are also affected by biotic factors, namely the properties of species, interspecific competition, grazing, and allelopathy. Shading by riparian vegetation is an important factor in lotic ecosystems, and it also affects the distribution and abundance of aquatic plants ; thus, macrophyte diversity and their abundance reflect the quality of an ecosystem as a whole.

In recent decades, aquatic vegetation has undergone significant changes. Anthropogenic changes associated with climate change have contributed to the general decline of Diversity macrophyte species diversity and led to the homogenization of vegetation in many rivers worldwide . Such changes in macrophyte communities may also have implications for other organisms due to their crucial role within river ecosystems . The effects of anthropogenic activities on macrophyte diversity depend on the type of activity, its frequency and intensity, and the resistance of the ecosystem to a single load . Intensive agricultural activity is associated with physical modifications of aquatic habitats, such as channelization of stream reaches, which alters environmental conditions that are important for the biotic communities.

2.7 Macrophytes management

Macrophytes require managing when either too much or too little vegetation, for a given use, exists in a lake or river. Therefore, the initial step in a management programme will be to determine the types and quantities of macrophytes present, whether the macrophyte populations are expanding or declining, and the relationship(s) between the vegetation and the lake uses. Generally, uses of aquatic habitats can be divided into those that depend on the presence of vegetation (e.g., assimilative capacity, lakeshore and streambank erosion control, sediment stabilisation, nutrient stripping (a temporary function in many lakes; Moore et al. 1984; Morency & Edwards 1985), commercial macrophyte harvesting, wildlife and fisheries habitat, the conservation of rare plant species, etc.) and those that do not either directly (e.g., waterskiing, hydro-electric generation, cooling, irrigation, barge transportation, etc.) or indirectly (e.g., bilharzia-free habitat; see Van Schayck 1985) require the presence of plants. For uses depending on the presence of vegetation, determination of the optimal quantity and type of desired plants can be difficult, and precise answers are often unknown. Even the relationship between macrophytes and fish production, possibly the most studied macrophyte-use interaction, is relatively poorly quantified (Tevyashova & Tevyashova 1973; Swales 1982; Shireman et al. 1983; Tucker et al. 1983; Durochee 1984; Mikol 1985; Theurer et al. 1985; and Ploskey 1982) although the difficulty in defining this relationship may simply reflect the recent recognition that in some situations there appears to be no relationship between macrophyte abundance and fish biomass (Shireman et al. 1985)

Watershed management for macrophyte enhancement includes lake isolation , the control of non-point nutrient run-off , the control of sewage discharge (Phillips 1984), and the various methods of eutrophication control generally considered in lake restoration programmes). In-lake management for enhancement includes the planting of "desirable" macrophytes the construction of shallow areas for emergents (Kelcey 1984); contouring and decreasing the invert slope (batter) of lake sides (Bjugstad et al. 1983) or rivers (Jackson & Van Haveren 1984); selective macrophyte control (Denike & Geiger 1974) phytoplankton control by the introduction of algal herbivores (Crisman 1981) the construction of constant-level polders in pumped-storage reservoirs and lakes or rivers subject to dewatering or low-flows (Thomas et al. 1984; Richards & Lake 1985); the use of baffles to dampen wave action and thereby reduce sediment resuspension; and construction of slow-flow oxbows beside power canals and aqueducts to serve as plant refuges. Many macrophyte recessions have been reported for marine and brackish habitats (Kirkman 1978; Orth & Moore 1983, 1984; Cambridge & McComb 1984) and for freshwater systems (Dzwonko & Plazinska 1977; Jupp & Spence 1977; Phillips et al. 1978; Moret 1982; Moss & Leath 1982; Taylor 1983; Wharfe et al. 1984; Bonner et al. 1985; Hejny 1985). This phenomenon is apparently happening more frequently and becoming more important to management (Liddle & Scorgie 1980; Hanna 1983; Ikusima 1983). Examples range from the classic recession resulting from water level manipulation of previously stable lakes for hydroelectric or irrigation purposes (Quennerstedt 1958), to the decline of rare species and their replacement by more successful aliens as, e.g., has occurred in many New Zealand lakes (Johnstone 1972; Graham 1976; Coffey 1980; Howard-Williams & Vincent 1983). Therefore, the problem of macrophyte enhancement, like macrophyte control, may be either a total-vegetation problem or a species-specific problem.

2.8 Restoration of macrophytes

The macrophyte vegetation of shallow soft water lake sand wetlands is very different from that of both acidic and alkaline waters. Very characteristic are macro-phytes with an extensive root system and short leaves in a rosette, e.g., Isoetes lacustris (quillwort). These isoetids often form dense stands on the lake sediment. The highest macrophyte diversity in these lakes or wetlands can be found in the Atlantic regions of Europe, including the Netherlands. Furthermore, soft water macrophytes occur scattered throughout the world in mountainous areas, especially Isoetes species(Keeley et al., 1983; Gacia et al., 1994). Restoration of the acidified soft water lakes, which are acidified and eutrophicated as a consequence of atmospheric ammonia deposition, requires radical measures. In such cases nitrogen saturation and accumulation of organic matter in the catchment soil and the lake sediment strongly inhibits recovery. The removal of sapropelium layer in several soft water lakes in the Netherlands (Brouwer et al., 1996b) did not lead toan increase of pH and ANC. A slight decrease in pH observed in these lakes in the years following sediment removal might be due to oxygenation of the sediment after removal of accumulated, anoxic, organic matter, and a reduction of alkalinity generating processes inorganic anaerobic sediments.

The availability of nitrogen decreased somewhat after sediment removal, but ammonium remained the dominant N source. The CO_2 concentrations increased somewhat in the first year following sediment removal but decreased in the years thereafter. Some re-establishments of soft water macrophytes from the seed bank occurred in the year following sediment removal. These plants, however, declined rapidly in the following years and *J. bulbosus* and *Sphagnum* species became the dominant macro-phytes. In periods with low CO_2concentrations in the water layer, almost complete die-off of these species occurred. The removal of accumulated organic matter from the sediment of several dozens of acidified soft water lakes in the Netherlands without further measures against acidification did not lead to recovery of the water quality and vegetation. Moreover, it led to the exhaustion of the seed bank that is mostly present in the sediment.

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CHAPTER III

Spatial pattern of foliar hydrogen peroxide concentration and its implication in riparian vegetation management

3.1 Introduction

The study was conducted along the Hii river in Shimane prefecture. In both locations the permits for sampling were approved by the concerned authorities for the surveying and sampling. The aerial photos were taken during the survey which is a part of the base line survey. During the field work the samples of leaves and soil and other necessary information was carefully gathered to arrive at the conclusions.

3.2 Observation site

Field observations were conducted along the Hii River in western Japan. The Hii River, originating in the Chugoku Mountain range, flows into Shinji Lake and Naka Lake downstream before draining into the Sea of Japan. The river extends approximately 153 km and covers approximately 2017 km^2 of the total basin area. Eighty percent of the basin is covered with forest, and 20% of the downstream area is covered with rice fields and residential areas. The Hii River emits a water source to Funayuzan (1,143 m in height) located at the prefecture boundary between Shimane and Tottori prefectures, and along the way, it flows northward while joining the branches such as Kimagikawa, Akigawa, Kuno, Tataya River, Akagawa, it flows to the east in the Izumo Plain, flows through Shinji Lake, Nakaumi, and flows into the Sea of Japan through the water supply line. It is a river with a 153 km extension of the mainstream and a catchment area of 2,540 km$^{2.}$

The geology of the catchment area accounts for about 70% of the granitic rocks of the third Phase of the Cretaceous and the weathering is progressing. For this reason, many earth and sand are flowed from the upstream part, soils flowing down accumulate on the river bottom of the downstream plain part, causing the falling ability to be insufficient, and the river bottom is higher than the dam wall inside. In addition, downstream Shinji Lake and Nakaumi have small water level difference with the Sea of Japan from a few cm to 10 cm, and the river width of the Ohashi River connecting the two lakes is narrow, creating a situation where drainage is bad at the time of the flood. Looking at the water quality of the Hii River has COD value of 75% and the BOD 75% value (Hii River Mainstream), 2.7 mg / L in the central part of the water supply channel and 4.1 mg / L in the Lake Nakaumi , 4.9 mg / L of the Ohashigawa estuary, 5.1 mg / L of Shinji Lake, 0.8 mg / L downstream of the Hii River (Shinabashi bridge), 0.8 mg / L upstream of the Hii River (Satokuma Ohashi). Especially, Lake Shinji has a high value of 5.0 mg / L or more.

In contrast to most Japanese rivers, this river is characterized by sandy channels including riparian zones, except for the upstream reaches, because of a large discharge of sand due to iron mining many years ago. Although, the riparian zone of this river was once moderately vegetated, vegetation coverage has increased enormously, particularly in recent years, and is dominated by *P. australis* and *P.karka* as herbs and *Salix* spp.and *J.mandshurica* as trees. This vegetation coverage has become one of the most important issues in the management of this river (Izumo River Management Office, Shimane, Japan). Observations were conducted at 13.8 km to 15.6 km from the river mouth and along one tributary that flows into Shinji Lake (See Figure 1). Riparian vegetation in this area is located at elevations depending on species-specific needs, and the vegetation is often colonized in the layer along the slope of the riparian zone. On 13 October 2016, the location and elevation at the normal water level for all trees taller than 1 m were measured species-by-species on the right-hand-side bank from 13.9 km to 15.1 km from the river mouth, which is within the river reach.

Japanese rivers are steep and the floodplain substrate conditions are coarse and infertile. Therefore, only native plant species could grow on this substrate. However, due to rapid urbanization and human interference, the soil characteristics of these river floodplains have changed in recent years. In many floodplains, native plant species have been replaced by exotic invasive species and thick forest and vegetation has been created. Though forestation in river channel and floodplain has some good points (e.g., erosion control), it creates water blockage and increase flood magnitude. We investigated causes of forestation in Hii River where the number of *Salix pierotti* and *Juglans ailantifolia* have been increased lately. Soil nitrogen concentration was found to be higher in comparison to other Japanese river floodplains. *Phragmites spp*. Had higher biomass production. *Salix* were found to distribute closer to the water line whereas, *Juglan spp.*were distributed on the higher margin of the floodplain.

However, in the recent study it has been noticed that woody species growing in the sandbar in and along the river way, the formation of the bushes and forests are proceeding has been reported in various places in Japan. This is deeply related to the decrease in the scale and frequency of flood disturbance due to ensuring the stability of river channels and river improvement for the purpose of improving flood safety. The river's forestation is beneficial from the standpoint of river improvement, such as expansion of green spaces in rivers, erosion control of riverbeds and the like. On the other hand, the forest group causes a rise in the water surface due to a decrease in the flood flowing ability, and there is concern that the flood safety degree will decrease, such as generating a local high-speed flow. There is also great fear that the original riverbank ecosystem established under a unique environment in which disturbance caused by flooding, destruction of vegetation, bare ground, recovery of vegetation is repeated, will disappear as the forestation progresses. Indeed, it has been reported that destroying of native plants is caused by invasion of invasive alien species trees with vigorous fertility in riverbeds. In Hii River (*Phragmites karka*) which produces as much as 8000 g of organic matter annually grows abundantly, and the largest production is done in domestic rivers. In addition, the seeds of Onigurumi (*Juglans ailantifolia*) and Yanagi willow (*Salix pierotii*) mass-produced in the upstream area have settled on the banks, and the degree of tree planting has been increasing year by year. It is obvious that such a situation will greatly hinder the river management itself in the near future.

It is a common recognition both in Japan and abroad the fluctuation of plants on ordinary riverbanks depends greatly on the form of the sand flowing into the riverbank. However, Hii River is a sand river, which has different characteristics from other rivers. In this research, we aim to conduct experiments and analyzes to grasp and evaluate the actual condition of vegetation environment and forestation in Hii River. The location of the observation site is shown in **Figure 3.1**.

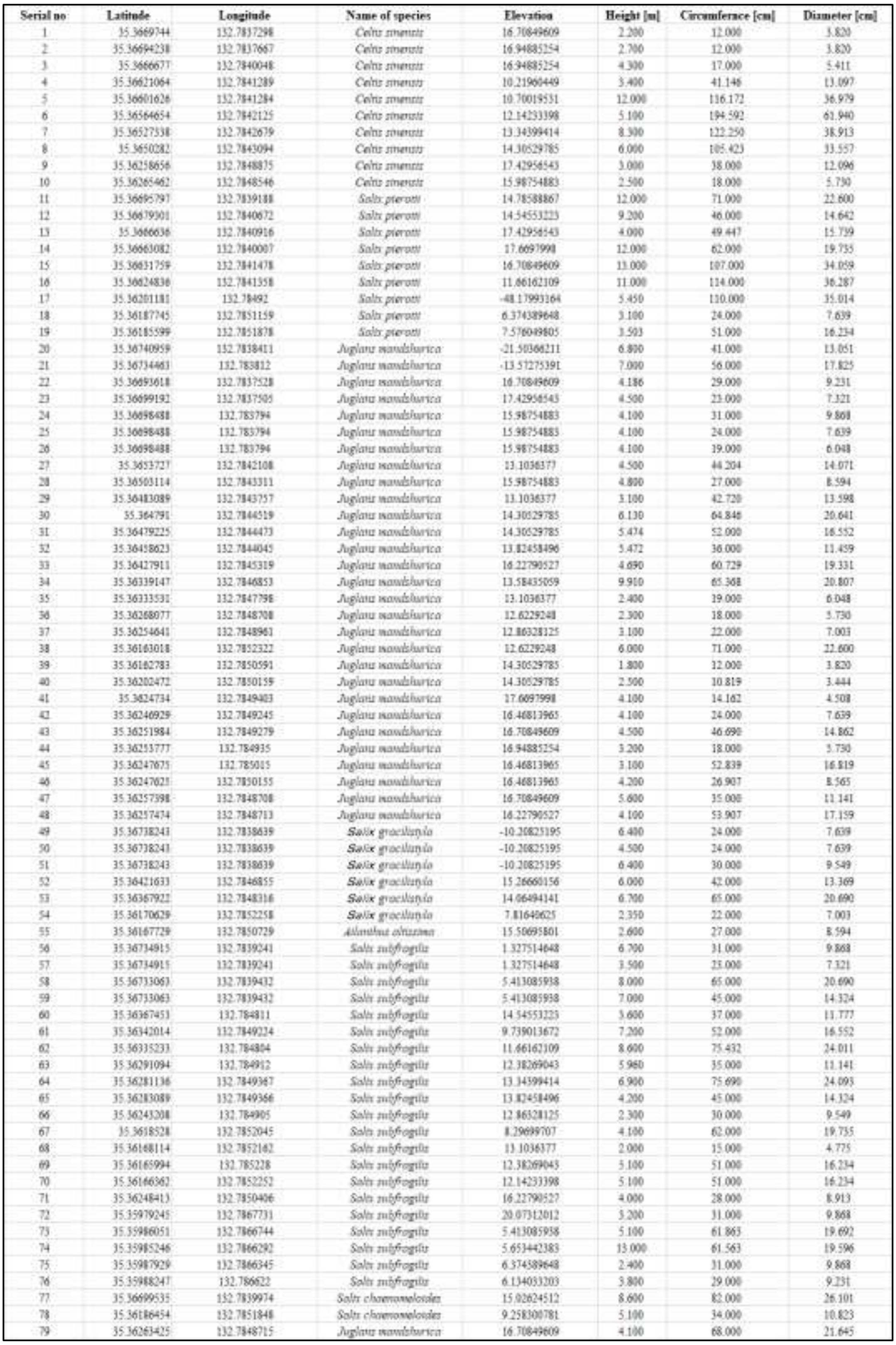
Figure 3.1. Location of observation site

3.3 Methodology

3.3.1 Soil and leaf sampling

As for tree leaf sampling, typical homogeneous areas densely populated by different species were selected after surveying the riparian zone along the river reach. Several leaves under different shading conditions were carefully collected. The locations of all species were recorded using GPS (Garmin eTrex, Garmin Ltd. Olathe, Kansas, United States), and aerial photos along the river line were taken using a drone (DJI Spreading Wing S900,DJI Japan Corporation,Tokyo,Japan). The leaf samples were sealed in plastic bags and then transported in an icebox filled with dry ice −70 °C) to the laboratory, where the samples were frozen at −80 °C until analysis. Sediment to a depth of 30 cm was sampled from the ground surface at each location and then tightly sealed in plastic bags for transport to the laboratory. The moisture content was determined as weight loss after drying at 105 °C for 24 h until no further change in weight was recorded. All aboveground shoots in the quadrat were harvested, and belowground biomass was excavated until all biomass was retrieved. These samples were oven dried for three days (or more) at 70 °C until no change in weight was recorded, and the dry weight was obtained. Also, the tree species were located along the river line and the data of the tree species location was tabulated in **Table 3.1**.

Table 3.1. Tree species location



Serial no	Latitude	Longitude	Name of species	Elevation	Height [m]	Circumference [cm]	Diameter [cm]
1	35.3669744	132.7837298	*Celtis sinensis*	16.70849609	2.200	12.000	3.820
2	35.36694238	132.7837667	*Celtis sinensis*	16.94885254	2.700	12.000	3.820
3	35.3666677	132.7840048	*Celtis sinensis*	16.94885254	4.300	17.000	5.411
4	35.36621064	132.7841289	*Celtis sinensis*	10.21960449	3.400	41.146	13.097
5	35.36601626	132.7841284	*Celtis sinensis*	10.70019531	12.000	116.172	36.979
6	35.36564654	132.7842125	*Celtis sinensis*	12.14233398	5.100	194.592	61.940
7	35.36527338	132.7842679	*Celtis sinensis*	13.34399414	8.300	122.250	38.913
8	35.3650282	132.7843094	*Celtis sinensis*	14.30529785	6.000	105.423	33.557
9	35.36258656	132.7848875	*Celtis sinensis*	17.42956543	3.000	38.000	12.096
10	35.36265462	132.7848546	*Celtis sinensis*	15.98754883	2.500	18.000	5.730
11	35.36695797	132.7839188	*Salix pierotii*	14.78588867	12.000	71.000	22.600
12	35.36679301	132.7840672	*Salix pierotii*	14.54553223	9.200	46.000	14.642
13	35.3666636	132.7840916	*Salix pierotii*	17.42956543	4.000	49.447	15.739
14	35.36663082	132.7840007	*Salix pierotii*	17.6697998	12.000	62.000	19.735
15	35.36631759	132.7841478	*Salix pierotii*	16.70849609	13.000	107.000	34.059
16	35.36624836	132.7841358	*Salix pierotii*	11.66162109	11.000	114.000	36.287
17	35.36201181	132.78492	*Salix pierotii*	-48.17993164	5.450	110.000	35.014
18	35.36187745	132.7851159	*Salix pierotii*	6.374389648	3.100	24.000	7.639
19	35.36185599	132.7851878	*Salix pierotii*	7.576049805	3.503	51.000	16.234
20	35.36740959	132.7838411	*Juglans mandshurica*	-21.50366211	6.800	41.000	13.051
21	35.36734463	132.783812	*Juglans mandshurica*	-13.57275391	7.000	56.000	17.825
22	35.36693618	132.7837528	*Juglans mandshurica*	16.70849609	4.186	29.000	9.231
23	35.36699192	132.7837505	*Juglans mandshurica*	17.42956543	4.500	23.000	7.321
24	35.36698488	132.783794	*Juglans mandshurica*	15.98754883	4.100	31.000	9.868
25	35.36698488	132.783794	*Juglans mandshurica*	15.98754883	4.100	24.000	7.639
26	35.36698488	132.783794	*Juglans mandshurica*	15.98754883	4.100	19.000	6.048
27	35.3653727	132.7842108	*Juglans mandshurica*	13.1036377	4.500	44.204	14.071
28	35.36503114	132.7843311	*Juglans mandshurica*	15.98754883	4.800	27.000	8.594
29	35.36483089	132.7843757	*Juglans mandshurica*	13.1036377	3.100	42.720	13.598
30	35.364791	132.7844519	*Juglans mandshurica*	14.30529785	6.130	64.846	20.641
31	35.36479225	132.7844473	*Juglans mandshurica*	14.30529785	5.474	52.000	16.552
32	35.36458623	132.7844045	*Juglans mandshurica*	13.82458496	5.472	36.000	11.459
33	35.36427911	132.7845319	*Juglans mandshurica*	16.22790527	4.690	60.729	19.331
34	35.36339147	132.7846853	*Juglans mandshurica*	13.58435059	9.910	65.368	20.807
35	35.36333531	132.7847798	*Juglans mandshurica*	13.1036377	2.400	19.000	6.048
36	35.36268077	132.7848708	*Juglans mandshurica*	12.6229248	2.300	18.000	5.730
37	35.36254641	132.7848961	*Juglans mandshurica*	12.86328125	3.100	22.000	7.003
38	35.36163018	132.7852322	*Juglans mandshurica*	12.6229248	6.000	71.000	22.600
39	35.36162783	132.7850591	*Juglans mandshurica*	14.30529785	1.800	12.000	3.820
40	35.36202472	132.7850159	*Juglans mandshurica*	14.30529785	2.500	10.819	3.444
41	35.3624734	132.7849403	*Juglans mandshurica*	17.6697998	4.100	14.162	4.508
42	35.36246929	132.7849245	*Juglans mandshurica*	16.46813965	4.100	24.000	7.639
43	35.36251984	132.7849279	*Juglans mandshurica*	16.70849609	4.500	46.690	14.862
44	35.36253777	132.784935	*Juglans mandshurica*	16.94885254	3.200	18.000	5.730
45	35.36247675	132.785015	*Juglans mandshurica*	16.46813965	3.100	52.839	16.819
46	35.36247625	132.7850155	*Juglans mandshurica*	16.46813965	4.200	26.907	8.565
47	35.36257398	132.7848708	*Juglans mandshurica*	16.70849609	5.600	35.000	11.141
48	35.36257474	132.7848713	*Juglans mandshurica*	16.22790527	4.100	53.907	17.159
49	35.36738243	132.7838639	*Salix gracilistyla*	-10.20825195	6.400	24.000	7.639
50	35.36738243	132.7838639	*Salix gracilistyla*	-10.20825195	4.500	24.000	7.639
51	35.36738243	132.7838639	*Salix gracilistyla*	-10.20825195	6.400	30.000	9.549
52	35.36421633	132.7846855	*Salix gracilistyla*	15.26660156	6.000	42.000	13.369
53	35.36367922	132.7848316	*Salix gracilistyla*	14.06494141	6.700	65.000	20.690
54	35.36170629	132.7852258	*Salix gracilistyla*	7.81640625	2.350	22.000	7.003
55	35.36167729	132.7850729	*Ailanthus altissima*	15.50695801	2.600	27.000	8.594
56	35.36734915	132.7839241	*Salix subfragilis*	1.327514648	6.700	31.000	9.868
57	35.36734915	132.7839241	*Salix subfragilis*	1.327514648	3.500	23.000	7.321
58	35.36733063	132.7839432	*Salix subfragilis*	5.413085938	8.000	65.000	20.690
59	35.36733063	132.7839432	*Salix subfragilis*	5.413085938	7.000	45.000	14.324
60	35.36367453	132.784811	*Salix subfragilis*	14.54553223	3.600	37.000	11.777
61	35.36342014	132.7849224	*Salix subfragilis*	9.739013672	7.200	52.000	16.552
62	35.36335233	132.784804	*Salix subfragilis*	11.66162109	8.600	75.432	24.011
63	35.36291094	132.784912	*Salix subfragilis*	12.38269043	5.960	35.000	11.141
64	35.36281136	132.7849367	*Salix subfragilis*	13.34399414	6.900	75.690	24.093
65	35.36283089	132.7849366	*Salix subfragilis*	13.82458496	4.200	45.000	14.324
66	35.36243208	132.784905	*Salix subfragilis*	12.86328125	2.300	30.000	9.549
67	35.3618528	132.7852045	*Salix subfragilis*	8.29699707	4.100	62.000	19.735
68	35.36168114	132.7852162	*Salix subfragilis*	13.1036377	2.000	15.000	4.775
69	35.36165994	132.785228	*Salix subfragilis*	12.38269043	5.100	51.000	16.234
70	35.36166362	132.7852252	*Salix subfragilis*	12.14233398	5.100	51.000	16.234
71	35.36248413	132.7850406	*Salix subfragilis*	16.22790527	4.000	28.000	8.913
72	35.35979245	132.7867731	*Salix subfragilis*	20.07312012	3.200	31.000	9.868
73	35.35986051	132.7866744	*Salix subfragilis*	5.413085938	5.100	61.863	19.692
74	35.35985246	132.7866292	*Salix subfragilis*	5.653442383	13.000	61.563	19.596
75	35.35987929	132.7866345	*Salix subfragilis*	6.374389648	2.400	31.000	9.868
76	35.35988247	132.786622	*Salix subfragilis*	6.134033203	3.800	29.000	9.231
77	35.36699535	132.7839974	*Salix chaenomeloides*	15.02624512	8.600	82.000	26.101
78	35.36186454	132.7851848	*Salix chaenomeloides*	9.258300781	5.100	34.000	10.823
79	35.36263425	132.7848715	*Juglans mandshurica*	16.70849609	4.100	68.000	21.645

3.3.2 Determination of photosynthetic pigments

Approximately 25-50 mg of fresh leaf tissues were prepared in three replicates with a leaf tissue punch. The pigments were extracted with N,N-dimethylformamide for 24 hours and measured with a spectrophotometer (Shimadzu, UV Mini 1210). Chlorophyll a and b and the carotenoid concentrations were calculated based on Wellburn (1994).

3.3.3 Plant tissue preparation for the stress assay

Stress assay molecules such as hydrogen peroxide (H_2O_2), catalase (CAT), and ascorbate peroxidase (APX) were determined spectrophotometrically (Shimadzu UV mini 1210). Plant leaves were extracted by grinding the freeze-dried (with liquid nitrogen) samples (~500 mg) with ice-cold 50 mM phosphate buffer at pH 6.0; polyvinylpyrrolidone (PVP) was added for to mask the effect of phenolic compounds in the plant materials. The extract was centrifuged at 5000 rpm at 40 °C for 15 min, and the supernatant was separated and incubated at −80 °C until further analysis. For each analysis, every extract was performed in triplicate, and the results were recorded on a fresh weight basis.

3.3.4 H_2O_2 Concentrations

The content of H_2O_2 was determined using a preprepared standard curve for a known concentration series. The reaction mixture contained 750 µL of enzyme extract and 2.5 mL of 1% $TiSO_4$ in 20% H_2SO_4 (*v*/*v*). The mixture was centrifuged at 5000 rpm for 15 min at room temperature, and the intensity of the yellow color developed was measured spectrophotometrically at 410 nm based on Jana and Choudhuri (Jana & Choudhuri, 1982). The results are presented as µmol/g FW.

3.3.5 CAT activity

CAT activity (EC 1.11.1.6) was measured according to(Aebi, 1984). In brief, 100 µL of 10 mM $H_2O_2$and 2.0 mL of 100 mM potassium phosphate buffer (pH 7.0) were added to a cuvette, and 500 µL of enzyme extract was added to initiate the reaction. The reduction in absorbance at 240 nm was recorded for 3 min at 10-s intervals. CAT activity was calculated using the extinction coefficient of 40 Mm/cm, and the results are presented as µmol/min/g FW.

3.3.6APX activity

APX activity (EC 1.11.1.11) was determined according to Nakano and Asada (Nakano & Asada, 1981). The reaction mixture contained 100 μL of enzyme extract and 200 μL of 0.5 mM ascorbic acid in 50 mM potassium phosphate buffer (pH 7.0). The reaction was started after adding 60 μL of 1 mM H_2O_2. The decrease in absorbance at 290 nm was recorded every 10 s. APX activity was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1}, and the results are presented as µmol/min/g FW.

3.3.7 Total carbon, total nitrogen and total phosphorus in sediment

Total carbon (TC) and total nitrogen (TN) in sediment were assessed using a CHN elemental analyzer (Yanako CHN coder MT-5 and Autosampler MTA-3, manufactured by Yanako CO., Ltd., Kyoto, Japan). Sediment subsamples of each section were dried at a temperature of 105 °C to determine the water content. After grinding to a powder, total phosphorus (TP) in the sediment was determined by digesting the samples with HNO_3-$HCLO_4$(Alpha, 1995), and the molybdenum blue colorimetric method was applied.

3.4 Results

3.4.1 H_2O_2 concentration related to site elevation

The *P. australis*, *P. karka* and *S. pierotii* exhibited a significant increase in H_2O_2 with increasing elevation from 0.3 m to 4 m (*P. australis* $F = 507.8$, $p < 0.05$; *P. karka* $F = 39.2$, $p < 0.05$; and *S. pierotii* $F = 142.5$, $p < 0.05$). Conversely, *J. mandshurica* showed the opposite trend, where the H_2O_2 concentration gradually decreased with increasing elevation ($F = 15.62$, $p < 0.05$) as shown in **Figure 3.2(a) and Figure 3.2 (b)**. The highest H_2O_2 concentration (47.1 µmol/g FW) was found in *S. pierotii* located at 4 m from the normal water level. *P. karka* had a relatively lower concertation of the same at all elevations, with a minimum value of 2.14 µmol/gFW at 1 m elevation. Among all four species, the herb species *P.australis* and *P.karka* displayed lower H_2O_2 concentrations than did the tree species *J. mandshurica* and *S. pierotii*.

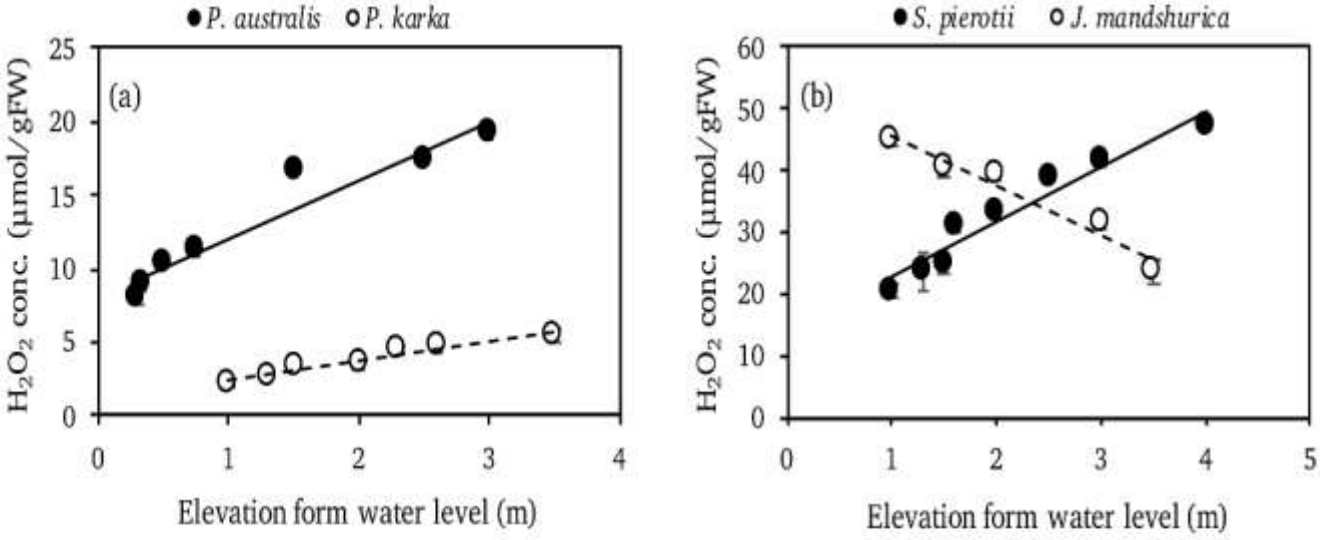


Figure 3.2. Variations in foliar hydrogen peroxide concentrations with respect to elevation for (a) herbaceous species, and (b) tree species.

3.4.2 H_2O_2 concentration related to edaphic condition

The H_2O_2 concentration gradually decreased with increasing soil moisture content for all of the examined species, except for *J. mandshurica* (Pearson's correlation; *P. australis* $R = 0.90$, $p < 0.01$; *P. karka* $R = 0.69$, $p < 0.05$; and *S. pierotii* $R = 0.94$, $p < 0.01$) as shown in **Figure 3.3 (a)** and **Figure 3.3 (b)**. H_2O_2 concentration in *J. mandshurica* decreased with increasing soil TN concentration (Pearson's correlation; $R = 0.84$, $p < 0.01$), and there was no significant trend for the other species (Pearson's correlation; *P. australis* $R = 0.41$, $p < 0.01$; *P. karka* $R = 0.19$, $p < 0.05$; and *S. pierotii* $R = 0.35$, $p < 0.01$) as shown in **Figure 3.3 (c)** and **Figure 3.3 (d)** . Regarding soil TP, there was no specific trend in H_2O_2 concentration for the herb species (Pearson's correlation; *P. australis* $R = 0.23$, $p < 0.01$; *P. karka* $R = 0.84$ $p < 0.01$) as shown in **Figure 3.3 (e)**. However, the H_2O_2 concentration in the tree species increased significantly with soil TP for *S. pierotti* (Pearson's correlation; $R = 0.96$, $p < 0.01$) and decreased for *J. mandshurica* (Pearson's correlation; $R = 0.78$, $p < 0.01$), respectively as shown in **Figure 3.3 (f)**. .

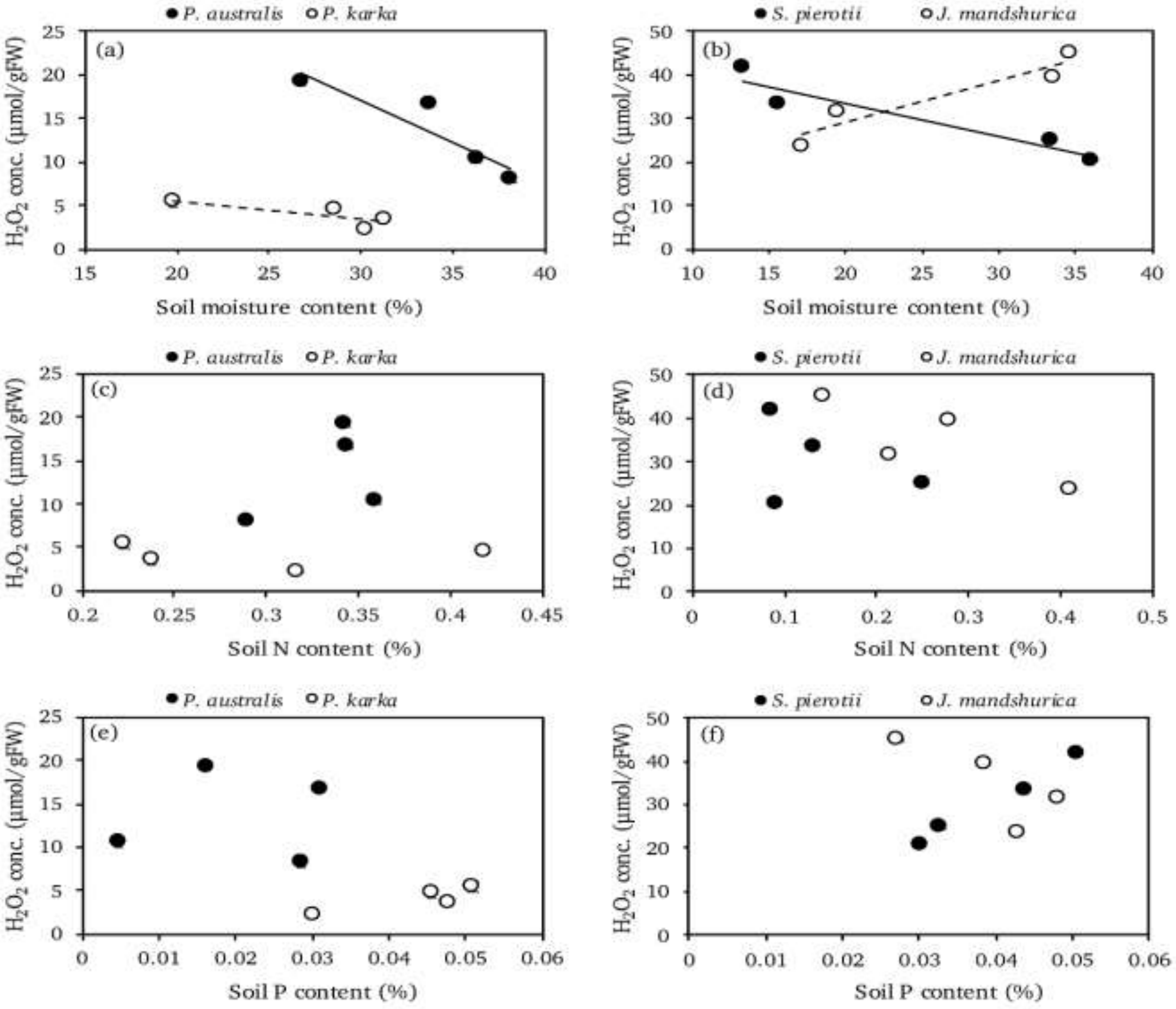


Figure 3.3. Species-specific foliar H_2O_2 concentrations with respect to (a-b) soil moisture, (c-d) total nitrogen (TC) content, and (e-f) total phosphorous (TP) content of soil.

3.4.3 Chlorophyll content and H_2O_2 concentration

Total chlorophyll contents and ration of chlorophyll a and chlorophyll b were differed between herb species and tree species. The total chlorophyll concentrations were higher for the herb species than that for the tree species as shown in **Figure 3.4 (a)** and **Figure 3.4 (b)**. In terms of the ratio of chlorophyll a and chlorophyll b, negative trends were observed for H_2O_2 content for all species (Pearson's correlation; *P. australis* $R = 0.68$, $p < 0.01$; *P. karka* $R = 0.86$, $p < 0.05$; *S. pierotii* $R = 0.81$, $p < 0.01$; and *J. mandshurica* $R = 0.82$, $p < 0.01$) while positive trends were observed for total chlorophyll content as shown **Figure 3.5 (a)** and **Figure 3.5 (b)**.

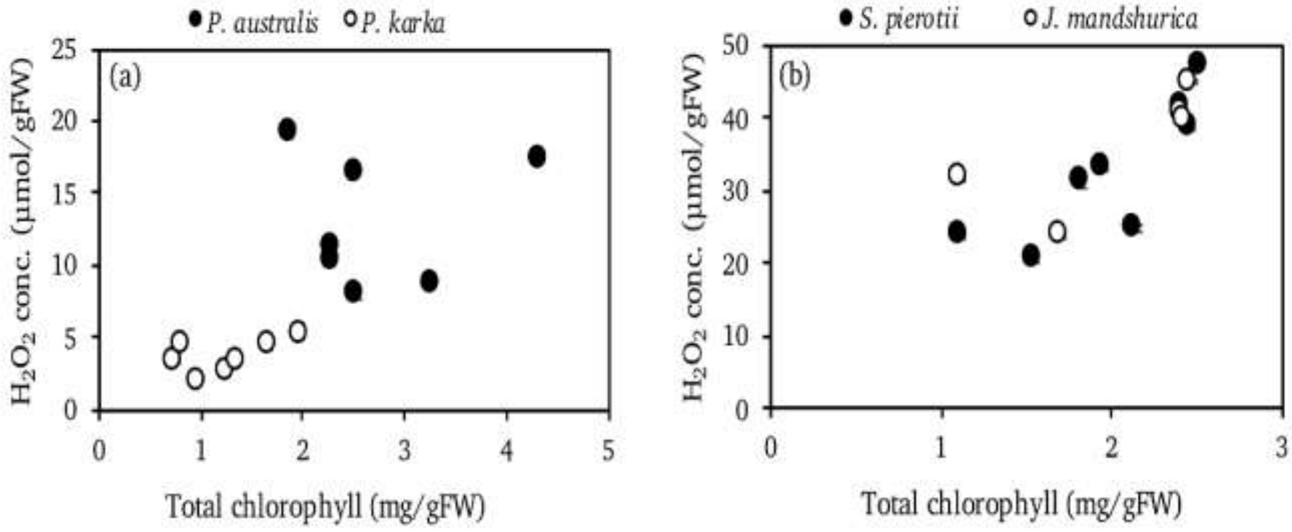


Figure 3.4. Foliar H_2O_2 concentration of (a) *P. australis* and *P. karka*, and (b) *S. pierotii* and *J. mandshurica* as a function of foliar total chlorophyll content.

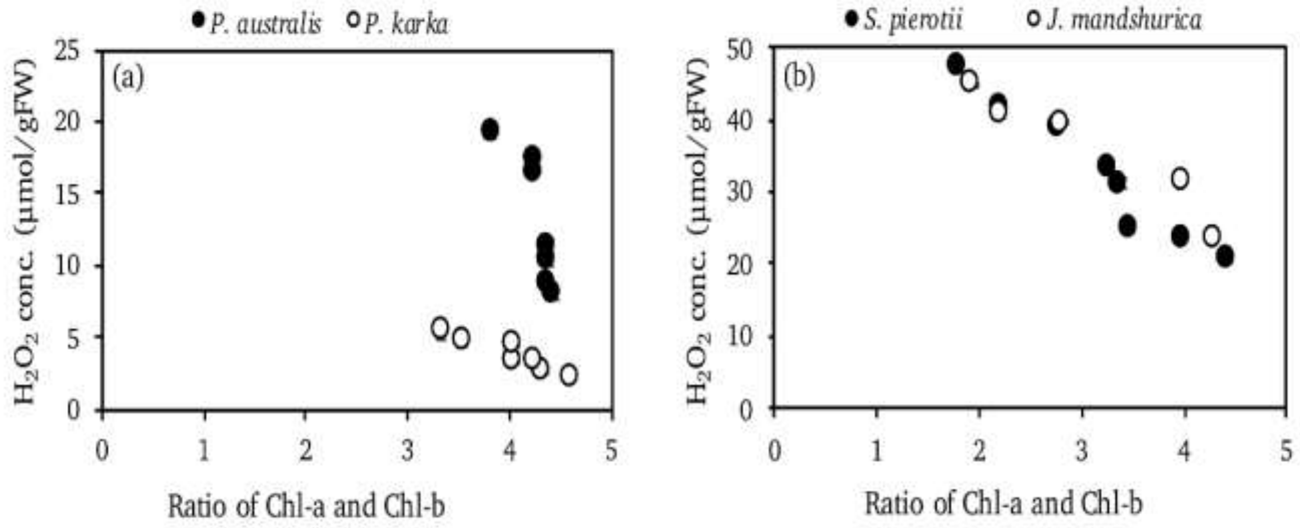


Figure 3.5. H_2O_2 concentration of (a)*P. australis* and *P. karka*, and (b)*S. pierotii* and *J. mandshurica* as a function ratio between chlorophyll a and chlorophyll b content of leaves.

3.4.4 CAT activity of plants

When the respective CAT activities values are plotted as a function of H_2O_2 concentration, clear positive correlations are found for all four species (Pearson's correlation; *P. australis* R = 0.85, $p < 0.05$; *P. karka* $R = 0.84$, $p < 0.05$; *S. pierotii* $R = 0.90$, $p < 0.05$; and *J. mandshurica* $R = 0.89$, $p < 0.05$) as shown in **Figure 3.6 (a)** and **Figure 3.6 (b).** with the highest value (3.73 µmol/min/g FW) was found in *P. australis* at 3 m and the lowest (0.43 µmol/min/g FW) was found in *S. pierotii* at 1 m.

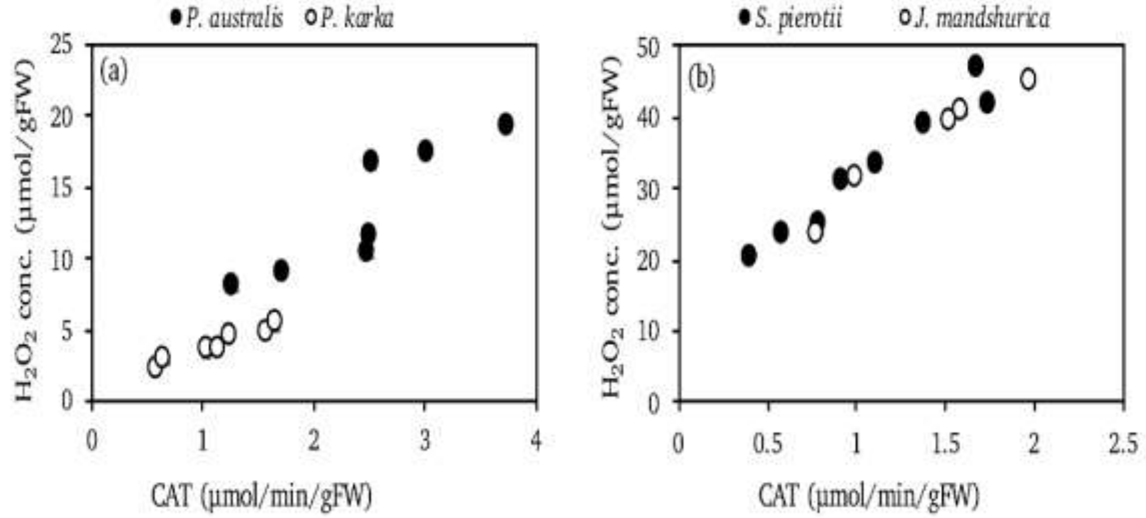


Figure 3.6. Foliar H_2O_2 concentration of (a) *P. australis* and *P. karka*, and (b) *S. pierotii* and *J. mandshurica* as a function of foliar catalase (CAT) activity

3.4.5 Site elevation and plant distribution

Total biomass of both *P. australis* and *P. karka* decreased significantly with increasing elevation (Pearson's correlation; *P. australis* $R = 0.95$, $p < 0.05$ and *P. karka* $R = 0.71$, $p < 0.05$) as shown in **Figure 3.7**. Compared to *P. karka*, *P. australis* had a higher decreasing rate. However, the below ground biomass of both species did not exhibit a difference with the elevation, though the *P. karka* exhibited slight increment in biomass at higher elevation (3.5 m). The distribution of two tree species *S. pierotii* and *J. mandshurica* exhibited two different trends with the elevation from the normal water level. The number of *S. pierotii* decreased with elevation (Pearson's correlation; *S. pierotii* $R = 0.99$, $p < 0.05$), steadily and whereas *J. mandshurica* increased up to 3 m elevation and then decreased at higher elevations. Both species exhibited same tree density at their maximum existence elevations as shown in **Figure 3.8**.

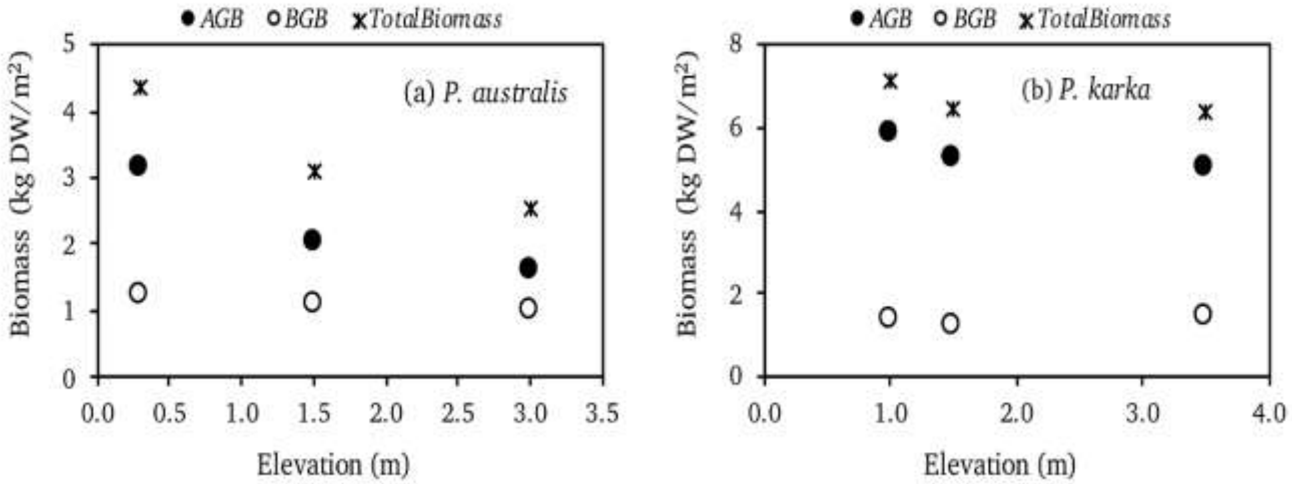


Figure 3.7. Distribution of the aboveground, belowground, and total biomass of *P. australis* and *P. karka* with respect to elevation from normal water level.

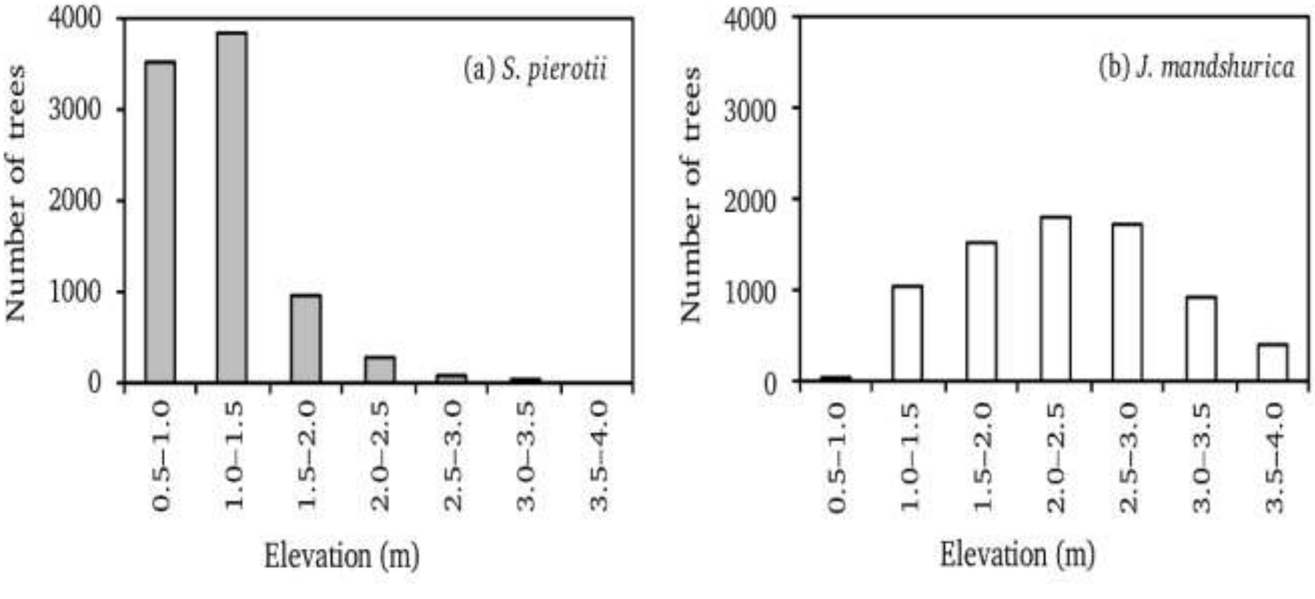


Figure 3.8. Distribution of the tree species, (a) *S. pierotii*, and (b) *J. mandshurica* with elevation from the normal water level. Number of trees represent tree count in each location.

3.5 Discussion

Riparian vegetation can be found at species-specific elevations, and colonization often occurs in the layer along the slope of the riparian zone (Gregory et al. 1991; Naiman and Décamps 1997). Regardless, the mechanisms underlying this phenomenon have not been clarified to date. Riparian vegetation is highly dependent on flood flows; vegetation is flushed by such flows (Asaeda et al. 2011a; Friedman and Lee 2002) and severely destroyed by gravel deposition during floods (Asaeda and Sanjaya 2017; Sanjaya and Asaeda 2017). At the same time, however, seeds are spread downstream by flood water (Hernandez-Leal et al. 2019; Nilsson et al. 2010), followed by colonization at sites with favorable conditions (Herberg et al. 2017; Rashid et al. 2013; Rashid et al. 2019). In the Hii River, the riparian zone has become covered with thick vegetation in the last 10 years. Typical herb species include *P. karka*, which is particularly rich along this river, and *P. australis*, which is abundant all over Japan. Major tree species are *S. pierotii*, a species related to *Salix subfragilis*, that is a dominant species along Japanese rivers, and *J. mandshurica*, another species common to Japanese river systems (Koarai 2015). A number of studies have indicated that *P. australis* prefers areas close to water (Asaeda et al. 2005; Benoit and Askins 1999). Although often found close to water, the preferred zone of *P. karka* has not yet been determined. *Salix* spp. normally colonize moisture-rich soils (Azami et al. 2012; Nakamura et al. 2002; Niiyama 2008a, b), and *J. mandshurica* normally colonizes riparian zones with a little high elevation; overall, seed dispersal highly depends on river flows (Stromberg et al. 1996; Trémolières et al. 1998).

3.5.1 Site elevation and vegetation colonization

In the study area, soil moisture gradually declined with elevation in the observed range between 0 to 4 m. The riparian zone substrate in this river uniformly sand with D_{50}=0.1-0.5mm, that had been transported from upstream and accumulated for centuries. Thus, there is no effect of substrate particle size on the colonization of each species. The surrounding area of the river is flat and nearly the same elevation as the river, except for levees between the river and a rice field. Most of the area is used as a rice field and is impounded with water. Therefore, the moisture gradient is generated along the ground surface from the river to the levee by the difference in elevation between the ground surface and the seepage connecting the rice field water to the river water. The seepage deepens from the ground surface on the slope of the levee. The capillary height in the area is approximately 0.5 m.

Riparian vegetation plays a vital role in protecting banks from erosion, controlling flow velocity during heavy floods, increasing riparian infiltration, trapping, and stabilizing sediment (Gurnell 2014). Thus, the distribution of species-specific elevations in the riparian zone is due to the effect of the soil moisture content (Takahashi and Nakamura 2010; Ye et al. 2019); for example, *P. australis* requires soil moisture at 26% to 38%, *P. karka* at 19% to 30%, and *S. pierotti* at 13% to 36%. Except for *J. mandshurica*, the H_2O_2 concentration was significantly lower with higher moisture content for each species. This result indicates that a higher level of moisture in a species-specific range is preferable for these species.

Many studies have reported that reed communities always inhabit a river bank close to the water (Hudon et al. 2016; Volesky et al. 2017), whereas trees occupy higher elevations (Gurnell et al. 2016). Stomata close in response to reduced soil moisture, which then decreases photosynthetic activity during exposure to high solar radiation, which accelerates accumulation of H_2O_2 (Chaves and Oliveira 2004).

J. mandshurica displayed an opposite trend in terms of the relationship between H_2O_2 concentration and moisture content. Nonetheless, the moisture contents of the sites containing this species were nearly the same as those of *S. pierotti* and in the same range of the other species. Therefore, a high H_2O_2 concentration was not due to a low soil moisture concentration. Furthermore, there was no clear trend in TN concentration with elevation or species-specific site distribution, though there was a clear increasing trend for TP concentration with elevation. The ratio of C/N was within 8.6 to 12.9 at all elevations and species-specific sites, with total nitrogen mainly depending on natural sources (Davidson and Janssens 2006; Lal 2004). Additionally, in contrast to other species, *J. mandshurica* normally grew at the sites already colonized by other species. These trees are tall and highly competitive with other species, though *J. mandshurica* need a relatively fertile substrate (Hättenschwiler 2005; Westman 1981). In addition, their seeds are large compared to those of other species and have a strong shell, and they are more tolerant to low soil moisture conditions. Thus, it appears that for *J. mandshurica,* the specific distribution of H_2O_2 contents and the growth at a certain elevation are not due to moisture content but to a sufficient amount of nitrogen.

3.5.2 Soil nutrient and plant stress

Higher soil nutrient content leads to reduced abiotic stress, and thus plant diversity and coexistence may increase due to less competition (Kepfer-Rojas et al. 2019). However, the opposite scenarios have also been reported. Increasing nutrient content can favors competitive species that are capable of efficiently capturing resources (Dingaan et al. 2017). Plant growth in riparian ecosystem depends on nutrient-rich organic matter and on the dissolved nutrients in the water (Asaeda et al. 2011b). In this study, H_2O_2 concentration in *J. mandshurica* decreased with increasing soil TN which suggests that this herb was favored soil TN. However, H_2O_2 concentrations in other species were very weakly correlated with soil TN. However, increased soil TP appeared to be stressful for both tree species, whereas the herbs were not stressed by the availability of the same.

3.5.3 Site elevation and plant stress

The results of this study indicate that with increasing oxidative stress, the chl *a* and chl *b* ratio correlated negatively with the H_2O_2 content in all species. Chlorophyll *a* is the main pigment responsible for the photochemical reaction, and chl *b* supports chl *a* by supplying more light energy. Under slight darkness, when chl *a* performs poorly, chl *b* has a major role in supplying more light energy to chl *a* to maintain efficient photosynthesis. Thus, chl *b* is more subjected to stress under low-light conditions. Previous studies have suggested that plants adapted to shade tend to have a lower chl *a* and *b* ratio, suggesting a lower amount of light-harvesting proteins and an association with the reaction center complex (Leong et al. 1985). However, stomata close associated with low-moisture conditions, resulting in a carbon dioxide deficit and a surplus of light energy. This process increases oxidative stress and reduces the total chlorophyll concentration and the ratio of chl *a* and *b*, thus this scenario is directly related to the oxidative stress generated by a surplus of ROS. Indeed, it has been clearly recognized that H_2O_2 is a potent inhibitor of photosynthesis because even at a minimal level, it can reduce CO_2 fixation by half through the enzymes of the Calvin cycle (Foyer and Shigeoka 2010).

3.5.4 Plant stress and antioxidant activities

In our study, all four plant species showed a strong positive correlation between hydrogen peroxide generation and antioxidant activity. This result clearly indicates that scavenging activity is initiated with hydrogen peroxide generation. Although H_2O_2 can act as a signaling molecule, CAT directly transforms hydrogen peroxide into H_2O and 1/2 O_2, which is important for detoxification (Cuypers et al. 2011).

3.5.5 Tissue H_2O_2 concertation in vegetation management

Vegetation management requires monitoring of plants. Common approaches are adaptive management to understand the condition of plants, where the growth and prosperity or shrinkage of stands are monitored for a long period. It is extremely time consuming and costly. The current approach used in the study quantifies the environmental stress in relatively no time, which involves a very simple methodology of analysis there by helping to quantify the environmental stress associated with different species and to understand the level of risk associated with each species and manage them accordingly (Asaeda et al. 2020). This method also helps to restore the species that are on a verge of extinction by examining the favorable location of colonization quantifying the environmental stress in a short period.

3.6 Conclusion

Recently managing the vegetation seems to be a potential problem in several places around the globe. Although, there are several approaches that are being tested on trial-and-error bases there is not any suitable method developed yet to help manage the biodiversity. There are several traditional techniques which are being followed by several vegetation management authorities but unfortunately, they are time consuming and the costly methods.

The results of the current study show the effect of elevation gradient and moisture variation on the abundance of different species along a riparian zone. Species-specific zones of colonization or recruitment were clearly revealed using hydrogen peroxide as an environmental stress quantification index. Additionally, because most plant species face environmental stress, the current study proposes a rapid, efficient, and reliable monitoring system for vegetation management. With this method, only a relatively simple chemical analysis is involved when assessing oxidative stress, which is measured in the form of the hydrogen peroxide content. In measuring oxidative stress, it is important to truly understand the intensity of the stress, predict probable outcomes, and take appropriate measures to protect vegetation accordingly.

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CHAPTER IV

Impact of regulated water levels on willows (*Salix subfragilis*) at a flood-control dam, and the use of hydrogen peroxide as an indicator of environmental stress

4.1 Introduction

Case study was conducted along the tributaries of Miharu dam , Fukushima , Japan . Japan with a steep slope of the country needs to build a dam to stably acquire water resources. Currently there are few large rivers without dams in Japan.

The Japanese have stopped small and medium rivers from the old days, to build a reservoir, and use the water for agriculture. In recent years, a multipurpose dam with several uses such as flood control function and irrigation, water supply and sewage system, industrial water, hydraulic power generation etc. are being constructed to prevent flooding. However, construction of the dam has problems. One of them is to greatly influence the ecosystem. First, since the vast construction of the dam requires extensive land, it is necessary to cut down the forests of the construction site. As a result, creatures living around the dam will lose their housing and will have to move to other areas. Also, if the river is blocked by the dam and the continuity is lost, it will affect the movement and breeding of aquatic life using it. Furthermore, when the supply flow rate to the downstream side decreases due to the reservoir of the dam, the flooding of sandbanks and riverbanks decreases, many plants grow and the forestation within the river way is promoted. Accumulation of nutrient salts by biomass increased with tree planting, reuse of nutrient salts produced by decomposition of carcasses, and repetition of this circulation promote eutrophication in the lake. The problems brought about by the construction of the dam are varied in this way.

In addition, invasion of alien species also has a major influence on Japanese ecosystems. Along with the globalization of the economy, these have intruded intentionally, such as relinquishing breeding, and intentionally invade the home country beyond the original habitat intentionally with the movement of goods. In June 2004, the "Act on Prevention of Damages on Ecosystems by Specified Alien Species" (abbreviated as "Outbred Organism Act") was promulgated. Under this Act, it designates foreign organisms that may cause damage to ecosystems or the like to secure biodiversity as specified alien species, restrict their imports, regulate their handling, and take measures appropriately. Due to invasion of alien species, problems such as the reduction and extinction of the number of endemic species due to predation, the destruction of the ecosystem base, the destruction of the native species by the alien species, and the genetic disturbance caused by crossing are becoming serious. In recent years, social interest has been increasing due to problems such as food damage of agricultural crops caused by excessive breeding of alien species and infectious diseases of animal media.

Until now, the main reason behind the dam construction projects reasons such as securing the amount of water in the lake, clarifying the amount of water storage possible, preventing eutrophication due to the death of trees, maintaining the quality of the lake, and decreasing fallen trees caused by the waves Trees have been removed. However, in recent years there is a movement to minimize the removal of trees in the dam lake from the viewpoints of nature conservation, soil stabilization by trees, economic cost reduction, The Miharu dam that I covered is one of them. The Miharu dam is a dam of the seasonal level fluctuation system, it lowers the water level during summer where rain is heavy and prevents the flood by adjusting the water discharge amount to raise the water level in the winter with little rain. This is completely opposite to the natural condition where the water level rises during the summer of the lakes in nature and the water level declines in winter.

Also, this dam has the characteristic that a front reservoir with a gentle slope is installed. This pre-reservoir has the role of preventing sediment flowing from the river and suspended matter, which causes deterioration of water quality, to flow into the main dam, and at the same time plants are in place where plants are inhabited by the accumulated sand. It is ecologically and physiologically important to study the dynamics of living things in the special environment of such a Miharu dam **Figure. 4.1**. Observation from the construction of the Miharu Dam so far revealed that the *Salix subfragilis* spread out around the reservoir. alien species such as angulatus (*Sicyos angulatus*) are increasing.

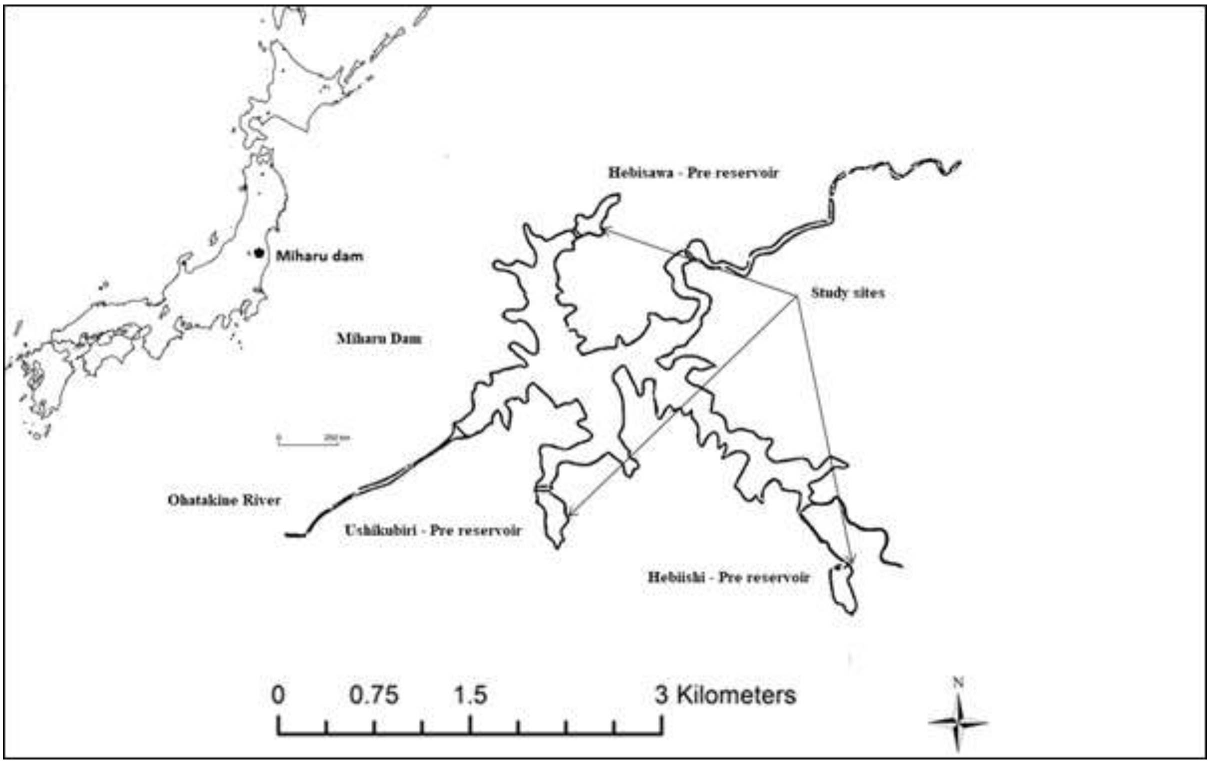

Figure 4.1. Location of the observation site

In this research, we aimed to analyze the environmental stress response of *Salix subfragilis* due to water level fluctuation and stress caused due to the invasive species(*Sicyos angulatus*).

4.2 Observation site

Miharu dam is the research site of this study, which is in Miharu Town, Tamura-gun, Fukushima Prefecture, and is a multipurpose dam under the direct control of the Ministry of Land, Infrastructure, Transport and Tourism built in Otakine River, the right branch of the Abukuma River. It is located about 10 km upstream from the junction of the Abukuma River, the catchment area is 226.4 km ^{2}, and the flooded area is 2.9 km^2. Construction of a rolling work started in March 1990, and reached the discharge water level in December 1997, normal dam operation began in April 1998.

The Miharu dam will reduce the flooding of the river by lowering the water level of the dam to EL.318.0 m between 11th June to 10th October during the flood period, alleviating flood damage in the Otakine River and Abukuma River basin. In addition, since the average annual rainfall around Miharu dam is as small as 1200 mm (average of 1800 mm in Japan), during the non-flood period from October 11th to June 10th , the EL is always full level EL 326.0 m by raising the water level to the vicinity, they are carrying out various water-saving functions such as securing water for living, agriculture, and industrial use. There is a difference of about 8.0 m in the water level between flood and non-flood, and the water level fluctuates relatively quickly within 2 weeks.

Hebisawa reservoir

This has abundant willow species (*Salix subfragilis*), and *Sciyos angulatus* are growing. The distance between two lake shores during flooding is about 120 m in wide places. The big difference with other spots is that the river is diverging into two, and a flatland is formed between the river and the river. In addition, it was also confirmed that a cormorant weed (*Trapa japonica*) which is a year-old grass plant grows in the part of the lake.

Ushikubiri reservoir

Salix subfragilis species and *Sciyos angulatus* are also grown in the reservoir in the pre-cattle creek river reservoir. The width in between the two lake shores at the time of flooding is near about 95 m, which is considered to be the point where the slope is the steepest as a whole from the distance and altitude of the shore as compared with other reservoirs. Also, there were many crawlers in the lake.

Hebiishi reservoir

Salix *subfragilis* and *Sciyos angulatus* have also grown in the Hebiishi pre reservoir. There were many flood places, where are there are no any *Sciyos angulatus*. The width of the shore lane at the time of flooding is nearly 200 m at a wide point, which is the widest compared to other reservoirs.

4.3 Methodology

In July 2008, several 10 m × 10 m quadrats were established on the slopes of the three front-reservoirs of Miharu Dam (six at Hebisawa (Hs), seven at Ushikubiri (Us), and six at Hebiishi (Hi)) and equally distributed over the drawdown zones. The abundance and location of each tree species were recorded, as well as the tree height and diameter at the breast height (DBH, 130 cm from the ground surface) of all individual trees in each quadrat. In June 2016, the location, height, and DBH of all individual trees in the drawdown zones of the three front reservoirs were recorded, and aerial photos were taken (50 m height). The number of remaining trees in each quadrat was then counted.

Sediments were collected from the ground surface at the center of each quadrat during each sampling period, and moisture content was determined as the weight lost after drying at 105°C for 24 hr. Particle sizes were determined by mechanical sieving, in accordance with American

Society for Testing and Materials protocols (ASTM D422-63, 2002). Unshaded leaves of *S. subfragilis* were sampled twice in both 2016 and 2017 (June 28 and October 3, 2016; June 6 and August 22, 2017) at approximately 15 locations in the drawdown zone. Equally distributed sites at different elevations were established in the drawdown zones of the Hebisawa and Ushikubiri front reservoirs.

On October 4, 2016, sampling was conducted at the Hebiishi front-reservoir. In the middle of the Hebiishi front-reservoir sampling site, there was a pond ringed with several individual *S. subfragilis*, which remained inundated throughout the flood season; leaves of these *S. subfragilis* were also sampled. During the October 2016 sampling event, leaves adjacent to sampled ones were selected and covered with a black sheet for 30 min to simulate shaded conditions prior to sampling, and were then carefully sampled to avoid exposure to light. Leaf sampling was also conducted in trees covered by *S.angulatus* near to the non-covered sampled trees; for these trees, the proportions of the canopy covered by S. angulatus were estimated from photographic images. Leaf samples were sealed in plastic bags and stored in an icebox filled with dry ice before being transported to the laboratory, where samples were frozen until analysis.

4.3.1 Determination of H_2O_2, CAT, APX, and POD via assays

For H_2O_2, POD, APX, and CAT assays, approximately 100 mg of fresh plant leaves were extracted in ice cold phosphate buffer (50 Mm, pH 6.0) that contained polyvinylpyrrolidone. The extract was centrifuged at 5000 rpm for 20 minutes at 4°C. The supernatant was collected and stored at -80°C for further analysis. For analysis of H_2O_2 concentrations, a 750 µl aliquot was mixed with 2.5 ml of 0.1% titanium sulfate in 20% (V/V) H_2SO_4 . The mixture was then centrifuged at 5,000 rpm at 20°C for 15 min. The intensity of the yellow color was measured via a spectrophotometer at 410 nm. Concentrations of H_2O_2 were estimated using standard curves for known concentrations of H_2O_2, and the results were expressed as µmol/g FW. POD (EC 1.11.1.7) was assayed according to the method described by, and absorbance was recorded at 470 nm at 15 s intervals for 3 min using an extinction coefficient of 26.6 Mm–1 cm–1. APX (EC 1.11.1.11) activity was assayed using the method described by. Decreasing absorbance at 290 nm was recorded every 15seconds, and APX activity was determined using an extinction coefficient of 2.8 Mm^{-1} $cm^{-1.}$ CAT activity (EC 1.11.1.6) was determined following the method described by and was calculated using an extinction coefficient of 40 $Mm^{-1}cm^{-1}$. All CAT, APX, and POD activities were expressed as µmol/min/g FW.

4.4 Results

4.4.1 Relationship between quadrant elevation and number of trees

The **Figure 4.2 (A)** shows the relationship between the quadrat elevation and the number of trees in a quadrat in 2008. Tree densities at Ushikubiri and Hebiishi were not correlated with elevation (R = 0.015 and R= 0.045, respectively), whereas a weak correlation was detected between trees and elevation at Hebisawa (R = 0.666). No significant differences were detected for tree densities among the three sites (F = 0.001, P>0.05; F = 0.007, P>0.05; F = 0.007, P>0.05). Among all the three sites, the lowest and highest number of trees which ranged from 20 at 318 meters elevation and 130 at 324 meters elevation.

Survival rates of S. subfragilis trees within each quadrat over the 2008–2016 periods are shown in **Figure 4.2 (B)** as a function of elevation. A clear decreasing trend in tree survival was observed with increasing elevation (R = –0.906, P<0.05 at Ushikubiri; R = –0.445, P<0.05 at Hebisawa; and R = –0.527, P<0.05 at Hebiishi). Survival rates were below 0.4 among trees at 1 m above NWL during flood season. Among the three front reservoirs, survival rates were generally lowest at Hebisawa, followed by Hebiishi and Ushikubiri.

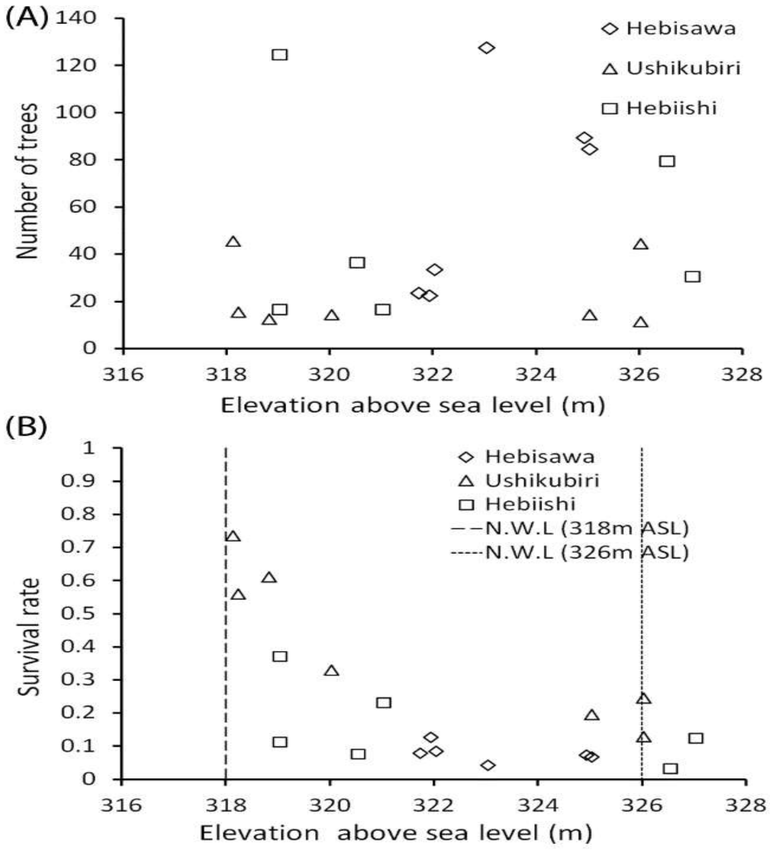

Figure 4.2

(A) Relationship between the elevation of quadrats and the number of *Salix subfragilis* trees in a quadrat, in 2008. The Y-axis represents the number of trees per quadrat.

(B) The survival rate of *S. subfragilis* trees (Y-axis) over the period from 2008 to 2016. The long-dashed line indicates the normal water level at 318 m above sea level during high flood risk season; the short-dashed line indicates normal water level at 326 m above sea level during non-flood seasons. Each marker indicates one quadrat.

4.4.2 Effect of hydrogen peroxide concentration on *Salix subfragilis*

The concentrations of H_2O_2 in *S. subfragilis* leaves collected from trees growing at different elevations are shown in **Figure 4.3**. H_2O_2 concentrations were low in the June 28, 2016, and June 06, 2017 samples (20–35 µmol/gFW), but were much higher in the October 3, 2016, and August 22, 2017samples. However, at the inundated sites in October 2016, H_2O_2 concentrations were as low as the levels recorded in the June samples. Concentrations of H_2O_2 were also low in the leaves of trees growing at less than 0.5 m above the non-flood season water level. Moreover, no significant differences were detected in H_2O_2 concentrations in leaves collected in June 2016 and June 2017 at the front reservoirs of Hebisawa and Ushikubiri (Hebisawa T = 2.36, $P>0.05$; Ushikubiri T = 2.77, $P>0.05$).

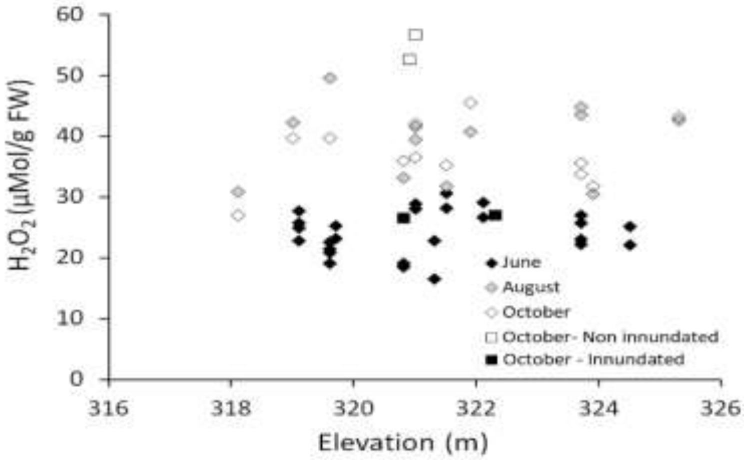

Figure 4.3. H_2O_2 concentrations in *Salix subfragilis* leaves at different elevations (regardless of location and sampling year). 'June', 'August', and 'October' indicate samples collected at Hebisawa and Ushikubiri reservoirs during 2016 and 2017. 'October - non-inundated' and 'October – inundated' indicate samples collected at the Hebiishi reservoir

4.4.3 Effect of soil moisture on *Salix subfragilis*

Concentrations of H_2O_2 in the October 2016 samples are presented as a function of soil moisture content in **Figure 4.4**. H_2O_2 concentrations significantly decreased with increasing soil moisture content (F =0.0068, $P<0.05$). Wherein least H_2O_2 concentration was observed at 20% moisture and highest H_2O_2 concentration at 60% moisture.

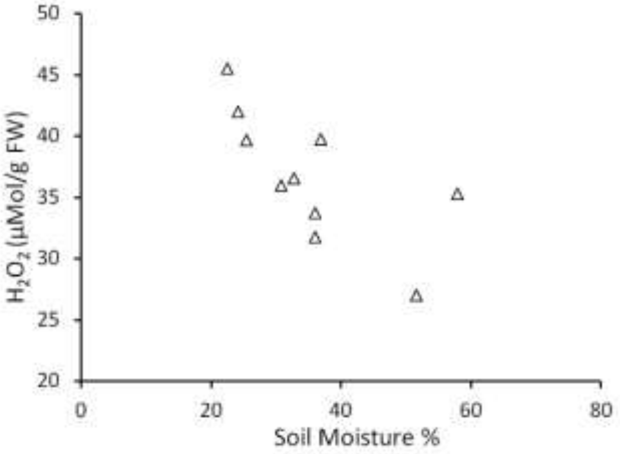

Figure 4.4 Foliar H_2O_2 concentration in *Salix subfragilis* in October 2016, as a function of soil moisture content

4.4.4 Effect of coverage by *Sciyos angulatus L.*

Figure 4.5 shows the H_2O_2 concentration as a function of the coverage ratio by S.angulatus. H_2O_2 concentration significantly enhanced with more than 50% of S.angulatus coverage ($F=5.835$, $p<0.05$).

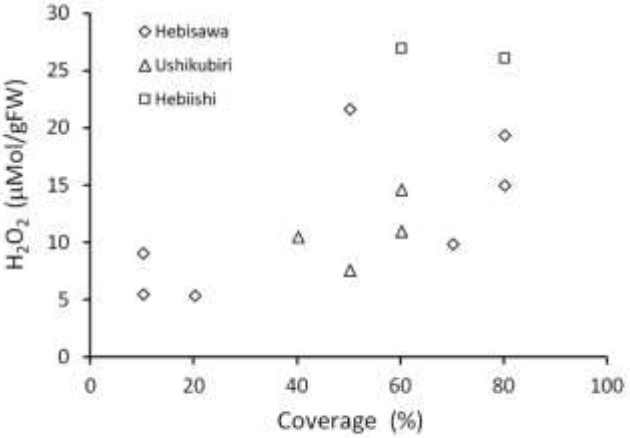

Figure 4.5 Excessive H_2O_2 concentrations in *Salix. subfragilis* leaves covered by *S. angulatus*, compared to uncovered leaves (Y-axis), as a function of coverage percentage (X-axis).

4.4.5 Effect of light and dark treatment on *Salix subfragilis* leaves

H_2O_2 concentrations of 30 minutes of darkness treated leaf samples are shown in **Figure 4.6**.

The darkness treated samples indicates the same level of June samples, revealing that no excessive electron generation occurred without photosynthesis activities.

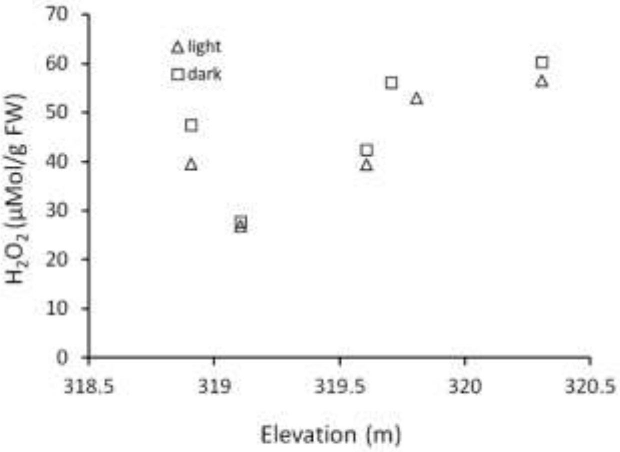

Figure 4.6 H_2O_2 concentrations (Y-axis) of 30 min dark-adopted (dark) and light-exposed (light) leaves collected from trees adjacent to quadrats, at different elevations (X-axis).

4.4.6 Effect of antioxidant activities

Antioxidant (CAT, APX, and POD) activity levels are presented in **Figure 4.7(A–C)**,expressed as a function of H_2O_2 concentration. In general, antioxidant activity increased proportionally, and significant correlations were obtained with H_2O_2 concentration ($R_{CAT} = 0.79$ for CAT; $R_{APX} = 0.82$ for APX; and $R_{POD} = 0.79$ for POD).

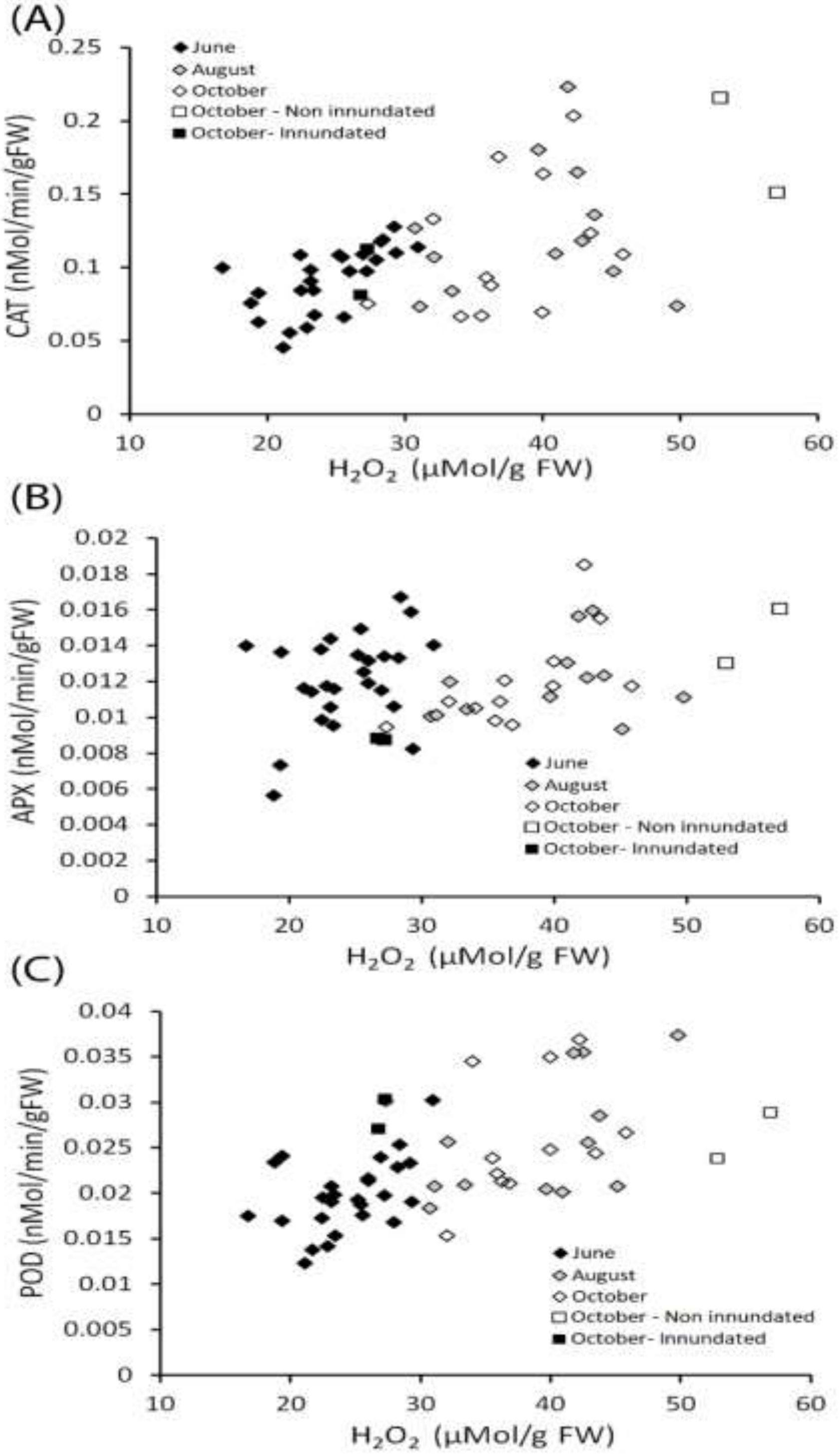

Figure 4.7

(A). Foliar CAT activity as a function of H_2O_2 concentration (regardless of sampling year and location).
(B) Foliar APX activity as a function of H_2O_2 concentration (regardless of sampling year and location).
(C) Foliar POD activity as a function of H_2O_2 concentration (regardless of sampling year and location). ‘Inundated’ and ‘non-inundated’ indicate samples collected at the respective site of the Hebiishi reservoir.

4.5 Discussion

4.5.1 Role of soil moisture for the survival of *S.Subfragilis*

Salix subfragilis disperses seeds in the middle of May, which corresponds to the period of water-level drawdown. The *S. subfragilis* colony that was established on the slope of front reservoirs of Miharu Dam following the first inundation in 1997 is of roughly the same age; since that time, however, virtually no new colonies have developed on the slope except for along the shoreline of the non-flood season impoundment. This population of *S. subfragilis* is, therefore, composed primarily of same-age individuals.

By 2007, however, many individuals in the previously healthy population, composed of 5–6 m tall trees at 10 years of age, had died within 10 years of establishment, regardless of how densely or sparsely colonized the populations, and the previous Salix stands are now converting to grassland. The normal life span of *S. subfragilis* in this area exceeds 30 years, suggesting that the high rates of mortality of young trees in this population was abnormal. *Salix subfragilis* exhibit intensive self-thinning strategies during the growing process, but self-thinning typically occurs within several years after germination, not at 10 years of age. As such, self-thinning does not seem to be a reasonable explanation for the high mortality rate.

Salix subfragilis normally grows along or close to shorelines. Trees growing in the drawdown zone, however, are left exposed several meters above the water level within 2 weeks during the flood risk season, due to the drawdowns. Comparisons of oxidative stress levels in *S. subfragilis* between June immediately following the drawdown of the water level and October (2016) or August (2017) (i.e., a 3–5month period) indicated that Salix stress levels were significantly higher in October and August than in June in both 2016 and 2017. Due to the sandy substrate, it takes approximately 3 months for soil moisture levels to respond to the changes in water levels. Thus, soil moisture content in June remained high, whereas in October and August, most of the excess moisture had evaporated or been transpired by vegetation, reducing soil moisture content to extremely low levels, with the exception of soil close to the shoreline. The relatively low mortality of Salix growing within 1 m from the shoreline compared to the gradually increasing mortality rate of Salix further away suggests that water stress due to low soil moisture content played a large role in the high *S. subfragilis* mortality rates. Particularly high mortality was observed at the Hebisawa front reservoir, which is located in a flat, south-facing area, and thus receives a high degree of solar radiation during summer; this area is more thickly vegetated with shrubs and forbs compared to the Ushikubiri and Hebiishi front reservoirs, which are in narrow, north-facing valleys that receive relatively less sunlight. As such, moisture content was lower in the soils at Hebisawa due to the higher rates of evapotranspiration than occurs at the other two sites, indicating that the *S. subfragilis* growing at Hebisawa suffered more severe water stress.

4.5.2 Effect on photosynthetic pigments

The high concentration of ROS causes the oxidative damages of biomolecules, proteins, lipids, DNA, etc., resulting in cell death. Associated with the reduction of the soil moisture, the stomata close due to the shortage of water, then the uptake of CO_2 decreases, resulting in the reduction of carbon synthesis. As it was the electron accepter, then the reduction of carbon synthesis leads to the acceleration of the O_2^- generation, one of ROS. O_2^- is transformed to H_2O_2 by the activities of the super oxide dismutase (SOD). Therefore, the H_2O_2 concentration increases due to the water stress at the dry site compared to the wet site.

Though the growth rate was not measured in the field, chlorophyll concentrations of leaves had a clear negative relationship with H_2O_2 concentration, indicating the adverse condition for the trees there. H_2O_2 concentration in June was low, indicating the low water stress then. The H_2O_2 concentration in October 2016 and August 2017, although it is deviated, is always higher than that of June samples. The soil moisture is high in June, then is gradually declined with time, while it kept as high at the inundated pond in Hebiishi front-reservoir. Nearly the same level of H_2O_2 was obtained in October 2016 samples at inundation site of Hebiishi front-reservoir, June samples. At the same time, basal H_2O_2 concentration in June, ~25µmol/gFW, is attributed to all other oxidative stresses, likely photosynthesis, respiration, etc. The difference in H_2O_2 between June and October or August samples, ~20µmol/gFW, is attributed to the fraction due to the water stress.

These results suggest that the water stress of *Salix subfragilis* becomes higher with the decreasing soil moisture(Azami et al., 2013), or with the length of exposed period after the drawdown. The intensity of the water stress is more or less equivalent to the level of other oxidative stresses. The oxidative stress due to other than water shortage is similar through June to October, although ages of leaves are different. The water stresses affected *S. subfragilis* more significantly with prolonged exposed period. The delay of the recovery of the water level in these days seems to have affected trees and increased the mortality rate. June samples show the small dependence on H_2O_2 concentration regardless of chlorophyll concentration. The oxidative stress with June samples are not water deficit, likely H_2O_2 was not generated intensively organelles related to chloroplast at the time. With August and October samples, on the other hand, chlorophyll concentration did not change much regardless of H_2O_2 concentration. Differently from light stress, in which too much energy is received by light harvesting pigments, surplus of electron is caused by the reduction of carbon dioxide supply. Thus, chlorophyll seems not be damaged much due to increasing ROS concentration.

Under drought stress, chlorophyll a and b ratio increase with the loss of pigments in the light harvesting chlorophyll a and b protein. Chlorophyll more significantly reduces than carotenoid as carotenoids protect chloroplast from stress-induced oxidative damages. These mechanisms avoid the photo-inhibition and oxidative stress.

4.5.3 The effect of a long period inundation on the early mortality of *Salix subfragilis*

These stands were inundated seven months during non-flood season in a year. Although *Salix subfragilis* inhabit on the moist soil, the long period inundation may be stressful for the Salix to hasten the mortality.

The H_2O_2 concentration of leaves at the inundation site in October 2016, though they were inundated throughout a year, was maintained at the stress level of inundation. Its nearly same level as the level of June (2016 and 2017) samples indicates that the stress due to inundation was less than water stress, as it is different from the case of low plants completely submerged in water. As all trees were more than 10 years old, and even the lowest branch and most of leaves were above the water level of the non-flood season and in the atmosphere. The submergence of seven months in winter does not generate the severe anoxic condition and hydrogen sulphite in the sediment. Sufficient amount of oxygen is transported through vascular system, and thus they are not much affected by the submergence of stems and roots in terms of oxygen supply.

4.5.4 Effect of Antioxidant activities

Antioxidants, APX, CAT, and POD, activities had different trends with respect to H_2O_2 concentration between June samples and August or October samples, though all of them scavenge H_2O_2. APX activity was higher with June samples compared to August or October samples, while the relationship was relatively consistent for POD activities. Locations of these antioxidant enzymes were different between each other; compared to widely generated APX, the generation of CAT and POD are limited, majorly peroxisome for CAT, and cell wall, cytosol, and vacuoles for POD. The major oxidative stress of June samples were some of various stresses rather than water deficit, which likely activated mainly APX of the organelles. However, for August and October samples, half of H_2O_2 was generated by water deficit, highly associated with chloroplast and cell walls. Thus, the share was APX activities seems to be relatively low compared to June samples. In contrast, POD activities of August or October samples likely became higher compared to June samples.

4.5.5 Effect of coverage by *Sicyos angulatus L.*

Concentrations of H_2O_2 were substantially higher in *S. subfragilis* whose canopy was at least 50% covered by *S. angulatus*. Several individual trees in Ushikubiri and Hebiishi front reservoirs that have fallen were thickly covered with *S. subfragilis.* At present, no allelopathic activities have been reported for *S. angulatus*, and the weight of even extensive *S. angulatus* cover is light enough for *S. subfragilis* to endure. *Salix subfragilis* did not exhibit any excessive stress relating to exposure to solar radiation, suggesting that 50% *S. angulatus* coverage incurs severe shading stress in *S. subfragilis*. Intensive colonization of *S. angulatus* on *S. subfragilis* also had strong effects on *S. subfragilis* mortality.

4.6 Conclusion

Flexible management-based methods are commonly used in the ecological management of riparian systems, where long-term monitoring of vegetation is conducted to identify stressors. *Salix subfragilis* trees growing in this area have been monitored for 8 years in order to gain a better understanding of the mechanisms driving their high mortality. However, the responsible stressor was not identified solely from monitoring, as the change in *Salix* health was due to prolonged stress conditions. In this study, one ROS, H_2O_2, was identified as the primary cause of the increase in *Salix subfragilis* mortality based on relatively straightforward analyses, and our results suggest that H_2O_2 levels can be used as an effective indicator of environmental stress levels and plant condition.

Salix subfragilis are inundation-tolerant species often found along reservoir shorelines and are capable of colonizing the drawdown zones of flood-control dams, which otherwise would remain devoid of vegetation. These individuals are vulnerable to water stress during periods of water drawdowns, however. Oxidative stress levels of the inundated trees included in our study were as low as those of trees recently exposed following inundation, suggesting that one potential measure for reducing stress levels in *Salix subfragilis* would be to supply additional water to the colony. Several studies have shown that *Salix subfragilis* require high soil moisture in order to germinate but we recommend that soil moisture content be maintained at appropriate levels even for mature trees.

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CHAPTER V

Evaluation of habitat preferences of invasive macrophyte *Egeria densa* in different channel slopes using hydrogen peroxide as an indicator

5.1 Introduction

The mid-streams of large Japanese rivers were characterized as gravel beds during the post-World War II era. Fine sediment beds were extremely limited. Thus, the ecosystems of gravel beds, characterized by rich hyporheic flows and biota, such as insect larvae and salmonid fish, were maintained for long periods (Hauer et al., 2016). Except for some emergent species, *Phragmites japonica* (Asaeda et al. 2009), almost no submerged macrophyte colonies existed in the main streams of major rivers (Kadono, 2004). Since then, dams and weirs have been frequently constructed and most of the waterways have been regulated. Therefore, almost all the gravel particles introduced upstream are trapped before entering the midstream, thus the supply of gravel to the midstream and downstream is completely curtailed. In addition, gravel was mined for use as construction materials from the 1960s to 1970s. The amount of gravel, therefore, substantially reduced in the midstream, compared to that of previous years (Asaeda and Sanjaya, 2017). In contrast, fine sediment inflows continued from the mid to downstream catchments. They were transported and settled, filling interstices on downstream gravel beds. Thus, the midstream beds are now partially covered with fine sediments that bury stones. .

In the past two decades, invasive macrophyte *Egeria densa* began to form colonies in many rivers (MLIT, 2018). It often covers extensive areas of the channel bed and completely changes the ecosystem there (Collier et al. 1999; Yarrow et al., 2009). Financially, this causes substantial losses to inland fisheries, particularly in the yield of Ayu fish (*Plecoglossus altivelis*), a grazer of benthic algae (Asaeda et al., 2018). *E. densa* was cultivated in aquariums in the early 19th century, but it was disposed into natural freshwater bodies and became naturalized in the 1940s. However, it had not spread into rivers as they were gravelly in those days and were not in a suitable condition to support submerged macrophytes, however, it has been found in some lakes of western Japan since the 1970s (Kadono, 2004). Besides Japan, *E. densa*, spread widely in other continents (Champion and Tanner, 2000; Santos et al. 2011). It affected stream ecosystems extremely, retarding flow velocity, increasing sedimentation (Collier et al. 1999), and exile of native species (Santos et al., 2011; Gillard et al. 2017). Therefore, though the effects were particularly eminent in Japanese gravel rivers, the invasion of *E. densa* is a worldwide problem.

As several changes occur simultaneously in natural rivers, it is not easy to elucidate the primary reason that prevented earlier macrophyte colonization or their increase today. The habitat preferences of macrophytes are normally evaluated by monitoring their growth rate or biomass (Barko et al., 1991; Riis et al., 2012; O'Hare et al. 2018). However, there are various potentially influential factors in the natural environment and each factor changes from time to time during the period of the macrophytes' growth. The existing conditions are, thus, considered to be a result of the integrated environmental conditions experienced previously, and the casual observation that is mainly practiced in vegetation management is not necessarily appropriate for evaluating their habitat preference.

In natural water, macrophytes are subjected to environmental stresses, such as flow velocity, high solar radiation, excessive high or low temperature, etc. In cell organelles, then, reactive oxygen species (ROS) are generated based on the intensity of the stresses, photosynthesis, and metabolic activities (Zaman and Asaeda, 2013; Asaeda and Rashid, 2017; Parveen et al., 2017a). A part of these ROS is scavenged relatively quickly by antioxidant activities, and the homogeneity of ROS in tissues is maintained by a balance between the ROS and the antioxidants. However, under excessive stress, this balance collapses as oxidative stress surpasses the antioxidant capacity of the plant. The existence of ROS in plant tissues leads to oxidative stress, and when critical levels are exceeded, the plants tend to deteriorate (Sharma et al., 2012; Choudhury et al., 2017).The most common ROS is hydrogen peroxide (H_2O_2), which is generated by the superoxide dismutase by in the superoxide (Sharma et al., 2012; Asada, 2006). The H_2O_2 concentration is relatively stable and can be easily analyzed chemically (Satterfield and Bonnell, 1955; Zhou et al., 2006). The amount of tissue H_2O_2 concentration, therefore, has potential for use as an indicator to monitor the instantaneous environmental stress intensity on macrophytes (Asaeda et al., 2018).

During the daytime, the total amount of H_2O_2 generated in plant tissues is, therefore, primarily the sum of the H_2O_2 generated in response to environmental stress, photosynthesis, and other nonstress metabolic products. This process can be generalized as the following simple equation:

$$H_2O_{2(\text{Total})}=H_2O_{2(\text{Photosynthesis})}+H_2O_{2(\text{Metabolic byproduct and respiration})}+\sum H_2O_{2(\text{Environmental stress})} \quad (1)$$

Although there are some interactions between the different environmental stressors and opposing trends in some combinations of stressors (Rivero et al. 2014), the share of H_2O_2 concentration of stresses is separated from metabolic, respiration, and photosynthesis produced H_2O_2 (Mittler, 2002). However, the plant oxidative stress is determined by cumulative H_2O_2 content present in the cells regardless of the source.

Several types of stressors are acting on submerged macrophytes in natural rivers. In the relatively steep non-polluted rivers, the major stressors include the mechanical stress introduced by high current velocity/turbulence, solar radiation, and temperature (Yarrow et al., 2009; Riis et al., 2012). As these are based on different physical quantities, it is difficult to compare the magnitude of each stressor on the submerged macrophytes. However, it is possible to differentiate photosynthesis produced H_2O_2 from the total accumulated H_2O_2 by dark adapting the plants (Asaeda et al. 2018). In addition, when other stresses are eliminated under controlled conditions in the laboratory, it is possible to quantify each type of stress by the produced H_2O_2.

Considering the facts that, it can be hypothesized that 1) there is a relationship between induced H_2O_2 concentration in *E.densa* and the intensity of each stress given by the habitat metrics, such as water velocity, temperature, and the solar radiation of the habitat. 2) *E. densa* growth reduced and is deteriorated in the condition in which H_2O_2 concentration exceeds a threshold value. 3) The habitat metric condition for *E. densa*, therefore, remains to be a H_2O_2 concentration less than the threshold value. Then, the habitat preference and adaptability of *E. densa* is studied in terms of H_2O_2 formation under varying riverine conditions and the controlled conditions in the laboratory, focusing on obtaining empirical relationships of factors on the tissue H_2O_2 contents.

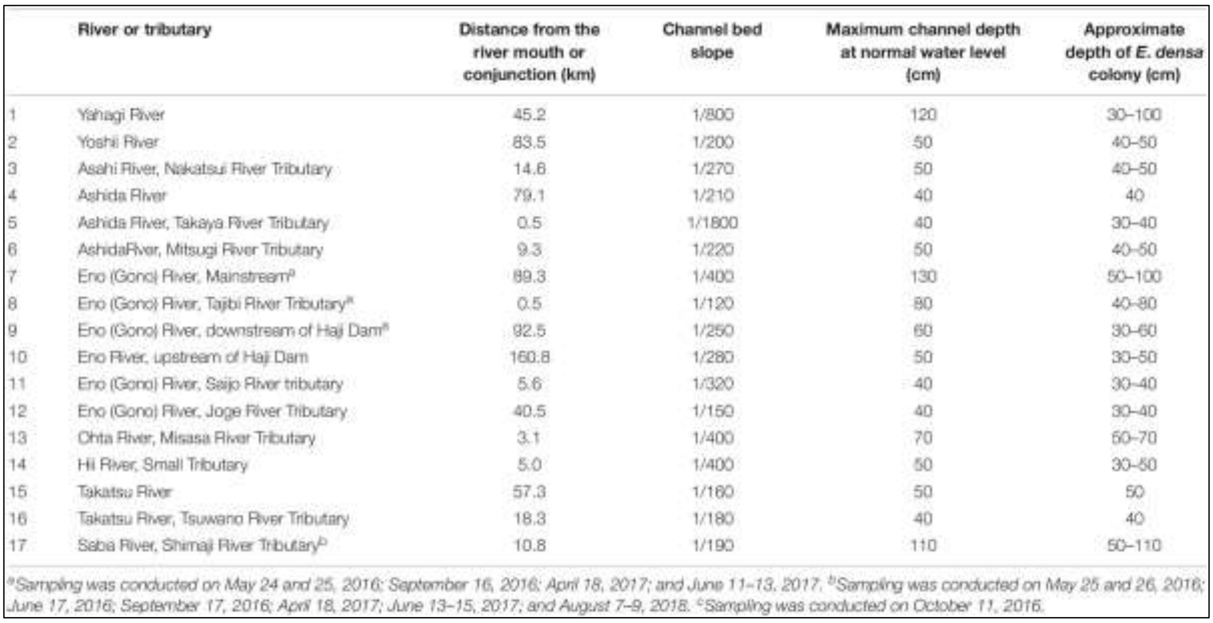
As the tissue presence of H_2O_2 can be used to evaluate the plant condition, and the plant H_2O_2 content can be evaluated in a short period, it has the potential to be adopted in macrophyte monitoring practices. In the present study, we focused the H_2O_2 production of *E. densa* over various field and laboratory conditions. However, the methodology is widely applicable for various types of macrophyte managements, such as the identification of the optimum condition in the endangered species' restoration, or in the extermination of alien species.

5.1Methodology

5.2.1 Field observations

Several rivers that are highly colonized by *E. densa* were selected from the species distribution records in Japan (MLIT, 2018). In 2016 and 2017, observations were conducted in rivers for location data of *E. densa* colonization. Sampling activities were conducted on fine days (days with clear sky and no rain expected) in different seasons from Eno (Go), Saba and Hii Rivers, including their tributaries **Table 5.1**. In each river, the surveys reached approximately 20 to 50 km from the upstream to the downstream area, and five to ten sites where more than one third of the bed was covered with pure *E. densa* colonies were selected for the study, including the most upstream colony in the main channel.

Table 5.1 : River channel data where large colonies of *E. densa* were found



	River or tributary	Distance from the river mouth or conjunction (km)	Channel bed slope	Maximum channel depth at normal water level (cm)	Approximate depth of *E. densa* colony (cm)
1	Yahagi River	45.2	1/800	120	30–100
2	Yoshii River	83.5	1/200	50	40–50
3	Asahi River, Nakatsui River Tributary	14.8	1/270	50	40–50
4	Ashida River	79.1	1/210	40	40
5	Ashida River, Takaya River Tributary	0.5	1/1800	40	30–40
6	AshidaRiver, Mitsugi River Tributary	9.3	1/220	50	40–50
7	Eno (Gono) River, Mainstream[a]	89.3	1/400	130	50–100
8	Eno (Gono) River, Tajibi River Tributary[a]	0.5	1/120	80	40–80
9	Eno (Gono) River, downstream of Haji Dam[a]	92.5	1/250	60	30–60
10	Eno River, upstream of Haji Dam	160.8	1/280	50	30–50
11	Eno (Gono) River, Saijo River tributary	5.6	1/320	40	30–40
12	Eno (Gono) River, Joge River Tributary	40.5	1/150	40	30–40
13	Ohta River, Misasa River Tributary	3.1	1/400	70	60–70
14	Hii River, Small Tributary	5.0	1/400	50	30–50
15	Takatsu River	57.3	1/160	50	50
16	Takatsu River, Tsuwano River Tributary	18.3	1/180	40	40
17	Saba River, Shimaji River Tributary[b]	10.8	1/190	110	50–110

[a]Sampling was conducted on May 24 and 25, 2016; September 16, 2016; April 18, 2017; and June 11–13, 2017. [b]Sampling was conducted on May 25 and 26, 2016; June 17, 2016; September 17, 2016; April 18, 2017; June 13–15, 2017; and August 7–9, 2018. [c]Sampling was conducted on October 11, 2016.

At each sampling site, there were several *E. densa* patches, and each patch was composed of several plants. Thus, more than 5 samples were collected from overlying shoots of different plants of a same patch in light-exposed (under natural conditions) and dark-adapted conditions to differentiate photosynthesis generated H_2O_2 from environmental factors and metabolism induced H_2O_2 of tissues. The dark exposure treatment was performed by placing a black plastic sheet (3 m × 3 m) floating over the *E. densa* colonies for 30 min. The 30 min pre-dark period was determined from laboratory experiments, which were conducted to determine the optimum pre-darkness duration. The plastic sheets were tied to fixed metal poles that were inserted in the riverbed, allowing the sheets to float on the water surface without causing mechanical disturbances to the macrophytes or altering the water flow. The PAR intensity under the sheet was found to be zero. The light-exposed samples were collected adjacent to the darkness treated samples. The collected samples were put in resealable plastic bags and quickly stored in a cool box containing dry ice until they were transferred to the laboratory to be stored at -80 ºC. Biomass was obtained from a 50 cm× 50 cm quadrant of each sampling point.

At each sampling point, the water velocity was measured with an ultrasonic velocimeter and recorded for more than 1 min (Tokyo Keisoku Co. Ltd., Japan), at 20% (reference velocity) and 80% (depth of the colony) of the total water depth. Turbulence velocity component was calculated as a root mean square deviation from the mean velocity, from the velocity record. Photosynthetically active radiation intensity (PAR intensity) in the water was measured with a portable quantum flux meter (Apogee, MQ-200, USA) at 10 cm depth intervals.

On October 23, 2018, the lateral configuration of a channel was investigated to derive the effect of the *E. densa* colony on the environment, including the distributions of depth, sand depositions thickness, *E. densa* biomass, particle size, and the *E. densa* burying condition in trapped sediments at a point 45 km upstream from the river mouth of the Yahagi River. During the summers of 2016, 2017, and 2018, surveys were conducted from the upstream to the downstream areas in other rivers where the existence of *E. densa* was recorded, then the locations and the depth of the rivers were recorded, and the channel slope was obtained from a topographic map (GSI, 2018).

5.2.2 Laboratory experiments

The pre-darkness period in the field observation was investigated as follows. The apical cuttings with an average length of 10 cm were obtained from the stock culture and planted in two tanks (50 cm × 35 cm × 35 cm) with thoroughly washed commercial river sand (90% of <0.2 mm particle size; washed using tap water several times until all organic materials are washed away and finally, washed using distilled water to further remove nutrients). In each tank, twenty *E. densa* cuttings were planted and maintained in a temperature-controlled room maintained at a constant 23±3 °C temperature. Each tank was exposed to approximately 100 $\mu mol/m^2/s$ PAR with a 12 h/12 h light and dark period. Nutrients were supplied via a 5% Hoagland nutrient solution. After a two-month acclimatization period, one of the two tanks was covered entirely by a black plastic sheet, providing darkness. *E. densa* tissues from different samples were collected at 10 min intervals for 2 h and then collected at 6, 12, and 24 h. Light-exposed samples were collected, simultaneously. To avoid the stress of cutting, the tissues were collected from fresh tips during each sampling activity. The experiment was conducted in triplicates with different samples. The H_2O_2 concentrations of tissues were then analyzed. The H_2O_2 concentration of *E. densa* gradually declined with the dark duration, taking the lowest value at 30 min, then slightly increasing later for all cases. Therefore, in the field experiment, 30 min of darkness was adopted for the dark-adapted samples.

The effect of temperature on H_2O_2 generation was investigated, using four tanks similar to those in the previous experiments. After an acclimatization period of 2 months, the temperature regimes of the tanks were set to 10, 15, 25, and 30 °C, respectively, using an aquarium water temperature controlling system (Aquarium cooler ZC-100α, Zensui Corporation, Japan). Light intensities, 220, 320 and 680 $\mu mol/m^2/s$ were obtained with the combination of several LED lamps. Then, a 5 m long flume equipped with a straightening plate at the upstream end was used to check the velocity effect. The central part was lightened with 200 µmol/m^2/s of PAR intensity by a LED lamp. The velocity was adjusted at 23 cm/s to maintain the low turbulence intensity condition, less than 2 cm/s of turbulence velocity, following Asaeda et al. (2018), and the normal flow velocity of the *E. densa* habitat (Champion and Tanner 2000). The temperature conditions were maintained for 7 days, and plants were sampled for the chemical analyses. The experiment was conducted in triplicates with different samples for each condition.

5.2.3 Chemical and biomass analyses

The tissue H_2O_2 was estimated calorimetrically using spectrophotometry (Asaeda et al., 2018). Plant chemicals were extracted into ice-cold phosphate buffers (50 mmol/L, pH 6.0) by crushing approximately 100 mg of the plant in the presence of polyvinylpyrrolidone (PVP). The extractions were centrifuged at 5000 *g* for 15 min at 4 °C. The enzyme extraction of 750 µL was then mixed with 2.5 mL of 0.1% titanium sulfate in 20% (v/v) H_2SO_4, and the mixture was centrifuged at 3500 rpm for 15 min at 20 °C. The optical absorption at a wavelength of 410 nm was measured using spectrophotometry (UV-1200, UV-Visible Spectrophotometer, Shimadzu, Japan), and the H_2O_2 concentrations (µmol/gFW) were estimated using a standard curve.

The dry weight of biomass was estimated by oven drying the collected biomass samples at 70 °C for 72 h or until the weight became stable. The dried biomass was weighed and expressed in units of gDW/m^2.

5.2.4 Statistical comparison

The linear or power law (for solar radiation intensity) correlation between parameters was tested by Pearson's correlation analysis and the statistical significance between observations were tested with Student's t-test. The statistical comparisons of field data were performed to obtain the relationship between the H_2O_2 content and the external factors (such as velocity, turbulence velocity, light intensity, biomass, and depth), and the relationship between the factors (turbulence and mean flow). For H_2O_2 concentration and velocity or temperature relationship, statistical comparisons were performed for the different study sites and/or sampling time groups, which have different temperatures and solar radiations, to obtain the interaction between stresses and the H_2O_2 concentration. The gradient of the regression line was obtained for the whole set of data in the relation between each stress component and H_2O_2 concentration. Then, for each study site and sampling time groups, statistical analysis was conducted to check the significance of the regression. For the relationship between H_2O_2 concentration and ligh1t intensity, the power law regression of the excessive H_2O_2 concentration of the light exposed samples and dark-adapted samples was conducted to obtain the light intensity at zero H_2O_2 concentration. Then the data scattering was compared with the standard deviation.

5.3 Results

The relationship between H_2O_2 concentration and water temperature, obtained from both laboratory experiments (PARs were 220, 320 and 680 μmol/m^2/s with 0 cm/s of velocity; 200 μmol/m^2/s of PAR and 23 cm/s of flow velocity), and dark-adapted condition of the field observation (PAR=0 μmol/m^2/s) is shown in the Figure 1. The recorded temperatures of field studies ranged between 10–25 ℃ depending on the sampling seasons and rivers, and the fluctuations were observed to be 1–2 ℃ in the same sampling condition groups. Thus, the extrapolated regressed lines of each group to zero velocity were used here **Figure 5.1**. H_2O_2 concentration has a negative correlation with temperature in the 10 to 30 ºC temperature range and was regressed to lines with a gradient of -0.316 μmol/gFW/degree, for all light intensity groups. The regression equations are shown in **Figure 5.1** compared to observed data. (R=0.982, P<0.01 for 220 μmol/m^2/s PAR; R=0.963, P<0.01 for field observation, 0 μmol/m^2/s PAR). There was no overlapping among data from different groups. Thus, the effect of interaction between temperature and light intensity is sufficiently small.

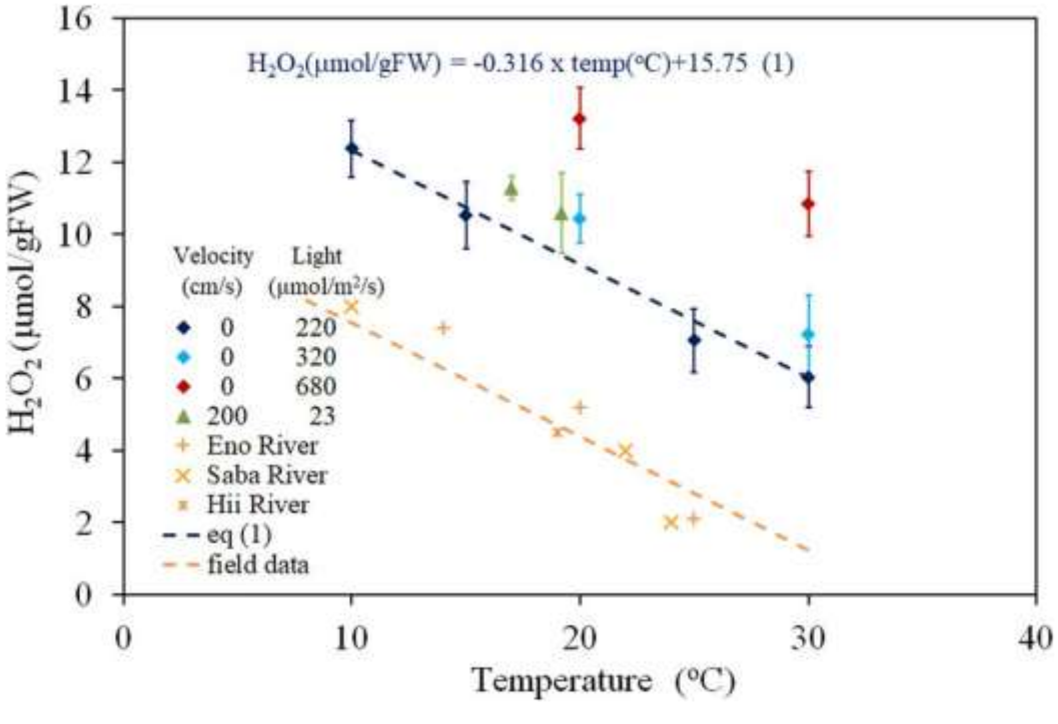


Figure 5.1 The concentration of H_2O_2 in *E. densa* tissue as a function of water temperature. The error bars represent the standard deviation (n=3). Eno, Hii and Saba Rivers data are values extrapolated to zero velocity of the regression line with -0.316 (µmol/gFW cm/s) gradient, shown by dashed lines in **Figure 5.2.**

Figure 5.2 presents the H_2O_2 contents of the field observation samples with respect to turbulence velocity. Under light exposure, the H_2O_2 contents are always higher than those in the corresponding dark-adapted samples by 5-10 µmol/gFW. However, the scattering was greater than that in the dark-adapted samples. Dark-adapted samples are composed of different temperature groups, which depend on the sampling time and rivers.

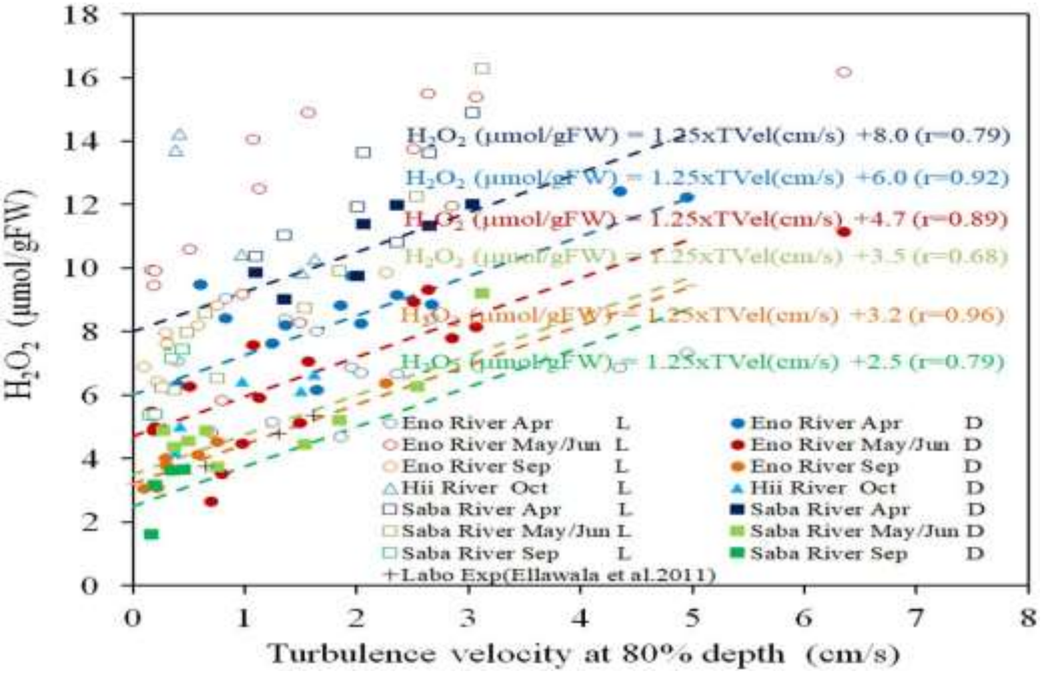


Figure 5.2 The concentration of H_2O_2 in *E. densa* tissue as a function of turbulence velocity at 80% depth in light-exposed (L) and dark-adapted (D) conditions. The 'Labo Exp' represents data from the laboratory experiment (Ellawala et al. 2011). The dashed lines indicate the linear regressed lines with the gradient of 1.25 µmol/(gFW(cm/s)) of the dark-adapted conditions of the same colored symbols of as those of the sampling rivers and seasons, respectively. H_2O_2 is given in units of µmol/gFW, and while the *T Vel* (turbulence velocity) are in cm/s. The dates May, Sep (September), and Oct (October) represent data obtained in 2016 while Jun (June) and Apr (April) represent data of 2017. Eno, Saba, and Hii represent Eno, Saba, and Hii rivers including their tributaries, respectively

The H_2O_2 contents of dark-adapted samples were highly correlated with the turbulence velocity, with a gradient of 1.25 μmol/gFW(cm/s)) (*R*=0.796, *P*<0.01). The regression lines with the same gradient are shown in **Figure 5.3** for the dark-adapted samples of different sampling time and river groups, compared to observed data. For each group, the H_2O_2 contents were highly regressed to the lines (*R*= 0.917, 0.885, 0.964 for April, May/June and September sampling at the Eno River, respectively, 0.76 for the Hii River, and 0.84, 0.68, and 0.63, respectively, for the sampling during each season respectively at the Saba River for all *P*<0.01). This finding indicates that the H_2O_2 concentration dependence on turbulence velocity is independent of temperature.

There is a significant positive correlation between the mean flow and turbulence velocity (*R* = 0.722, *P* < 0.01), as shown in **Figure 5.3**. Also, the correlation between the H_2O_2 content of dark-adapted samples with the mean velocity is also positive and significant (*R* = 0.571, *P* < 0.01). The relationships can be explained with linear regression equations (equation (2) for the 0-60 cm/s mean flow velocity range). The line in **Figure 5.4** included the cases in which the turbulence intensity was particularly high because of the large gravel beds. Although these values provide slightly higher H_2O_2 concentrations compared to those at normal sites, the relatively proportional relationship with turbulence velocity indicates that the mean flow velocity is available as a reference of the mechanical stress due to flow velocity (Asaeda et al., 2017).

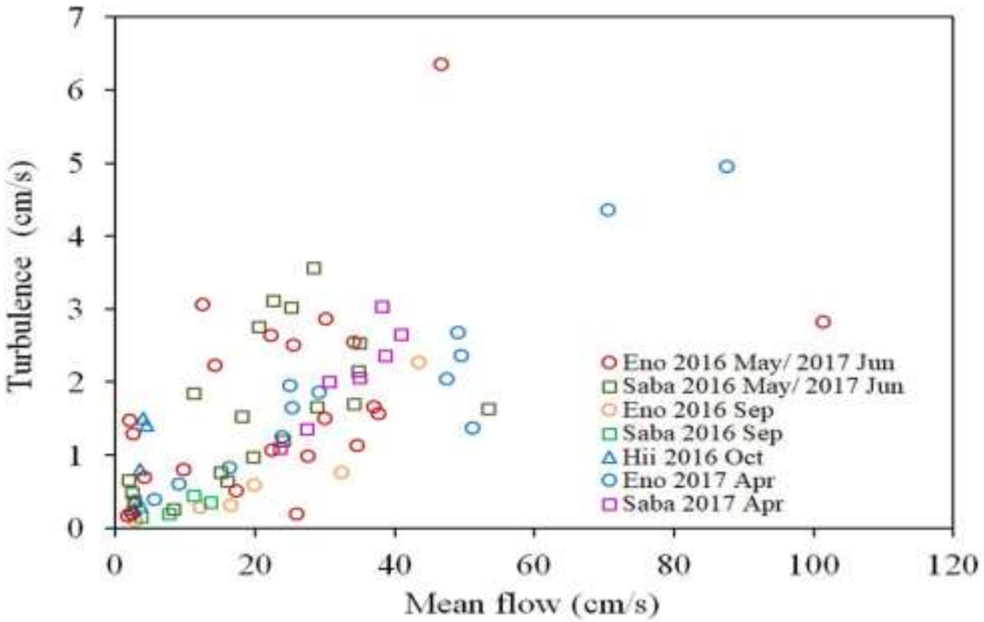


Figure 5.3 Relationship between the turbulence velocity and the mean flow velocity of each sampling site of different rivers. The sample collected month is represented by the month indicated in the legend. Eno, Saba, and Hii represent Eno, Saba, and Hii rivers, including their tributaries, respectively.

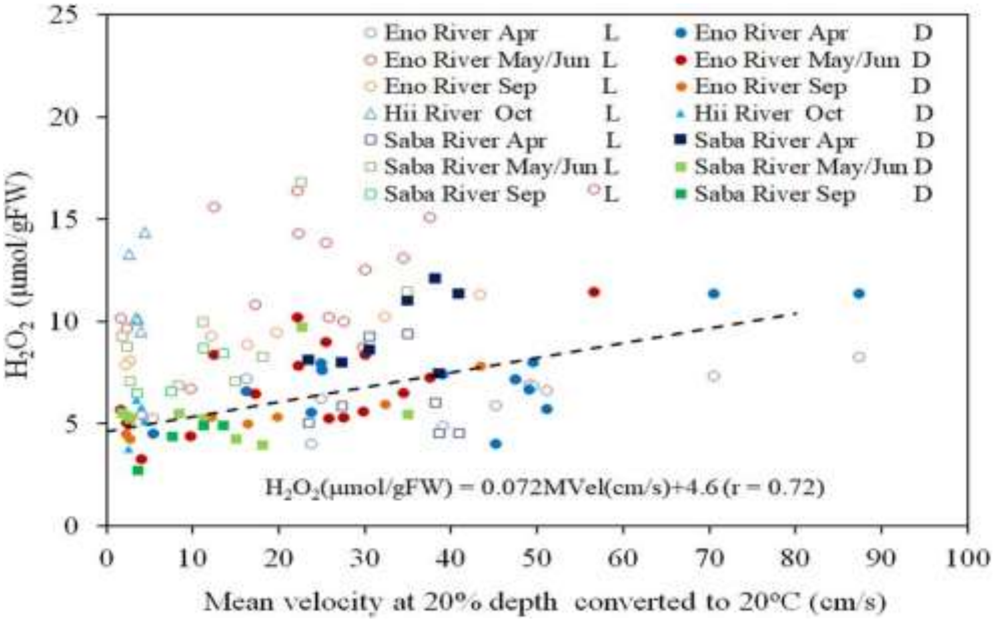


Figure 5.4 The concentration of H_2O_2, converted to 20 °C, in *E. densa* tissue as a function of the mean velocity at 20% deep thin light-exposed (L) and dark-adapted (D) conditions. The dashed line indicates the linear trend of the dark-adapted condition. The dates May, Sep (September), and Oct (October) represent data obtained in 2016 while Jun (June), Apr (April) represent data of 2017. Eno, Saba, and Hii represent Eno, Saba, and Hii rivers including their tributaries, respectively. H_2O_2 is given in units of μmol/gFW, and the *M Vel* (mean velocity) is in cm/s.

$$H_2O_2(\mu\text{molg/FW}) = 0.072MVel\,(\text{cm/s}) + 4.6 \qquad (2)$$

The H_2O_2 concentration of the light-exposed samples fluctuated heavily, but always exceeded the value of the corresponding dark-adapted samples. The excessive H_2O_2 content is light induced H_2O_2 content, postulated as a function of light intensity in **Figure 5.5.** There is a positive correlation between the light intensity and the H_2O_2 concentration, however, the increasing rate of the excessive H_2O_2 decreases with increasing light intensity. When the light intensity is lower than 40 µmol/m^2/s, the excessive H_2O_2 was nearly 0, similar to experimental results obtained by Hussner et al (2010) and Rodrigues and Thomaz (2010). Therefore, power low regression analyses were conducted for the excessive H_2O_2 concentration with respect to the surplus light intensity from 40 µmol/m^2/s, as equation (3) in the figure 5.5 ($R = 0.738$, $P < 0.05$). Although, the scattering is large, most of the data are distributed within the standard deviation from the equation (3) (1.57 µmol/gFW) without any systematic deviation regardless of rivers and sampling seasons, where the solar radiation intensity ranged from 40 to 600 µmol/m^2/s, and temperature from 10 to 25 °C.

$$H_2O_2 = [Light\ Intensiy - 40]^{\frac{2}{3}}/10 \qquad (3)$$

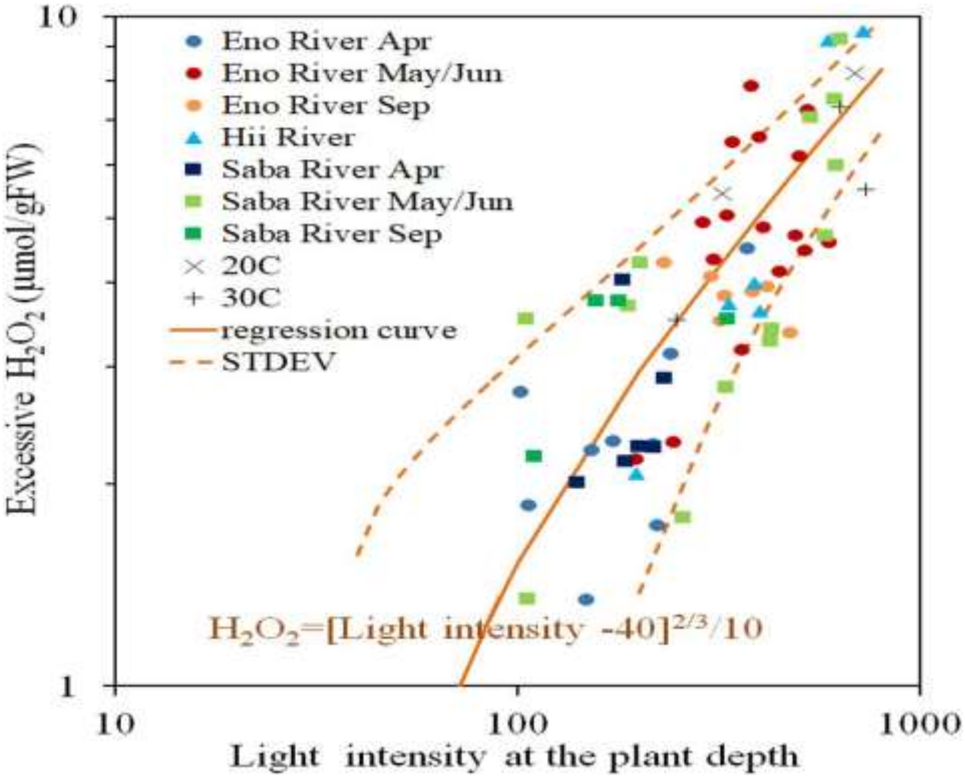


Figure 5.5 Photosynthesis-produced H_2O_2 concentration (Excessive H_2O_2) of *E. densa* tissue as a function of light intensity. The sample collected month is represented by the year and the month indicated in the legend. Eno, Saba, and Hii represent Eno, Saba, and Hii Rivers, including their tributaries, respectively. When the light intensity is lower than 40 µmol/m^2/s, the excessive H_2O_2 concentration was 0 µmol/gFW. The regression curve in the excessive H_2O_2 concentration and the excessive light intensity from 40 µmol/m^2/s condition is postulated by a rigid line, and the standard deviation is shown by the dashed line. "Light" is the light intensity at the sample depth (µmol/m^2/s), and the excessive H_2O_2 concentration, $H_2O_2\,rad$ (*Temp*), is expressed in µmol/gFW. The 'H_2O_2rad' denotes the light induced H_2O_2 content. The Exp 20 ºC and Exp 30 ºC represent the excessive H_2O_2 concentration quantified in experiments under 20 ºC and 30 ºC controlled temperatures.

5.3.1 Diurnal H_2O_2 variation of *E. densa*

In the diurnal observation, the light intensity during the 2018 sampling was higher for the 2018 observation day (~1500 µmol/m^2/s at the shoot height) compared to that for the 2017 observation day (<1000 µmol/m^2/s at the shoot height). This high solar radiation intensity condition continued for several weeks be12.1fore the 2018 observation day, while rainy or cloudy conditions had persisted for several weeks before the 2017 observation day. Therefore, samples from the 2018 observation were exposed to high solar radiation for several weeks, as opposed to samples from the 2017 observation **Figure 5.6 (A)**.

The diurnal variation of tissue H_2O_2 concentration followed the diurnal solar radiation intensity in 2017 and for healthy samples in 2018 S2 and S4 in 2018 **Figure 5.6 (B)**. Then, during the day, the H_2O_2 concentration rose up to nearly 16 µmol/gFW by noon and then, declined in the afternoon with the decline in solar radiation. In contrast, the values were substantially lower for the degraded samples in the 2018 observation. Then, samples in 2018 S2-2 and 2018 S3, showed less than 10 µmol/gFW of H_2O_2 concentration around noon. *E. densa* colonies remained healthy in the 2017 observation, while in the 2018 observation, shoots close to the water surface were degraded and appeared to be starting to die at S2-2 and S3.

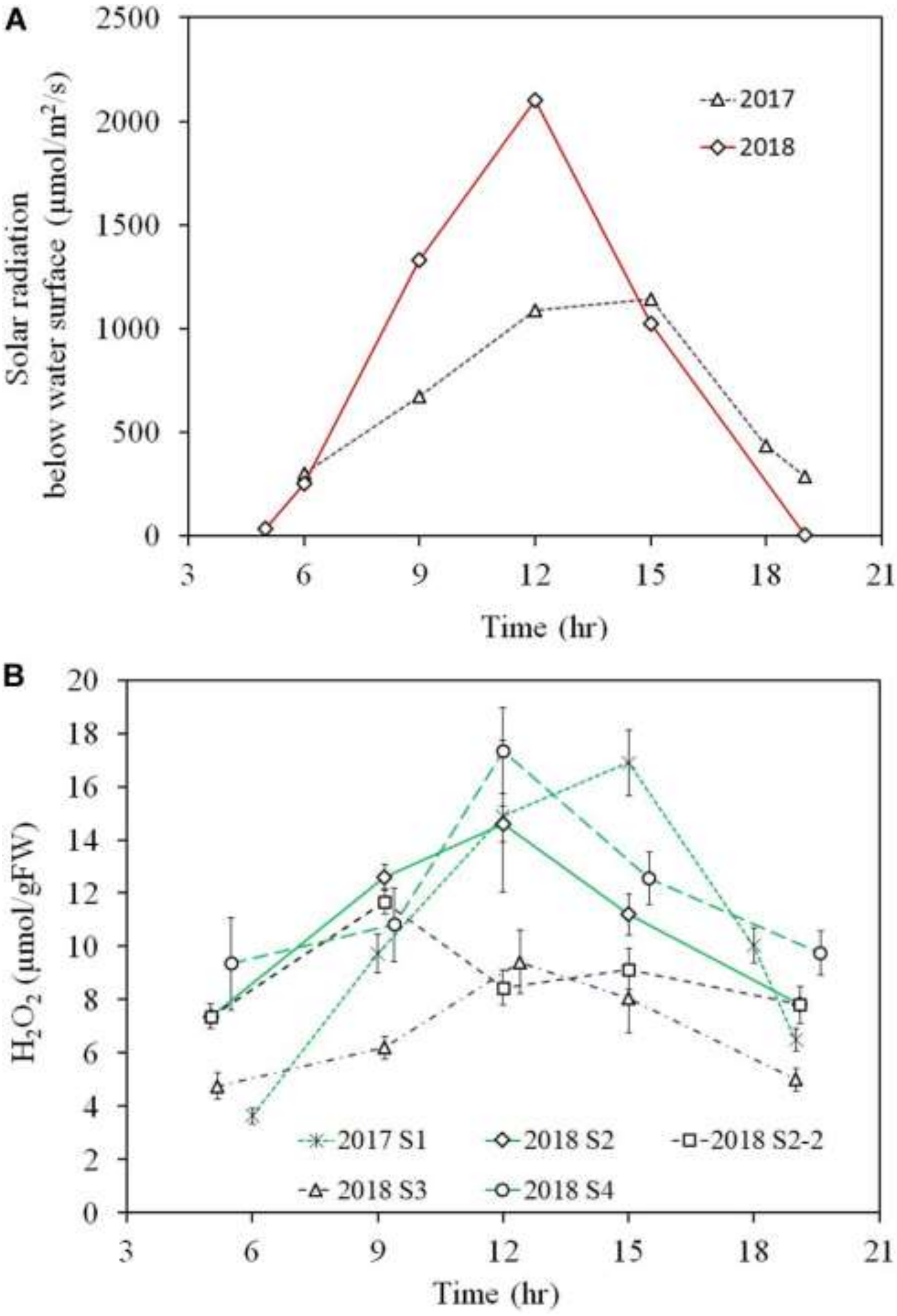


Figure 5.6

(A) Diurnal variation of light intensity just below the water surface of 2017 and 2018 observations

(B) *E. densa* tissue H_2O_2 concentration observed in the years 2017 (moderate radiation) and 2018 (excessive radiation). 2017 S1 represent the healthy shoots of 2017 observation. 2018 S2-2 and 2018 S3 represent degraded shoots under high solar radiation exposure at shallow water (<10 cm deep) in 2018, and 2018 S2 and 2018 S4 represent healthy shoots exposed to moderate radiation in deep depth sites (S2 30 cm deep, S4 50 cm deep) of the observation. The error bars represent the standard deviation**.**

As per **Figure 5.7 (A)** and **(B),** the H_2O_2 content for both the light-exposed and dark-adapted samples were negatively correlated with the biomass (R = -0.474, P <0.01 for light-exposed and R = -0.504, P < 0.01 for dark-adapted). It was observed that there were no samples with an H_2O_2 content exceeding the 16 µmol/gFW range. The approximate channel slope of the river in which large colonies of *E. densa* were formed in the running water was between 1/120–1/1800. No *E. densa* colony was found in the further upstream reaches, with channels steeper than 1/100, unless weirs were constructed to regulate the water flow.

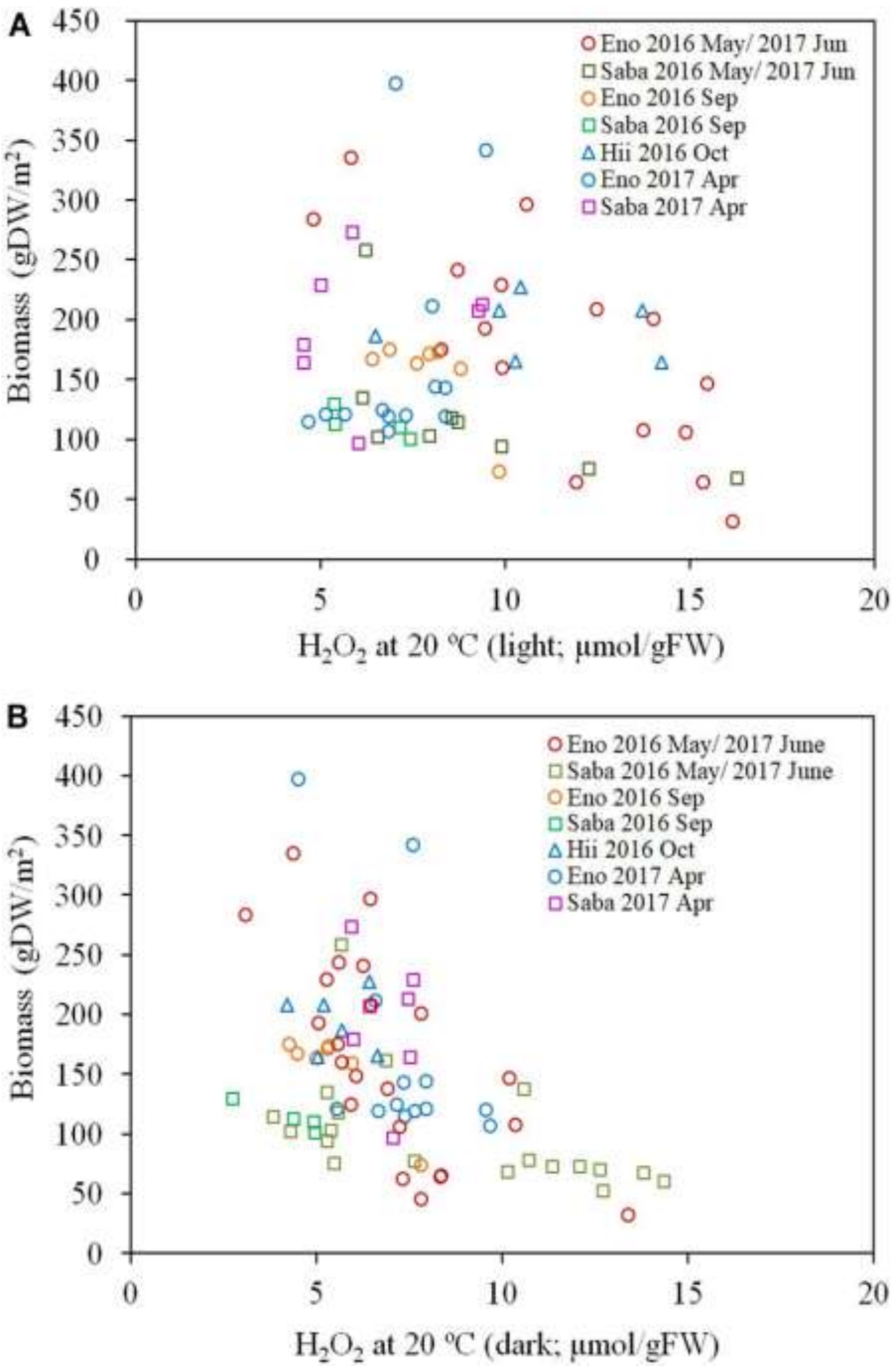


Figure 5.7

(A) Biomass of *E. densa* as a function of light exposed H_2O_2 concentration

(B) Dark-adapted H_2O_2 concentration was corrected to 20 ºC. The sample collected month is represented by the year and the month indicated in the legend. Eno, Saba and Hii represent Eno, Saba, and Hii rivers, including their tributaries, respectively.

The stress on plants generated by the flow velocity is more intensified at deeper sites; however, a high biomass concentration was found in deeper zones in the channel rather than in the shallow zones. The biomass of the colonies was highly correlated with the depth of the water column, peaking at a depth of 80 cm, and gradually declining as the depth increased further **Figure 5.8**. The biomass distribution exhibited a common trend irrespective of the river or the observation site.

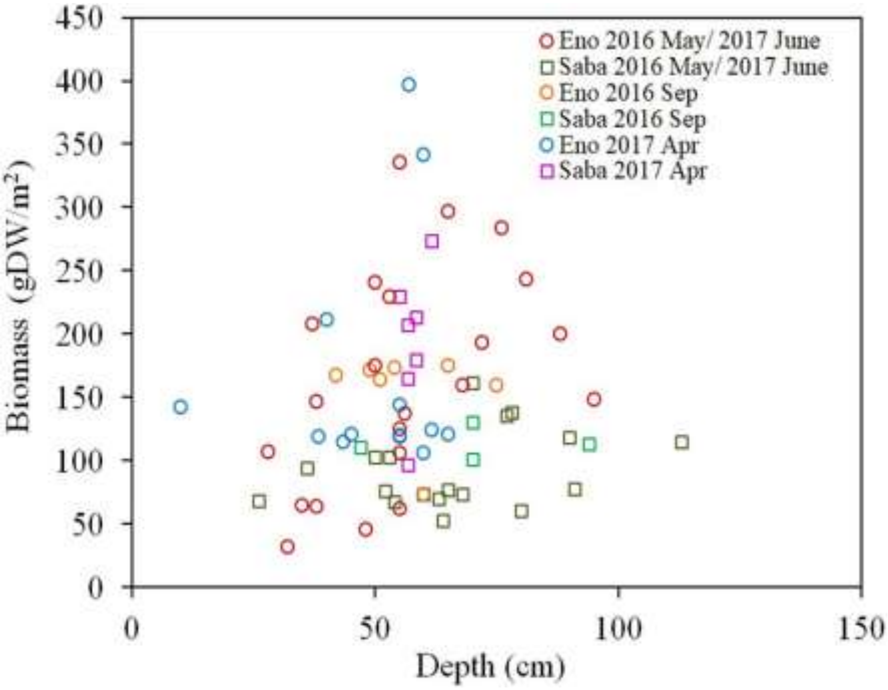


Figure 5.8 Biomass of *E. densa* as a function of water depth. The sample collected month is represented by the month indicated in the legend. Eno and Saba represent Eno and Saba rivers, including tributaries, respectively.

5.4 Discussion

5.4.1 Environmental stressors on *E. densa*

There are several types of stressors acting on submerged macrophytes in natural rivers. As these are based on different physical quantities, it is difficult to compare the magnitude of the effect of each stressor on the submerged macrophytes. The difference in H_2O_2 concentrations between the continuous light-exposed and 30 min pre-shaded samples differentiate the stress-induced H_2O_2 from photosynthesis generated H_2O_2. Based on the outcome of the laboratory experiment, we developed a relationship between temperature and the H_2O_2 generation in plant tissues. When the fractions of H_2O_2 corresponding to photosynthesis and temperature effects were eliminated from the continuous light-exposed samples of field studies, the observed trend of H_2O_2 was similar to the result of the oscillating grid laboratory experiment, in which the amount of H_2O_2 in macrophyte tissues was proportional to the root mean square velocity of the turbulence as is shown in **Figure 5.2** (Asaeda et al. 2017).

Under the zero-turbulence velocity, the tissue H_2O_2 concentration corresponds to a combination of photosynthesis, metabolic activities, and environmental stresses. The difference between light and dark treatment experiments distinguishes the amount of H_2O_2 generated by photosynthesis from the remaining stressors. Further, the variation in solar radiation intensity during the day is reflected in the H_2O_2 concentrations, which varied according to the light intensity from approximately 2 to 10 µmol/Gfw as shown in **Figure 5.5**. There were parallel relationships between the H_2O_2 concentration and the temperature for different light intensity groups with the same velocity as shown in **Figure 5.1**, which explains the independency of H_2O_2-temperature relationship trend from the light intensity but simultaneously elevates the trend due to excessive H_2O_2 production as shown in **Figure 5.2**. The temperature H_2O_2 dependency linear relationship provides the temperature generated H_2O_2 concentration, which is reportedly around ~10 µmol/Gfw in the *E. densa* habitat temperature range of 10 °C to 35 °C, in the present observation and previous reports (Hanamoto and Ikushima, 1988.; Yarrow et al., 2009; Gillard et al. 2017).

H_2O_2 concentration of dark-adapted samples had linearly increasing trends with respect to turbulence velocity, regardless of different sampling months and river groups, which differentiate the temperatures as shown in **Figure 5.1**. The H_2O_2 concentration extrapolated to zero velocity had the same relationship with the temperature dependency on the H_2O_2 concentration. Thus, the increasing rate of the H_2O_2 concentration with respect to turbulence effects indicates the turbulence induced H_2O_2.

These H_2O_2-solar radiation and H_2O_2-temperature relationships exhibit almost the same trends for photosynthetic rates obtained by the outdoor experiments with different temperatures and light intensities (Riis et al. 2012).Therefore, the H_2O_2 relationship with the light intensity and temperature conditions, can be considered as common trends for *E. densa*. In the practical application, H_2O_2 concentration can be applicable to determine the different types of stressors in the same manner and to compare their relative magnitudes.

Compared to the H_2O_2 induced by metabolic activities, which is ~4 μmol/Gfw given in the field experiments of dark-adapted samples at zero velocity, in the relatively steep non-polluted rivers, the major stressors include the mechanical stress introduced by high current velocity/turbulence, solar radiation, and temperature. The river water quality is relatively good, and there is no salinity in the midstream of Japanese rivers. However, in eutrophic water, organic matter accumulates at the bottom of stagnant zones, which creates an anoxic zone in the sediment layer; however, the bottom sediment anoxia contributed only ~5 μmol/Gfw of H_2O_2 (Parveen et al., 2017a). As for the biotic stress, toxic strains of cyanobacteria, *Microcystis*, for instance, generate only 1.5 μmol/Gfw of H_2O_2 (Amorin et al. 2017). Therefore, the amount of H_2O_2 generated by photosynthesis, temperature and flow velocity is relatively large compared to other stresses, and these stresses are considered as major stresses, which control its colonization.

The contribution of each stress to the total H_2O_2 of the plant can be distinguished with this method and can be adapted to determine the total level of environmental stress on macrophytes. The combination of different stresses sometimes imposes two opposing demands on the plant (Choudhury et al. 2017) or interact each other. However, the parallel relationship among the major stresses in natural rivers, namely, solar radiation, flow velocity, and water temperature and the interactive effects seems to be sufficiently small. The reason for the relatively lower interactive effects among stressors is not clear.

H_2O_2 is generated by the surplus number of electrons. In the photosynthesis process, the surplus amount of electrons are generated on the thylakoid membrane by strong energy (Asada, 2006), while the consumption of electrons is decreased under low temperature due to the suppressed CO_2 fixation by the inactivation of Rubisco in Calvin cycle (Nishiyama and Murata, 2014), or the mechanical damage of organelles in turbulent flow (Atapaththu et al. 2017). Therefore, the sites that can cause the electron surplus is different between stresses.

5.4.2 Threshold condition of *E. densa* mortality

E. densa colonies of 2017 observation remained healthy, while in the 2018 observation, shoots close to the water surface were degraded and appeared to be starting to die. The intensive oxidative stress caused by high solar radiation for several days should be the reason for the degradation of 2018 colonies. The results indicate the depression of plant metabolism owing to solar radiation exceeding the tolerable levels. When the H_2O_2 concentration became higher than 16 μmol/gFW in hypoxia and hydrogen sulfate exposure experiments for *E. densa*, plants deteriorated, and the total chlorophyll concentration and the H_2O_2 concentration substantially declined compared to other samples (Parveen et al., 2017 a, b, c). Also, with the exposure of Fe, *E. densa* exhibited lowest growth rate, chlorophyll content and photosystem efficiency at around 16 μmol/gFW of H_2O_2 content and beyond the level, healthy plants did not exist (unpublished data). Same as the other observations, the 2018 observations show the H_2O_2 level of colonies peaked beyond the 16 μmol/gFW during the daytime. Therefore, the H_2O_2 concentration of 16 μmol/gFW can be considered as a critical value for the survival of *E. densa*, regardless of the types of stressors. Exceeding the threshold level, would lead to the deterioration of plants due to oxidative damage.

5.4.3 Empirical expression of habitat preference and colonizable conditions simulation

The H_2O_2 concentration of macrophytes can be used to explain the expected macrophyte distribution in a river. When equation (4) is considered, the H_2O_2 generated by each stressor can be expressed as follows:

The total H_2O_2 concentration ($H_2O_2\,tot$) at a particular temperature ($Temp$) is given by:

$$H_2O_2tot\,(Temp) = H_2O_2rad\,(Temp) + H_2O_2vel\,(Temp) + H_2O_2met\,(Temp) \qquad (4)$$

where H_2O_2rad is the H_2O_2 generated by solar radiation exposure, H_2O_2vel is the H_2O_2 generated by the flow velocity, and H_2O_2met is the H_2O_2 generated by metabolism.

The light intensity at a particular depth of water, z, can be calculated using equation (5) (Middelboe and Markager, 1997):

$$I = I_0 \exp(-kz) \qquad (5)$$

where k is the attenuation coefficient in water, which was 0.035(/cm) in the observed rivers. The solar-radiation-induced H_2O_2 content has an increasing relationship with the light intensity, given by equation (3). Considering equation (3), the H_2O_2 concentration generated by the solar radiation under a particular temperature (within the temperature range 10 – 30 ºC), therefore, can be expressed as equation (6):

$$H_2O_2rad\,(Temp) = [I_0\exp(-kz) - 40]^{2/3}/10 \quad \text{for } I_0 \exp(-kz) \geq 40\ \mu\text{mol/m}^2\text{/s.}$$
$$H_2O_2rad\,(Temp) = 0 \text{ for } I_0 \exp(-kz) < 40\ \mu\text{mol/m}^2\text{/s.} \qquad (6)$$

Direct mechanical stress is generated by turbulence rather than the mean flow (Atapaththu et al., 2015; Asaeda and Rashid, 2017; Asaeda et al., 2017); however, there is a close relationship between these two quantities, particularly in straight uniform channels with uniform roughness. It is assumed that the mean velocity U (m/s) under the uniform flow is empirically given by Manning's law (equation (7)):

$$U = \frac{1}{n} R^{2/3} S^{1/2} \tag{7}$$

where R is the hydraulic radius, approximately given by the depth 'H' (m), the channel bed slope 'S', and Manning's roughness coefficient 'n'. The effects of longitudinal configuration, vegetation, etc., are added in 'n'. Therefore, considering equation (2), the H_2O_2 accumulation due to velocity stress and metabolism at a particular temperature (within the temperature range 10 – 30 ºC) can be expressed as follows (equation 8):

$$H_2O_2vel(Temp) = 0.072 \frac{1}{n} H^{2/3} S^{1/2} + 4.6 \tag{8}$$

Subsequently, the total H_2O_2 concentration within the temperature range 10 - 30 ºC can be given as follows (equation 9)

$$H_2O_2(Temp) = [I_0 exp(-kz) - 40]^{2/3}/10 + 0.072 \frac{1}{n} H^{2/3} S^{1/2} + 4.6$$

for I_0 exp (-kz) ≥10 μmol/m^2/s;

$$H_2O_2(Temp) = 0.072 \frac{1}{n} H^{2/3} S^{1/2} + 4.6$$

for I_0 exp (-kz) <10 μmol/m^2/s. (9)

Figure 5.9 shows the simulated H_2O_2 concentration generated by using equation (10), for different channel slopes and depths. I_0 is assumed to be 2000 μmol/m^2/s, as the highest solar radiation experienced in the observation area on a fine day during summer and when the canopy top was assumed to be located at the 80% depth, which is the average canopy height of the observed *E. densa* colonies. Manning's roughness coefficient for the channels is approximated at 0.08. Channel slope was obtained from the topographic map (GSI, 2019). The target area was characterized by steep basins, and the difference in elevation between the channel bed and the riparian zone is nearly constant thus the riverbed gradient is nearly the same as that of the riparian zone. The simulated results are consistent with the observed data of the rivers. The H_2O_2 concentration generally declines with depth, due to declining light intensity. In the steep channel, the H_2O_2 concentration increases again with a further increase in depth as the flow velocity rises. On gentler slopes, due to the lower flow velocity, H_2O_2 concentration is lower, and there is a wider range of depths in which *E. densa* colonies potentially form. However, the shallow depths become unsuitable for colonization of *E. densa* due to the higher light intensity.

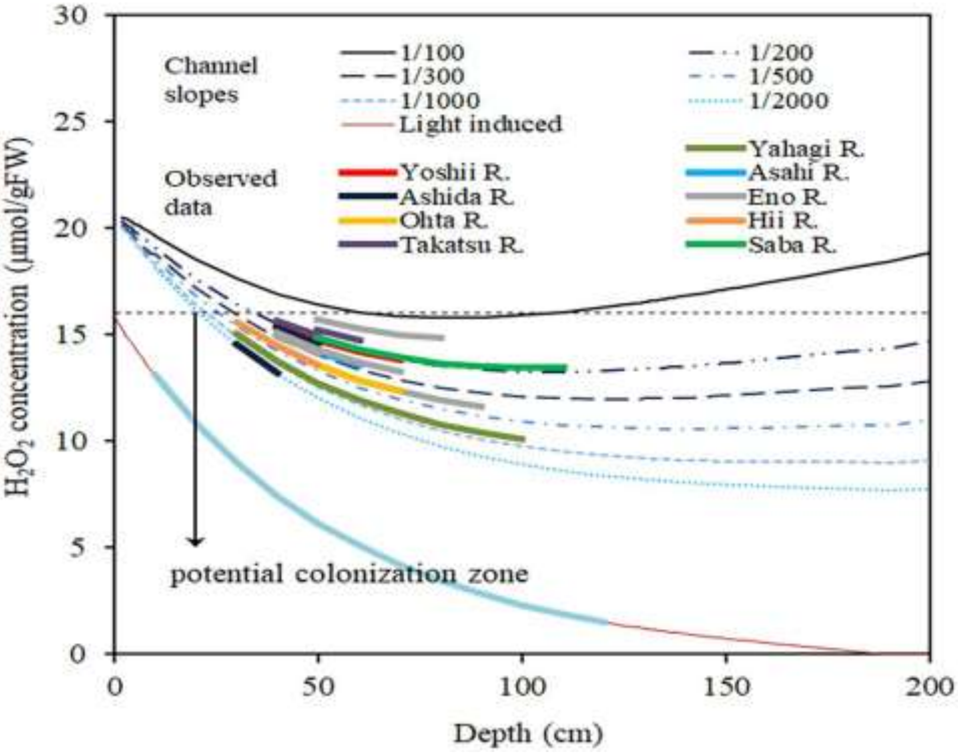


Figure 5.9 Simulated H_2O_2 concentration in the plant body of *E. densa* changes with the water column depth for different channel slopes and actual slope data of several Japanese rivers. The line 'Light-induced' represents the H_2O_2 concentration induced by light and change with the depth. The downward arrow 'potential colonization zone' represents the depth zone of different channel slopes suitable for *E. densa* colonization.

5.4.4 *E. densa* colonization in rivers

In rivers steeper than 1/100, the upstream to midstream areas are originally filled with gravel or boulders, and fine sediment beds are rare as they are easily flushed away during flooding. The flow velocity depends on the channel slope, depth, and roughness. Roughness is determined by the bed sediment size as well as other factors related to the channel configuration, such as longitudinal morphology and bars (Parker and Peterson 1980). The formation of *E. densa* colonies poses a physical disadvantage due to the high flow velocity and high light intensity as well as the high turbulence caused by the gravel bed as long as gravel sediments are supplied. However, when the gravel supply is decreased, the gravel-to-sand transition is extended further upstream (Singer, 2008), and the sand bed area increases in the former gravel zones. Sand is transported mostly as a suspended sediment load along the channel. *E. densa* communities accumulate suspended sediments efficiently, as they have a complex, dense stem structure that is widely distributed over the bed with thicker stems and denser whorls compared to similar native species, *Hydrilla verticillata*, and common native submerged species, *Elodea* sp., *Myriophyllum spicatum*, and *Potamogeton crisps* (Sand-Jensen, 1998; Vermaat, et al. 2000; Statzner et al. 2006). At the same time, water intake weirs have been frequently constructed along rivers in the last 30 years (personal communication). Then, stagnant water was produced in the upstream zone. Thick *E. densa* colonies were developed in the upstream area of many weirs in the observation (data are not shown). With the *E. densa* dispersal ability via fragmentation, it spread increasingly into the downstream (Casati et al. 2000, 2002; Redekop et al. 2016). Then, the dense shoot morphology leads to the formation of many sandy patches in the former complete gravel beds of the downstream. When fine sediments are supplied, it is easier to fix roots and take nutrients from the ground than from the stony bed (Barco, et al. 1991). Buried by a sediment layer, the *E. densa* complex shoots reinforce the sand layer, which is otherwise easily washed away by floods. Once the *E. densa* colonies develop, the sandy spaces increase widely on the gravel bed, accommodating more macrophytes, including other species. Subsequently, the ecosystem changes to a macrophyte-dominated ecosystem. A similar phenomenon was observed, after the occurrence of a large flood in the Yahagi river (in July 2017), sandy sediments accumulated in colonies burying *E. densa* up to 20 to 30 cm on a bed that was originally gravelly. The *E. densa* biomass was relatively dense, up to 150–400 gDW/m^2, in these sites, as the accumulated sand layer was reinforced with *E. densa* stem structures. This layer was thus rigid and could not be easily flushed away during moderate floods. In contrast to the original gravel bed, the sandy surface was smooth, and turbulence generation was also reduced. Further, in field observations, an accumulation of fine sediments was always found inside the *E. densa* colonies of all rivers.

5.5 Conclusion

The high concentrations of H_2O_2 introduced by high flow velocity and high solar radiation in summer inhibited the formation of large colonies in the gravel channel, owing to the high oxidative stress. The accumulation of H_2O_2 in *E. densa* showed a significant relationship for both flow velocity and solar radiation. The critical H_2O_2 concentration to maintain a healthy population of *E. densa* can be considered as 16 µmol/gFW, which corresponds to the termination of biomass accumulation. Under the strongest solar radiation on summer days, the H_2O_2 level often exceeds the critical condition, leading to the deterioration of *E. densa* and ultimately, the H_2O_2 concentrations decline as the plant tissues start to deteriorate. H_2O_2 concentrations of *E. densa* were estimated for channels with different slopes and different depths. The H_2O_2 concentration is higher than the critical value in shallow water and increases in steeper channels, exceeding the critical value at a channel slope larger than 1/100. Once colonized, *E. densa* accumulates sandy suspended sediment efficiently and creates a preferable environment for further colonization. The present methodology can be applied to predict the area that can be conveniently colonized by *E. densa* within a short period, which has been determined based on a prolonged monitoring activity.

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CHAPTER VI

Conclusion

Recently managing the vegetation seems to be a potential problem in several places around the globe. Although, there are several approaches that are being tested on trial-and-error bases there is not any suitable method developed yet to help manage the biodiversity. There are several traditional techniques which are being followed by several vegetation management authorities but unfortunately, they are time consuming and the costly methods.

The results of the current study show the effect of elevation gradient and moisture variation on the abundance of different species along a riparian zone. Species-specific zones of colonization or recruitment were clearly revealed using hydrogen peroxide as an environmental stress quantification index. Additionally, because most plant species face environmental stress, the current study proposes a rapid, efficient, and reliable monitoring system for vegetation management. With this method, only a relatively simple chemical analysis is involved when assessing oxidative stress, which is measured in the form of the hydrogen peroxide content. In measuring oxidative stress, it is important to truly understand the intensity of the stress, predict probable outcomes, and take appropriate measures to protect vegetation accordingly.

Flexible management-based methods are commonly used in the ecological management of riparian systems, where long-term monitoring of vegetation is conducted to identify stressors. *Salix* trees growing in this area have been monitored for 8 years in order to gain a better understanding of the mechanisms driving their high mortality. However, the responsible stressor was not identified solely from monitoring, as the change in *Salix* health was due to prolonged stress conditions. In this study, one ROS, H_2O_2,was identified as the primary cause of the increase in *Salix* mortality based on relatively straightforward analyses, and our results suggest that H_2O_2 levels can be used as an effective indicator of environmental stress levels and plant condition.

Salix are inundation-tolerant species often found along reservoir shorelines and are capable of colonizing the drawdown zones of flood-control dams, which otherwise would remain devoid of vegetation. These individuals are vulnerable to water stress during periods of water drawdowns, however. Oxidative stress levels of the inundated trees included in our study were as low as those of trees recently exposed following inundation, suggesting that one potential measure for reducing stress levels in *Salix* would be to supply additional water to the colony. Several studies have shown that *Salix* require high soil moisture in order to germinate but we recommend that soil moisture content be maintained at appropriate levels even for mature trees.

The high concentrations of H_2O_2 introduced by high flow velocity and high solar radiation in summer inhibited the formation of large colonies in the gravel channel, owing to the high oxidative stress. The accumulation of H_2O_2 in *E. densa* showed a significant relationship for both flow velocity and solar radiation. The critical H_2O_2 concentration to maintain a healthy population of *E. densa* can be considered as 16 μmol/gFW, which corresponds to the termination of biomass accumulation. Under the strongest solar radiation on summer days, the H_2O_2 level often exceeds the critical condition, leading to the deterioration of *E. densa* and ultimately, the H_2O_2 concentrations decline as the plant tissues start to deteriorate. H_2O_2 concentrations of *E. densa* were estimated for channels with different slopes and different depths. The H_2O_2 concentration is higher than the critical value in shallow water and increases in steeper channels, exceeding the critical value at a channel slope larger than 1/100. Once colonized, *E. densa* accumulates sandy suspended sediment efficiently and creates a preferable environment for further colonization. The present methodology can be applied to predict the area that can be conveniently colonized by *E. densa* within a short time period, which has been determined based on a prolonged monitoring activity.