

HOKKAIDO UNIVERSITY

Title	Frond size, shape and fertility of Thelypteris confluens (Thunb.) C. V. Morton in wetlands disturbed by human activities in Hokkaido, northern Japan
Author(s)	Tsuyuzaki, Shiro; Zhang, Xiaoli
Citation	Flora, 269, 151630 https://doi.org/10.1016/j.flora.2020.151630
Issue Date	2020-08
Doc URL	http://hdl.handle.net/2115/82321
Rights	© 2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Туре	article (author version)
File Information	Tsuyuzaki SFlora v.269_151630.pdf



Original Research

Frond size, shape and fertility of *Thelypteris confluens* (Thunb.) C. V. Morton in wetlands disturbed by human activities in Hokkaido, northern Japan

Shiro Tsuyuzaki $^{a,\,*}$ and Xiaoli Zhang a

^a Graduate School of Environmental Earth Science, Hokkaido University, Sapporo

060 0810 Japan

ARTICLE INFO	
Edited by	
Key words:	
Dimorphic fronds	
Disturbances	
Fertility	
Frond size and shape	
Number of veins	
Wetlands	

* Corresponding author institution: Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810 Japan *E-mail addresses*: tsuyu@ees.hokudai.ac.jp (S. Tsuyuzaki), and cyoujyouri@yahoo.co.jp (X. Zhang)

1 ABSTRACT

Thelypteris confluens (Thunb.) C. V. Morton exhibits dimorphism between sterile and fertile 2 fronds when fully matured. To assess the frond fertility patterns in this species, the frond size 3 and the shape and degree of fertile fronds were measured in individuals growing in disturbed 4 wetlands in Hokkaido, northern Japan, between late summer and early autumn in 2017 and 2018. 5 This species often establishes on human-disturbed wetlands in boreal regions. The measured 6 parameters were frond fertility, number of veins (NV), dry weight (DW), frond length (FL), blade 7 length (BL), blade width (BW), frond area and blade perimeter. The Dissection Index, aspect 8 ratio and specific frond area were calculated. The data were analyzed by generalized linear 9 models (GLMs) and standardized major axis tests to investigate the determinants of frond fertility 10 and the differences between the sterile and fertile fronds. All the GLMs for predicting frond 11 fertility contained NV, FL and BL, suggesting that NV had a specific role in fertility. Compared 12 with the use of destructive measurements, the use of nondestructive measurements in the GLMs 13 led to slightly lower prediction accuracies. The frond sizes and shapes in relation to fertility 14 were not strongly related to the disturbance rank scores, while the populations in highly disturbed 15 sites showed low numbers of fertile fronds. These findings strongly suggested that the shape-16 and size-related variables and NV affected frond fertility differently. NV provides more precise 17 measurements of frond maturity and population dynamics for nondestructive long-term 18 monitoring. 19

21 **1. Introduction**

22

More than 50% of wetlands have been lost worldwide since 1900, although this decrease is 23 24 underestimated (Davidson, 2014). The major causes of deterioration are derived from human activities, such as peat-mining, drainage construction and land-use change. Therefore, detecting 25 the effects of anthropogenic disturbances on populations and ecosystems is necessary to conserve 26 27 wetland ecosystems. Bioindicators, which include any taxonomic group with populations that reveal the qualitative status of the environment, are often used to evaluate human disturbance 28 29 intensity in wetlands and other ecosystem types (Taddeo and Dronova, 2018; Herlihy et al., 2019). However, the morphological and life-history traits of plants are differently affected by 30 environmental and regional differences (McIntyre et al., 1995; Chitwood and Shinha, 2016). 31

Thelypteris confluens (Thunb.) C. V. Morton, also known as marsh fern, is a deciduous 32 perennial fern often found growing in wetlands, including marshes, fens and bogs. This species 33 is a Euro-Siberian boreo-temperate plant (Schweingruber et al., 2020). This species forms a 34 caespitose population in moist, open sites in Japan (Iwatsuki, 1992) and has potential for use in 35 the phytoremediation of wastewater or soil because of its establishment in a wide range of 36 disturbed habitats (Anderson and Walsh, 2007). Thelypteris confluens forms dimorphic fronds: 37 the fertile fronds have thin and curling pinnae whereas the sterile fronds have flattened pinnae 38 (Kavak, 2014) when they produce sori between late summer and early autumn in Hokkaido, 39 Japan (Takita, 2001). Because frond fertility is a key to anticipating the population dynamics of 40 ferns, a suitable predictor of frond fertility is required (Tsuyuzaki, 2000). 41 Furthermore, nondestructive methods for monitoring population dynamics are preferable (Hoyo and Tsuyuzaki, 42 2013). When we can evaluate frond fertility without destructive sampling, we can monitor 43 population dynamics well over the long term. 44

Therefore, the objectives of this study were: 1) identifying the morphological variations in *Thelypteris confluens* established in variously disturbed wetlands by using destructive and nondestructive measurements, and 2) finding out the relationships between the morphological variations and frond fertility.

52 2.1. Study sites

53

Four wetlands, from north to south in Hokkaido, northern Japan, were selected for the 54 sampling sites: Sarobetsu, Nopporo, Utasai and Shizukari (Table 1). These four sites were 55 distributed in a cool temperate region. The mean annual temperatures at the four sites increased 56 from 6.1°C to 6.8°C with decreasing latitude. The annual precipitation ranged between 878 mm 57 58 in Sarobetsu and 1684 mm in Utasai and generally decreased from south to north. To evaluate the human disturbance intensity, a disturbance intensity rank was assigned based on a disturbance 59 gradient for ecological assessment of wetlands (Lopez and Fennessy, 2002), which was adjusted 60 to this study by using peat mining, drainage (including ditches), road pavement and a vegetation 61 buffer (Fig. 1). The chart lists 24 ranks of the intensities of human impacts based on 4 62 hierarchies (1st hierarchy = peat mining, 2nd = drainage, 3rd = paved road, and 4th = vegetation 63 buffer). 64

The Sarobetsu wetland (or mire) is located in the northernmost part of Hokkaido, facing the 65 Thelypteris confluens fronds were sampled in peatlands mined in 1977. The peat 66 Japan Sea. was mined down to 6 m in depth. The site was enclosed by unmined bogs dominated by 67 Sphagnum mosses, grasses and sedges, which functioned as a grassland buffer (Nishimura et al., 68 2009). The grasses, represented by Molinia japonica (Hack.) Hayata and Phragmites australis 69 (Cav.) Trinius ex Steudel., were well established in the site. This wetland was affected by 70 71 land-use change and conversion to pastures, with the construction of large drainage areas in the past (Ito and Wolejko, 1990). The foliage of Thelypteris confluens was sparsely distributed, and 72 there were relatively few fertile fronds. 73

Nopporo is located in central Hokkaido. In Nopporo, the samples were collected adjacent to a semi-natural swamp near a parking lot. This wetland was close to a metropolitan city, Sapporo, and therefore has been greatly damaged by urbanization. However, due to natural and transplanted forests, the study site has remained a vegetation buffer. Since the foliage is established densely in a small area, the total number of samples was the lowest. The Utasai bog is located in the southern part of Hokkaido. This wetland is surrounded by forests. A paved road with drainage areas was constructed across the wetland. *Thelypteris confluens* established in the drainage areas close to the paved road. Nonwetland and/or exotic species were common along the paved road.

Shizukari wetland, previously dominated by *Sphagnum* mosses, is located in the southern
part of Hokkaido. This wetland is 1 km from a coast and was not enclosed by any vegetation
buffers. The wetland was enclosed by farmlands, which had been converted from the wetland.

86

87 2.2 Sampling and analysis

88

Fronds were sampled from the four sites between late summer and early autumn in 2017 and 89 Two to seven fronds of various sizes were collected from each plant. When fertile 2018. 90 fronds were available, a few fertile were selected and collected along with a few sterile fronds. 91 Samples of the foliage were collected at intervals more than 3 m apart. Therefore, the numbers 92 of samples differed between the sites, depending on the distribution pattern and density of the 93 foliage. In total, 1104 fronds were collected, 1013 of which were used to measure all the 94 The silhouette of each sample was scanned by an image scanner at 400 dpi 95 variables. (CanoScan LiDE 30, Canon, Tokyo) after pressing the frond specimens. Frond length (FL), 96 blade length (BL), blade width (BW), frond area and blade perimeter were calculated by ImageJ 97 software (ver. 1.52q) (Schneider et al. 2012). The number of midrib branches of the fronds (NV, 98 number of veins) was counted visually (Sato, 1985ab). Then, the dry weight (DW) of the whole 99 blade was measured after drying at 60°C for more than 3 days. The Dissection Index, aspect 100 ratio and specific frond area were calculated based on these values. Two aspect ratios were 101 examined, BW/FL and BW/BL. The Dissection Index was calculated by P/($\pi \times$ frond area) 102 (Kincaid and Schneider, 1983). Therefore, aspect ratio and Dissection Index are composed of 103 two variables and express the frond shape. The FL, BL, BW, NV and aspect ratio were 104 measured or calculated nondestructively and the others variables required destructive sampling. 105

106 The differences in sizes or shapes between the fertile and sterile fronds were compared by 107 generalized linear models (GLMs) with the assumption of a Gaussian distribution (Dobson and

108 Barnett, 2018). To detect the effects of frond characteristics on frond fertility, GLMs were used with the assumption of a binomial distribution; fertility was used as the response variable and 109 frond characteristics and disturbance rank were used as the explanatory variables. The most 110 111 suitable models were selected by a backward procedure with Akaike's Information Criteria (AIC) (Aho et al., 2014). Interactions between the variables were not examined because the objective 112 was the selection of suitable parameters to predict frond fertility. 113 To investigate the effectiveness of using nondestructive measurements to predict fertility, GLMs were tested by NV, 114 FL, BL, BW, BW/FL, BW/BL and disturbance rank, and all the parameters from the four sites. 115 116 GLMs were also developed by using three sites excluding Nopporo because fertile fronds were least abundant in Nopporo. To check the accuracy of the discrimination of frond fertility by 117 destructive and nondestructive methods, the prediction accuracy was calculated as follows: 118 (number of correctly discriminated fronds)/(total number of samples) \times 100 (%). 119

The allometric relationship between the measured variables was selectively assessed by using standardized major axis tests (SMA) to observe the site-based differences in the allocation of the human disturbance rank scores (Warton et al., 2006). Since frond characteristics are related to BL in most examined ferns (Sato et al. 1989, Tsuyuzaki 2000), allometry was obtained between the BL and the other variables for fertile and sterile fronds. All the statistical analyses were performed by R software (version 3.6.2) (R Core Team, 2019) with the libraries MASS (Venables and Ripley, 2002) and smatr (Warton et al., 2012).

127

128 **3. Results**

129

The disturbance intensity ranks of the sites were ordered from high to low as follows: 22 in Nopporo, 20 in Sarobetsu, 10 in Utasai and 9 in Shizukari (Fig. 1). The numbers of sampled fertile fronds were 6, 38, 121 and 124 in Nopporo, Sarobetsu, Shizukari and Utasai, respectively, while the numbers of sterile fronds were 85, 259, 218 and 253. The ratios of fertile to sterile fronds were 7.1%, 14.7%, 55.5% and 49.0% in Nopporo, Sarobetsu, Shizukari and Utasai, respectively, and low ratios were observed in the highly disturbed sites. Of these samples, 0, 38, 121 and 124 fertile fronds were perfect, i.e., all the variables were measured, in Nopporo,

Sarobetsu, Shizukari and Utasai, respectively, while 42, 259, 196 and 233 sterile fronds were 137 perfect. Although frond fertility increased with increasing frond size (Fig. 2), the frequency 138 distribution patterns differed between the variables. In particular, most sterile fronds showed 139 140 low dry weight and blade perimeter length. Of the shape variables, BW/BL of aspect ratios was highly overlapped. This showed that, in relation to BW and BL, the frond shape did not differ 141 greatly between the fertile and sterile fronds. BW/FL and the specific frond area were smaller 142 for the fertile fronds than for the sterile fronds. These results indicated that the responses of the 143 shape variables to fertility were different from those of the size variables. 144

145 The best model selected by the AIC consisted of seven of the eleven destructive and nondestructive variables for predicting frond fertility at the four sites (Table 2). The selected 146 variables were NV, DW, FL, BL, frond area, Dissection Index and specific frond area. The two 147 aspect ratios, out of the shape parameters, were not selected in the model, while another shape 148 index, Dissection Index, remained. The variables selected for the models of three sites were the 149 same as those for the models consisting of the four surveyed wetlands. The human disturbance 150 rank scores were not included in these two models, indicating that the fertility patterns did not 151 differ between the surveyed sites. Of the nondestructive variables, NV, FL, BL, BW/FL and 152 BW/BL were included in the best models. These results meant that the shape index remained in 153 all the models. Other than the two models that used all the variables, the rank was not retained 154 in the models developed with the nondestructive variables. 155

NV, FL and BL were included in all the four models, indicating that these three size variables were required to predict the frond fertility. The models developed with all the variables showed higher accuracy of fertility prediction (i.e., > 92%) than the models made with nondestructive variables (ca 85%), although the difference was only 7%. The difference in accuracy was derived mostly from the prediction error of the sterile fronds. These results indicated that the sterile fronds showed more diverse sizes and shapes than the fertile fronds.

Except for the allometric relationship between BL and specific frond area for the fertile fronds, all the regression lines were significant (Fig. 3). The differences in the slopes of seven of the nine allometric relationships between BL and the other variables were significant. These results indicated that most frond size and shape variables were allometrically different between 166 the fertile and sterile fronds. When sterile and fertile fronds had the same BL, fertile fronds had higher DWs, BWs, frond areas, and higher values of the Dissection Index, the BW/BL ratio and 167 the specific frond area. NV was lower on the fertile fronds than on sterile fronds when BL was 168 169 equivalent. The BW/BL ratio decreased with increasing BL, indicating that the frond shape became slimmer with increasing BL. Additionally, the fertile fronds were broader than the 170 The SMA showed clear differences in the slopes between the fertile and sterile 171 sterile fronds. 172 fronds; that is, the SMA decreased with increasing BL for the fertile fronds but increased with increasing BL for the sterile fronds. This allometry pattern also indicated that the fertile fronds 173 174 were denser than the sterile fronds. Nonsignificant differences were observed between the BL and FL and between the BL and blade perimeter, showing that the allocation to these two 175 variables did not differ with frond fertility. Since the FL included the frond petioles, this also 176 showed that the allocation to the frond petioles did not change with frond fertility. 177

178

179 4. Discussion

180

The fertility patterns of Thelypteris confluens predicted by the GLMs did not differ between 181 the disturbance intensity rank scores, indicating that the fertility patterns predicted by the 182 measured variables did not differ along the surveyed disturbance intensity range. Since the 183 disturbance rank scores were different between the four sites, the site differences were also 184 unlikely to affect the frond size or shape in relation to fertility patterns. Most lowland wetlands 185 in Hokkaido have been disturbed to some extent by human settlements (Ito and Wolejko, 1990) 186 and all the surveyed wetlands were affected by human-related disturbances, i.e., peat mining, 187 drainage construction and/or pavement. The environment, including disturbances and stressors, 188 affects frond size and shape (Chitwood and Sinha, 2016). Although the physical disturbances, 189 such as trampling, directly affect the size and shape of leaves (Vujic et al., 2016), large-scale 190 disturbances, such as peat mining, drainage construction and road construction, broadly and 191 indirectly affect frond size and shape. These results suggested that the differences in 192 disturbance characteristics among the four studied sites did not greatly affect the size or shape of 193 fronds in relation to frond fertility. 194

195 Fertile fronds were less abundant in Nopporo and Sarobetsu where the disturbances had been more intense. Plants such as willows and poplars often undergo vegetative reproduction more 196 than sexual reproduction to reduce seedling mortality in disturbed and/or stressful habitats 197 198 (Thomas et al., 2012). Vegetative reproduction supports the population persistence of Drosera anglica Hudson on a postmined peatland in Sarobetsu mire, which is greatly disturbed by human 199 activities (Hoyo and Tsuyuzaki, 2015). This fern has an intrinsic restriction on fertile frond 200 production, which depends on the balance between habitat requirements and vegetative organ 201 production (Shapre, 2005). The populations in Nopporo and Sarobetsu seemed to use this 202 203 strategy, i.e., there was a low abundance of fertile fronds in highly disturbed habitats. The fertility ratio at the population level may be affected by the fate of population dynamics, although 204 205 frond measurements have not been investigated at this level.

The fertility of *Thelypteris confluens* was predicted well by using multiple variables. Of 206 the frond variables, NV, BL and FL were retained in all the GLMs to predict frond fertility. In 207 models with single measurement variables, NV was the most powerful variable for detecting the 208 frond fertility of ferns, such as five Polystichum ferns (Sato, 1985b) and three Dryopteris ferns 209 (Sato and Tsuyuzaki, 1988). NV predicts the frond fertility of Pteris mutilata Burn., which 210 develops dimorphic fronds depending on fertility, better than the other size and shape parameters, 211 i.e., FL, BL and aspect ratios (Tsuyuzaki, 2000). NV is tightly related to the frond maturation of 212 ferns, including Thelypteris confluens, as previously described. These results suggest that NV 213 has a specific role in frond fertility, probably because the length of veins characterizes the frond 214 shape (Cope et al., 2012) and therefore is related to the other frond size variables. The accuracy 215 216 of the prediction by nondestructive measurements was not very low, 85-86%, but was slightly lower than that by variables including destructive measurements. Because measuring many 217 parameters provides high identification accuracy, digital morphometrics with a computer has 218 been applied to various researches, including research in plant taxonomy and ecology (Klein et al., 219 However, such destructive measurements do not allow long-term monitoring of 220 2017). The nondestructive methods used in this study measured only FL, BL, BW and NV. 221 populations. 222 NV is easily counted and should be measured at any expense when nondestructive measurement is required. 223

224 When the Dissection Index was included in the best models (i.e., those developed with all the variables), the aspect ratios (BW/FL and BW/BL) were discarded from the models. 225 The BW/FL ratio, BW/BL ratio and Dissection Index quantify frond shape. These results suggested 226 227 that frond fertility was related to not only size but also shape. The slope of the allometric relationship between BL and specific frond area was negative for the sterile fronds and was not 228 significant for the fertile fronds. Frond mass per area, which is equal to 1/(specific frond area), 229 is dependent more on frond thickness than on dry weight for vascular plants, because dry weight 230 is a more stable measurement than weight (Wilson et al., 1999). The specific frond area of the 231 232 fertile fronds was lower than that of the sterile fronds because the weight of the sori was included in the measurement of the fertile fronds. This difference was observed in the fertile fronds 233 because of sorus formation, which also seemed to be related to NV. In other words, frond shape 234 may be a consequence of sorus development. The present study concluded that frond size and 235 shape were both related to frond fertility and differently affected frond fertility. 236

237

238 Acknowledgments

239

The authors sincerely thank all the members of our lab for field assistance. The authors thank the Subject Editor and anonymous reviewers for their helpful comments. This work is partly supported by the Japan Society for the Promotion of Science and the Nippon Flower Designers' Association.

244

245 **References**

- 246
- Aho, K., Derryberry, D., Peterson, T., 2014. Model selection for ecologists: the worldviews of
 AIC and BIC. Ecology 95, 631-636.
- Anderson, L., Walsh, M.M., 2007. Arsenic uptake by common marsh fern *Thelypteris palustris*and its potential for phytoremediation. Sci. Total Environ. 379, 263-265.
- Chitwood, D.H., Sinha, N.R. 2016. Evolutionary and environmental forces sculpting leaf
 development. Curr. Biol. 26, R297-R306.

- Cope, J.S., Corney, D., Clark, J.Y., Remagnino, P., Wilkin, P. 2012. Plant species identification
 using digital morphometrics: A review. Expert Syst. Appl. 39, 7562-7573.
- Davidson, N.C. 2014. How much wetland has the world lost? Long-term and recent trends in
 global wetland area. Mar. Freshw. Res. 65, 936-941.
- Dobson, A.J., Barnett, A.G. 2018. An introduction to generalized linear models (4th edition).
 Chapman & Hall, London.
- Herlihy, A.T., Sifneos, J.C., Lomnicky, G.A., Nahlik, A.M., Kentula, M.E., Magee, T.K., Weber,
 M.H., Trebitz, A. 2019. The response of wetland quality indicators to human disturbance
 indicators across the United States. Environ. Monit. Assess. 19, article 296.
- Hoyo, Y., Tsuyuzaki, S. 2013. Characteristics of leaf shapes among two parental *Drosera* species
 and a hybrid examined by canonical discriminant analysis and a hierarchical Bayesian model.
 Amer. J. Bot. 100, 817-823.
- Hoyo, Y., Tsuyuzaki, S. 2015. Sexual and vegetative reproduction of the sympatric congeners
 Drosera anglica and *D. rotundifolia*. Flora 210, 60-65.
- Ito, K., Wolejko, L. 1990. Vegetational changes in response to drainage at Sarobetsu Mire, N.
 Hokkaido, Japan. In: Whigham, D.F., Good, R.E., Kvet, J. (Eds.), Wetland Ecology and
 Management: Case studies. Tasks for Vegetation Science 25. Springer, Dordrecht. (pp. 131-134).
- 271 Iwatsuki, K. 1992. Ferns and fern allies of Japan. Heibonsha, Tokyo, p 214.
- Kavak, S. 2014. *Thelypteris palustris*. The IUCN Red List of Threatened Species 2014.
 e.T164136A42331187.
- Klein, L.L., Caito, M., Chapnick, C., Kitchen, C., O'Hanlon, R, Chitwood, D.H., Miller, A.J.
 2017. Digital morphometrics of two north American grapevines (*Vitis*: Vitaceae) quantifies
 leaf variation between species, within species, and among individuals. Front. Plant Sci. 8,
- 277 article 373.
- Kincaid, D.W., Schneider, R.B. 1983. Quantification of leaf shape with a microcomputer and
 Fourier transform. Can. J. Bot. 61, 2333-2342.
- 280 Lopez, R.D., Fennessy, M.S. 2002. Testing the floristic quality assessment index as an indicator
- of wetland condition. Ecol. Appl. 12, 487-497.

- McIntyre, S., Lavorel, S., Tremont, R.M. 1995. Plant life-history attributes: their relationship to
 disturbance response in herbaceous vegetation. J. Ecol. 83, 31-44.
- Nishimura, A., Tsuyuzaki, S., Haraguchi, A. 2009. A chronosequence approach for detecting
 revegetation patterns after *Sphagnum*-peat mining, northern Japan. Ecol. Res. 24, 237-246
- 286 R Core Team. 2019. R: A Language and Environment for Statistical Computing. R Foundation for
 287 Statistical Computing, Vienna, Austria.
- Sato, T. 1985a. Quantitative expression of fern leaf development in *Polystichum tripteron*(Aspidiaceae). Plant Syst. Evol. 150, 191-200.
- Sato, T. 1985b. Comparative life history of five Hokkaido *Polystichum* ferns with reference to
 leaf development in relation to altitudinal distribution. Bot. Mag., Tokyo 98, 99-111.
- Sato, T., Grabherr, G., Washio, K. 1989. Quantitative comparison of fern-leaf development and
 fertility with respect to altitude in the Tirol, central European Alps, Austria. J. Biogeogr. 16,
 449-455.
- Sato, T., Tsuyuzaki, S. 1988. Quantitative comparison of foliage development among *Dryopteris monticola*, *D. tokyoensis* and a putative hybrid, *D. kominatoensis*, in northern Japan. Bot.
 Mag., Tokyo 101, 267-280.
- Schneider, C.A., Rasband, W. Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image
 analysis. Nature Methods 9, 671-675.
- Schweingruber, F.H., Kučerová, A., Adamec, L., Doležal, J. 2020. Anatomic Atlas of Aquatic and
 Wetland Plant Stems. Springer, Cham p. 44
- Shapre, J.M. 2005. Temporal variation in sporophyte fertility in *Dryopteris intermedia* and
 Polystichum acrostichoides (Dryopteridaceae: Pteridophyta). Fern Gaz. 17, 223-234.
- Taddeo, S., Dronova, I. 2018. Indicators of vegetation development in restored wetlands. Ecol.
 Ind. 94, 454-467.
- Takita, K. 2001. Hokkaido Botanical Chart. Kato Archives, Kushiro. 1452 pp.
- Thomas, L.K., Tolle, L., Ziegenhagen, B., Leyer, I. 2012. Are vegetative reproduction capacities
 the cause of widespread invasion of Eurasian Salicaceae in Patagonian river landscapes?
 PLoS ONE 7, e50652
- 310 Tsuyuzaki, S. 2000. Characteristics of "number of veins" to estimate leaf maturity in Pteris

- 311 *mutilata* (Pteridaceae). J. Plant Res. 113, 415-418.
- Venables, W.N., Ripley, B.D. 2002. Modern Applied Statistics with S (4th edn.). Springer, New
 York.
- Vujic, V., Rubinjoni, L., Selakovic, S., Cvetkovic, D. 2016. Small-scale variations in leaf shape
 under anthropogenic disturbance in dioecious forest forb *Mercurialis perennis*: A geometric
 morphometric examination. Arch. Biol. Sci. 68, 705-713.
- Warton, D.I., Duursma, R.A., Falster, D.S., Taskinen, S. 2012. smatr 3 an R package for
 estimation and inference about allometric lines. Methods Ecol. Evol. 3, 257-259.
- Warton, D.I., Wright, I.J., Falster, D.S., Westoby, M. 2006. Bivariate line-fitting methods for
 allometry. Bot. Rev. 81, 259-291.
- Wilson, P.J., Thompson, K., Hodgson, J.G. 1999. Specific leaf area and leaf dry matter content as
 alternative predictors of plant strategies. New Phytol. 143, 155-162.

325 Table 1

Climatological and geographical characteristics (including the human disturbance intensity rank score) of the four surveyed wetlands. The climatological data are obtained from the Japan Meteorological Agency webpage (https://www.jma.go.jp/jp/amedas/) (accessed on March 25 2020). For information on the human disturbance rank scores, see Fig. 1.

330

Wetland	Sarobetsu	Nopporo	Utasai	Shizukari
Latitude (N)	45°06'	43°04'	43°63'	43°58'
Longitude (E)	141°43'	141°54'	140°34'	140°44'
Elevation (m)	8	8	27	10
Mean annual temperature* (°C)	6.1	7.5	7.5	7.8
Annual precipitation (mm)	878	1018	1684	1369
Human disturbance rank score	20	22	10	9

*: based on records from 2017 and 2018

Table 2

Frond fertility predicted by frond parameters and disturbance ranks by GLM with the assumption 334 of a binomial distribution. The values show the intercepts and estimates of the models. AIC 335 was used to select the parameters that led to the best GLMs by a backward method. All models 336 used all 11 measured variables and the disturbance rank scores. Three sites: data obtained from 337 Nopporo, where the lowest number of fertile foronds, were not used. **: significant at P < 0.01, 338 *: P < 0.05, NS: not significant. The numbers of fronds show the correct and incorrect 339 discrimination on the left and right sides of slash marks, respectively. The AIC values are 422.19 340 and 422.07 for the best models created with all the variables from four and three sites, 341 respectively. The AIC values are 675.19 and 674.31 for models created with nondestructively 342 sampled variables from four an three sites, respectively. ×: not used for models developed with 343 nondestructive measurements. -: discarded from the models according to the AIC values. 344

345

	Destructive		Nondestructive	
	Four sites	Three sites	Four sites	Three sites
Intercept	-3.881*	-3.894*	-9.526**	-9.473**
Number of veins	$+0.070^{**}$	$+0.070^{**}$	$+0.152^{**}$	$+0.151^{**}$
Dry weight	$+5.797^{*}$	$+5.830^{*}$	×	×
Frond length	$+0.079^{**}$	$+0.079^{**}$	$+0.122^{**}$	+0.124**
Blade length	-0.199**	-0.199**	-0.220^{*}	-0.224**
Blade width	-	-	-	-
Frond area	-0.035*	-0.036*	×	×
Blade perimeter	-	-	×	×
Dissection Index	$+0.233^{**}$	+0.233**	×	×
Aspect ratio				
Width/(frond length)	-	-	-22.198*	-21.517*
Width/(blade length)	-	-	$+8.430^{*}$	$+4.199^{NS}$
Specific frond area	-0.024**	-0.024**	×	×
Human disturbance rank score	-	-	-	-
Accuracy of fertile frond	225/17	225/17	164/24	163/24
Accuracy of sterile frond	713/58	671/58	706/119	664/120
Prediction accuracy (%)	92.6	92.3	85.9	85.2

346



Fig. 1. Hierarchical classification procedures used to score the human disturbance rank. The 361 hierarchical ranks are adjusted to the study sites. The following four types of human 362 disturbances are considered: peat mining, drainage and ditches, paved roads and vegetation 363 The vegetation buffers are classified into three categories: grassland, forest and no 364 buffers. buffer. Each numeral indicates the disturbance rank score. The scores of the four sites are 365 shown by the first two letters of the wetland names: Sa = Sarobetsu, No = Nopporo, Ut = Utasai 366 and Sh = Shizukari. 367 368





371 372

373

Fig. 2. Relative frequency distributions (%) of the size and shape variables in relation to frond fertility, examined for each of the four study sites. The X axis ranges between 0 and 1 for all the variables. The maximum value of each variable is shown in the parentheses on each panel. The dark gray and open bars indicate sterile and fertile fronds, respectively. The light gray bars indicate the overlaps between the fertile and sterile fronds. *: the variables are significantly different between the fertile and sterile fronds at P < 0.01 (GLM). NS: nonsignificant.



382

383

384

Fig. 3. Allometric relationships between blade length and the other nine variables in relation to 385 The differences in slopes are examined by standardized major axis tests. 386 frond fertility. The open and closed circles indicate fertile and sterile fronds, respectively. The black and gray lines 387 indicate the allometric regression lines for fertile and sterile fronds, respectively. F and S: the 388 slopes of the lines for fertile and sterile fronds, respectively. When the slopes of S and F are 389 indicated in black letters, they are significantly different at P < 0.01. When the slopes of S and 390 F are indicated in gray, they are not significantly different. All the regression lines are 391 significant at P < 0.01, except for the specific frond area of fertile fronds, for which the slope 392 value is shown in italics. 393