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Aquatic Botany 158 (2019) 103129

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Salinity tolerance of aquatic plants indicated by monitoring data from the Netherlands

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ARTICLE INFO

Keywords:
Brackish
Chloride
Distribution
Macrophytes
Inland waters
Water Framework Directive

ABSTRACT

Data on salinity tolerance of aquatic plant species are scarce, but required to better understand species' responses in multiple stressed environments. We analyzed data from a Dutch Water board in the province of Zeeland according to the occurrence of plant taxa in relation to salinity (chloride concentration). The dataset contained 862 samples of plants (altogether 46 taxa) and chloride concentrations. The smallest range was found for *Nuphar lutea* occurring in sites with 43 to 140 mg l^{-1} Cl^-. The widest range was observed for *Glaux maritima* occurring in sites with 710 to 22,000 mg l^{-1} Cl^-. Two species (*N. lutea and Potamogeton trichoides*) had an optimum lower than 100 mg l^{-1} Cl^-, while three taxa (*G. maritima, Salicornia* spp. and *Ulva* spp. (=non-filamentous)) had an optimum higher than 10,000 mg l^{-1} Cl^-. Change points (CPs) were determined on salinity class level along the gradient. These CPs are defined as the class, at which the largest change in occurrence is determined. The CP of taxa that are relatively sensitive, such as *Sparganium erectum* and *Carex riparia*, were below 200 mg l^{-1} Cl^-, while relative tolerant taxa, such as *Stuckenia pectinata* and *Ranunculus sceleratus*, had CPs at concentrations that were more than tenfold higher. The upper limit of the salinity range at which taxa occurred in the study area were generally similar to the few limits that were previously published. Implications for assigning species to water body types for implementing the Water Framework Directive in the Netherlands are discussed.

1. Introduction

Macrophytes are a vital part of freshwater and brackish ecosystems. They act as a refuge for a variety of organisms, take up nutrients and provide oxygen to the water. While several macrophytes are generalists in terms of preferred habitat types and chemical conditions (Poikane et al., 2018; Szoszkiewicz et al., 2006), the distribution of others is strongly limited e.g. by nutrients or alkalinity (Jenačković et al., 2016; McElarney et al., 2010). A chemical variable strongly determining macrophyte species distribution is salinity (Jenačković et al., 2016; Jeppesen et al., 2015). Some macrophytes can only tolerate relatively low levels ($< 300\,mg\,l^{-1}$ Cl^-), and are considered freshwater species (Oertli, 1964), while others can occur in a wide salinity range and tolerate brackish conditions of more than $300\,mg\,l^{-1}$ Cl^-. An example of such a highly tolerant species is *Stuckenia pectinata* (Barbour, 1970; Kantrud, 1990). Other species, such as *Ruppia cirrhosa* and *R. maritima,* are restricted to the brackish environment and generally do not occur in freshwater (Izzati, 2015; Orth, 1994; Remane and Schlieper, 1972). Thus, salinity is one of the basic chemical characteristics of surface water systems that drives plant distribution in coastal areas.

Salinity is defined as the amount of cations and anions in the water (Munns and Tester, 2008). Because about half of the total ion concentration in brackish and saline water consist of chloride ions (Cl^-), the chloride concentration is often considered an appropriate measure for salinity. Increased concentration of dissolved salts impose two different types of stress on macrophytes: osmotic and ionic stress (Jampeetong and Brix, 2009; Munns and Tester, 2008). Under osmotic stress, caused by an hypertonic environment, cells can undergo plasmolysis and show symptoms similar to drought stress (Nawaz et al., 2010; Touchette, 2007). Ionic stress causes high concentrations of Na^+ to diffuse into the plant cells, inhibiting the production of enzymes, and processes catalysed by these enzymes slow down (Jampeetong and Brix, 2009; Kronzucker et al., 2013). In addition, sea and brackish water contain 10 to 1000 times more sulphate than freshwater, which may be transformed into hydrogen sulphide under anoxic conditions. Hydrogen sulphide can cause phytotoxic effects (Koch et al., 1990; Lamers et al., 2013). Therefore, it is generally not clear what causes the negative effect of salinity in sea- and brackish water on macrophyte growth.

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https://doi.org/10.1016/j.aquabot.2019.103129
Received 21 September 2018; Received in revised form 21 June 2019; Accepted 23 June 2019

Overall, symptoms of salt stress range from reduced growth rates, shorter roots, decrease in size, decreasing leaf area, and reduced assimilation rates to death (Jampeetong and Brix, 2009; Munns and Tester, 2008).

Currently, water authorities throughout Europe are trying to restore the ecological and chemical status of surface waters according to the Water Framework Directive. Ecological status in lentic waters is determined by "Biological Quality Elements", including phytoplankton, macrophytes, benthic macroinvertebrates and fish. The presence and abundance of macrophyte species are used to determine if the status of surface waters is at an acceptable level, i.e. the "Good Ecological State" (GES) or "Good Ecological Potential" (GEP). The knowledge on ecological preferences of macrophytes, however, is still limited especially when compared to macro-invertebrates and fish (Schmidt-Kloiber and Hering, 2015). Furthermore, most of the relevant research is restricted to macrophytes in freshwater, resulting in a limited understanding of macrophyte tolerance to salinity. Moreover, reported salinity ranges and tolerance limits of macrophyte species strongly differ. *Stuckenia pectinata,* for example, has a reported tolerance limit higher than 320 mg l^{-1} Cl^{-}(Lyon and Roelofs, 1986a) or far beyond 12,000 mg l^{-1} Cl^{-} (Kantrud, 1990). These discrepancies are likely caused by the difference in salinity ranges studied by the various authors. The lack of insight on salinity tolerance of aquatic flora hampers the restoration of brackish waterbodies.

The aim of this paper is to analyse the salinity tolerance of aquatic plant taxa using field data from water bodies covering a wide salinity range. For this purpose, we used monitoring data from a water board (Waterschap Scheldestromen) in the South of the Netherlands province of Zeeland). Our study assesses the univariate response of macrophytes towards salinity. While we recognize that the occurrence of aquatic plants is not only determined by salinity, this study provides a first data-driven estimation of the range, optimal salinity and response of certain taxa towards salinity under field conditions in a salinity range of 4 to 22,000 mg l^{-1} Cl^{-}. We addressed the following questions: 1.) How are individual taxa distributed along the salinity gradient? 2.) What is the optimum, minimum and maximum salinity at which they were observed? 3.) How does the maximum observed salinity relate to previously published data? Finally, we discuss the implications of our results for the current Dutch assessment of brackish waters under the European Water Framework Directive.

2. Methods

2.1. Study area

This paper deals with water bodies in Zeeland, a province located in the south-west of the Netherlands consisting of a number of islands and peninsulas as well as a land-strip bordering Belgium. The inland waters of Zeeland are mainly used for draining agricultural land. The studied waters are mostly linear (~10 m wide), very shallow (~1 m) human-made drainage ditches (van Dam, 2013). Most of the ditches have a trapezium like shape or almost vertical embankment with woody bank fixations. In general, submerged aquatic vegetation (SAV) in these ditches, designated as waterbodies under the Water Framework Directive, are absent or rare (van Dam, 2013; Personal communication: Yvonne van Scheppingen). The emergent macrophyte *Phragmites australis*, on the other hand, is common.

In addition to ditches, our dataset contains some larger (~100 m wide) and deeper (~3 m) waterbodies (Maas, 1979) which are remnants of intertidal areas or were generated following past dike breaches by storm surges or for strategic purposes during war (De Kraker, 2006). These larger waterbodies are widenings within the total transection of the water system and are here referred to as "bays" (in Dutch "kreken", not to confuse with flowing creeks). These bays are located near the coast where strong salinity fluctuations (up to extreme yearly fluctuations of ~10,000 mg l^{-1} Cl^{-}) caused by rainfall, evaporation and tidal influences (van Dam, 2013). Due to the strong agricultural use of the surrounding land, the water is usually eutrophic and has a low transparency of around ~20–50 cm, which can be caused by floating inorganic particles as well by algae (van Dam, 2013). The range in nutrient concentrations is similar across the salinity gradient, although phosphate concentrations are often lower than under freshwater conditions (van Dam, 2013). The water level in our study systems is strongly controlled and lower during the winter period as compared to the summer period. During summer, the water is 20–50 cm higher than in winter to meet agricultural demands. The fish species *Cyprinus carpio* and *Abramis brama* dominate most water bodies.

2.2. Data source

The data were obtained from the water authority "Waterschap Scheldestromen" of Zeeland. Plant presence was recorded by two analysts, who survey around 80 or 120 locations every spring, summer and autumn from March to October in 2000–2015, of which ~39 locations are repeated every three years. Most of these repeatedly sampled locations lie close to coastal areas or near pumping stations and are highly brackish. This often leads to extreme fluctuations in salinity, which are notably different between years and months. Samples of the submerged plants are taken from the shore (littoral zone) with a "throwing rake", (two rakes attached in opposite to each other and connected to a rope). The rake is thrown in the water and pulled back scraping the bottom part of a waterbody. This is done every 5–10 m in a stretch of 100 m along the shore in accordance with the Dutch sampling protocol of the Water Framework Directive (Beers et al., 2014). In addition, emergent plants (helophytes) are recorded in the region between the summer and the winter water levels (area between summer water level and thrash line) over a stretch of 100 m. The plants are identified in the field to species level according to "Heukels Flora" (Meijden, 2005) and if necessary taken to the laboratory and identified there with "Water- en oeverplanten" (Pot, 2004). Water samples are taken by the laboratory of Eurofins in Zeeuws-Vlaanderen at the same location as the biological samples. The timing of sampling may differ which resulted in discarding of some of the plant data (see 2.3 data analysis). The water samples are analyzed for a range of parameters, such as Cl, BOD_5, N, P, NH_3, NO_3, Cu, SO_4, As, conductivity and chlorophyll-a concentration using Dutch (NEN) standards, European (EN) standards and international (ISO) standards. Cl, for instance, is measured according to the NEN-ISO 15923 (spectrometry). After both biological and chemical samples are taken and processed, the information is uploaded into the database of Waterschap Scheldestromen. The biological data used in this paper was extracted for the period from 01-01-2000 to 31-12-2016. The chemical data was extracted for the period 01-01-1990 to 10-2-2017. We did not use data from earlier than the year 01-01-2000, because from 2000 onwards sample methods were standardized for the European Water Framework Directive; and later then 31-12-2015. Data is not available online, but can be acquired by contacting Waterschap Scheldestromen.

The biological dataset used in this paper contained data on some taxa that were not classified as aquatic (Pot, 2004), such as *Ranunculus acris* or *Alnus* spp.; these were removed prior to analysis. After this, 46 taxa remained and the dataset contained helophytes, pleustophytes and hydrophytes as well as two algae, *Ulva* ssp. (=non-filamentous) and *Ulva* spp. (=filamentous). The reason for this separation is the change in nomenclature: *Ulva* spp. (=filamentous) was referred to as *Enteromorpha* spp. in the dataset.

2.3. Data analysis

First, we combined the plant samples with the chloride concentrations. As chloride concentrations may vary strongly in the water systems, we used the median chloride concentration based on the chloride concentration of the month of the plant survey and (if available) the

Table 1
Steps to calculate relative fractions (Rf) after Lyon and Roelofs (1986b) for a hypothetical example.

Salinity Class (c)	1	2	3	4	5	6
Plant recordings (x)	5	10	20	20	7	0
Plant surveys (p)	50	50	100	200	140	50
Fraction ($f_x = x / p$)	0.1	0.2	0.2	0.1	0.05	0
Sum of all fractions (f_{sum})	0.65					
Normalized ($y = f_x / f_{sum}$)	0.15	0.31	0.31	0.15	0.08	0

two preceding months (maximum n = 3). We opted for the median instead of the maximum, since either the salinity maximum or the minimum may limit distribution ranges (Remane and Schlieper, 1972). For every taxon, we excluded all records associated to chloride values below the 5% and above the 95% percentile and removed taxa with less than 5 samples. Plant samples without corresponding chloride data were removed from the data set. From this data set we selected all locations that were sampled once and if multiple samples were taken at a particular location we selected the sample with the lowest concentration, to be conservative (Table S1.; for the results when highest concentrations are selected, with only minor differences). This resulted in 862 sampled locations with a fairly constant concentration along the gradient (Fig. S1.).

We applied the procedure described by Lyon and Roelofs (1986b) to calculate the percentage occurrence of a given taxon within the salinity classes. This procedure considers that different numbers of samples were taken within the individual classes. A hypothetical example for a single taxon is given in Table 1. First, the fraction of records of the taxon within a given salinity class (f_x) is calculated (number of records (x) in the class divided by the total number of plant surveys (p) within that salinity class). This is done for all salinity classes, after which the fractions are summed (f_{sum}). Finally, the fractions were normalized so that the sum of normalized fractions for all salinity classes equals “1”.

To describe the distribution of taxa within the salinity gradient, we generated a table of species records within 101 salinity classes with steps of 199 mg l^{-1} Cl^-, i.e. 0–199, 200–399 mg l^{-1} Cl^-, etc. Thirty of these classes did not contain any occurrences and were omitted for the analysis. Based on this table, we calculated the chloride optimum for every taxon with Eq. (1), adapted from Lyon and Roelofs (1986b). The species optimum is the weighted average of the salinity concentration based on the normalized fractions.

$$S = \sum \mu_i \cdot y_i \tag{1}$$

S = Species optimum (mg l^{-1})
μ_i = Average chloride concentration in class i
y_i = Normalized fraction within class i

To determine, if a taxon’s response deviates from a non-responsive distribution, a modified chi-square goodness-of-fit-test (MX^2) was applied (Lyon and Roelofs, 1986b) (Eq. (2)).

$$MX^2 = \bar{y}^2 \cdot \sum \frac{(y_i - \bar{y})^2}{\bar{y}^2} \tag{2}$$

MX^2 = Modified Chi-square
y_i = Normalized fraction within class i
$\bar{y}$ = Average of the normalized fractions over all classes

We use MX^2 as a “salinity distribution proxy” as the MX^2 indicates the distribution over the total range of classes. When the number of records is distributed evenly among classes, indicating that the plant is indifferent to salinity, the outcome of MX^2 is “0”. A value close to “1” indicates that all records occurred within a single salinity class pointing at a high specificity. However, caution is required with the interpretation of the MX^2 value. Taxa occurring in a wide range of salinities but with a high number of records within a specific salinity class also get high MX^2 values.

We used the Cumulative-Sum-of-Squares (CuSumQ) Change-Point-Analysis (CPA) to determine, within which class the number of occurrences decreases or increases. We applied the At-Most-One-Change (AMOC) principle (Eq. (3)).

$$\Delta_i = y_i - \bar{y} \tag{3}$$

Δ_i = Difference between the average and normalized fraction within class i
y_i = Normalized fraction within class i
$\bar{y}$ = Average of the normalized fraction over over all classes

Once Δ_i for every y_ith value was determined, the cumulative sum was calculated. This was performed by a repetitive summing of Δ_i, until the sum of all differences is determined ($\Sigma\,\Delta_1$; $\Sigma\,\Delta_1, \Delta_2$; $\Sigma\,\Delta_1, \Delta_2, \ldots, \Delta_i$). Every sum was then squared and the Change Point (CP) was identified at the class (i) were $(\Sigma\,\Delta_1, \ldots, \Delta_i)^2$ is at maximum. The CP (class) is defined as the class at which the largest change in the normalized fraction occurs; this can be positive or negative.

Finally, we assigned the taxon to one or two of the following salinity classes as defined by Oertli (1964): 0–300 mg l^{-1} Cl^- = freshwater; 300–3000 mg l^{-1} Cl^- = oligohaline; 3000–10,000 mg l^{-1} Cl^- = mesohaline; 10,000–18,000 mg l^{-1} Cl^- = mixohaline. We assigned one class when the species optimum (S) and the CP fell within the same class and two classes when S and CP fell in different salinity classes.

2.4. Literature study

The maximum and single values describing salinity were extracted from varies literature sources acquired via Google scholar using the key-words “salinity”,” chloride”, “conductivity” in combination with “range” and/or “gradient” and the taxa names present in our data-set. The identified references used different formats for displaying the salinity range of taxa and were extracted as follows. In case salinity classes with a min-max range were given (for example: Izzati, 2015) we used the maximum values. Similar, for references containing plots/graphs the maximum value was extracted with WebPlotDigitizer (Rohatgi, 2018). For data given as “Mean ± SE” (for example: Bouzillé et al., 2001), the mean was used. Individual values of salinity without a range were also used (for example: *Phragmites australis*, Hart et al., 1991). Taxa identified to genus level in the present study were compared to the most common species in the area.

The identified studies used different units for indicating salinity: ppt, ‰, NaCl or ms cm^{-1}. These units were converted to mg l^{-1} Cl^- to enhance comparability. mmol l^{-1} Cl^- was converted through multiplying by the molar mass: mg l^{-1} Cl^- = mmol l^{-1} Cl^- ·35.5 mmol mg^{-1}. Although the ratio of ions fluctuates, we assumed Cl^- to make up 55% (weight; Sverdrup et al., 1942); therefore ppt, ‰ or mg l^{-1} sea salt was multiplied with 0.55. For NaCl used in laboratory experiments a similar approach was used, but multiplying with 0.61. Conductivity was converted to mg l^{-1} Cl^- using the linear relation presented in chapter 3. The found maximum values from literature were compared to the maximum values derived during this study. Altogether, we identified 43 relevant references.

3. Results

Chloride concentration was strongly related to conductivity, showing a linear relation: Chloride (mg l^{-1}) = 378 Conductivity (mS cm^{-1}) – 260 (R^2 = 0.88, n = 32,155 records) indicating that chloride is an appropriate measure of salinity in our dataset.

3.1. Taxon responses

The 46 taxa addressed differ strongly in their distribution along the salinity gradient (Fig. S2.). A summary of values describing the optima and response of species is provided in Table 2. The species’ optimum, as

Table 2
Summary of values describing salinity optima and ranges in mg l^{-1}. Total number of records (n), number of classes in which a taxon occurred (cf), species optimum (S) in mg l^{-1}, modified chi-square (MX^2), minimum (min) and maximum (max) salinity of occurrence in mg l^{-1}, class at which the Change Point occurred (CP), classification according to S and the CP within a salinity class (Class).

Taxon	n	cf	S	MX^2	min	max	CP	Class
Alisma plantago-aquatica	25	4	327	0.30	20	650	0-199	fresh-oligohaline
Aster tripolium	78	42	11,632	0.04	960	18,500	10,800-10,999	mixohaline
Azolla filiculoides	18	7	1035	0.18	180	1800	800-999	oligohaline
Bolboschoenus maritimus	278	46	5674	0.01	81	11,000	4400-4599	mesohaline
Callitriche spp.	140	10	761	0.11	35	1900	800-999	oligohaline
Carex otrubae	137	29	3848	0.03	77	8500	2400-2599	mesohaline
Carex riparia	19	2	123	0.66	25	250	0-199	freshwater
Ceratophyllum demersum	59	8	643	0.14	34	1528	400-599	oligohaline
Ceratophyllum submersum	22	8	901	0.12	54	1700	600-799	oligohaline
Elodea canadensis	27	3	272	0.34	24	510	200-399	freshwater
Epilobium hirsutum	274	29	3255	0.03	42	6200	4400-4599	mesohaline
Glaux maritima	21	18	15,805	0.08	710	22,000	9200-9399	mixohaline
Glyceria fluitans	7	2	333	0.49	55	590	0-199	fresh-oligohaline
Glyceria maxima	14	3	223	0.38	60	460	0-199	freshwater
Glyceria notata	6	2	180	0.55	56	250	0-199	freshwater
Iris pseudacorus	106	8	626	0.15	17	1600	400-599	oligohaline
Juncus articulatus	18	6	682	0.21	20	1200	400-599	oligohaline
Juncus effusus	41	4	350	0.30	18	840	0-199	fresh-oligohaline
Juncus gerardii	59	38	13,355	0.05	280	22,000	11,800-11,999	mixohaline
Juncus inflexus	93	22	3189	0.04	51	7200	3000-3199	mesohaline
Lemna gibba	12	4	1196	0.30	50	2168	400-599	oligohaline
Lemna minor	341	23	2168	0.04	36	4500	2600-2799	oligohaline
Lemna minuta	6	2	160	0.56	94	250	0-199	freshwater
Lemna trisulca	89	10	822	0.10	35	1900	600-799	oligohaline
Mentha aquatic	88	8	824	0.14	29	1800	0-199	fresh-oligohaline
Myriophyllum spicatum	21	5	530	0.21	29	1200	200-399	oligohaline
Nuphar lutea	6	1	78	0.99	43	140	0-199	freshwater
Nymphaea alba	26	2	168	0.54	22	400	0-199	freshwater
Phragmites australis	764	49	5423	0.01	4	11,000	9600-9799	mesohaline
Potamogeton crispus	15	4	414	0.28	30	700	400-599	oligohaline
Potamogeton pusillus	31	7	751	0.19	30	1300	600-799	oligohaline
Potamogeton trichoides	5	1	69	0.99	29	130	0-199	freshwater
Ranunculus aquatilis	11	3	275	0.33	34	480	0-199	freshwater
Ranunculus sceleratus	50	13	3190	0.08	38	6800	3000-3199	mesohaline
Rumex hydrolapathum	26	4	371	0.24	25	800	200-399	oligohaline
Ruppia cirrhosa	21	17	10,191	0.07	700	18,000	6600-6799	meso-mixohaline
Ruppia maritima	5	5	12,534	0.56	7100	13,768	9400-9599	meso-mixohaline
Salicornia spp.	31	26	14,233	0.05	1650	21,000	10,800-10,999	mixohaline
Schoenoplectus lacustris	35	11	1609	0.10	30	3100	1200-1399	oligohaline
Sparganium erectum	19	2	130	0.66	20	330	0-199	freshwater
Stuckenia pectinata	59	17	2786	0.07	64	4700	2000-2199	oligohaline
Typha angustifolia	69	9	2148	0.11	32	7200	1800-1999	oligohaline
Typha latifolia	46	8	784	0.14	35	1600	200-399	oligohaline
Ulva spp. *(=filamentous spp.)*	229	43	5992	0.02	130	11,500	4800-4999	mesohaline
Ulva spp. *(=non-filamentous)*	14	11	13,270	0.25	5200	17,000	14,800-14,999	meso-mixohaline
Zannichellia spp.	31	13	2738	0.09	51	7200	1600-1799	oligohaline

derived from Eq. (1), ranged between 78 mg l^{-1} Cl^- for *Nuphar lutea* with a min and max of 43–140 mg l^{-1} Cl^- and 14,233 mg l^{-1} Cl^- for *Salicornia* spp. with a min-max of 1650–21,000 mg l^{-1} Cl^-.

According to the MX^2 values, some taxa were relatively indifferent, including *P. australis* (0.01), *B. maritimus* (0.01) and *Ulva* spp. (=filamentous.) (0.02). Other taxa were more sensitive, although still occurring in a wide salinity range, e.g. *S. pectinata* (0.09) and *Zannichellia* spp. (0.1), Others were relatively responsive, e.g. *T. latifolia* (0.14) and *C. demersum* (0.14), or had a high preference for a certain location along the gradient, e.g. *Alisma plantago-aquatica* (0.30) and *Sparganium erectum* (0.66).

According to the CPA, 13 of the 46 taxa have a CP below < 199 mg l^{-1} Cl^-, the classes 200–399, 400–599 and 600–799 mg l^{-1} Cl^- contain five, four and five taxa, respectively. The remaining taxa have a higher CP. There are consistent differences between the CPs of taxa with different life strategies: the helophytes have a CP between 9400–9599 mg l^{-1}, the pleustophytes between 8400-8599 mg l^{-1} Cl^- and the hydrophytes between 3000–3199 mg l^{-1}. From these results it is obvious that the hydrophytes are the most strongly indicative to lower salinities and display a CP around 3000 mg l^{-1} Cl^-.

Ten of the 46 taxa were classified as preferring freshwater, 18 as oligohaline, seven as mesohaline and four as mixohaline. The remaining species were assigned to more than one class.

3.2. Taxon upper limit and literature study

The results achieved for the taxa addressed in this paper were compared with previous published salinity data (Table S2.). This comparison indicates that while most taxa fall within a similar range, we found that some taxa occur in waters with higher salinity than previously recorded. This may mainly be due to the scattered available information. Other taxa have a much wider reported tolerance range than what we found. These reported ranges are, however, often based on laboratory experiments and not by field observations, i.e. they refer to the fundamental rather than the realized niche.

4. Discussion

We determined the salinity range and the salinity optimum of 46 plant taxa along an environmental gradient from 4 to 22,000 mg l^{-1}

Cl^-. The observed upper salinity limits determined based on our field data-set covering a wide range of salinities are generally within the range of previously published data (34 of 46 taxa). In some cases (12 of 46 taxa), our field data indicated a higher upper limit, which may be the result of limited publications on the ranges of freshwater and oligohaline taxa.

4.1. Taxon responses

Our analyses point out that, some taxa are relatively indifferent towards salinity (i.e. they have a low MX^2); these taxa occur along a wide range of salinities with an even distribution among (often the higher salinity) classes. Examples are *A. tripolium, B. maritimus*, *C. otrubae, Ulva* spp. (=filamentous) and *P. australis*, all of which have the strongest increase in occurrence at salinities above 3000 $mg\,l^{-1}$ Cl^-. These observations are in general agreement to earlier findings, as most of these taxa were described to occur in meso- to mixohaline waters and have a tendency to occur in brackish environments (Chambers et al., 1998; Dijkema, 1990; Reed and Russell, 1978).

The occurrence of other taxa, such as *R. cirrhosa* and *Zannichellia* spp., strongly increase above 1000 $mg\,l^{-1}$ Cl^-, but have a slightly higher salinity distribution proxy than the previously described taxa indicating that their occurrence is limited to a smaller salinity range. The preference of *R. cirrhosa*, and *Zannichellia* spp. for brackish and saline environments is in accordance with other earlier reports (Herkül et al., 2018; Izzati, 2015; Orth, 1994; Orth and Moore, 1984). Other taxa, including *G. maritima, L. trisulca* and *R. sceleratus* showed similar distributions which is in accordance with the literature as well (Brock, 1981; Grillas, 1990; Haramis and Carter, 1983; Lyon and Roelofs, 1986a).

For some taxa, our results based on our field-data differed from what has been reported in the literature. For instance, the occurrence of *M. spicatum* was limited to a relatively small salinity range and observed only in waters up to 1200 $mg\,l^{-1}$ Cl^-, while literature reports its occurrence in mesohaline regions (Herkül et al., 2018; Orth, 1994). In addition, we also found taxa such as *A. filiculoides*, *C. demersum*, *C. submersum*, *G. fluitans*, *G. maxima*, *G. notata*, *P. crispus*, *P. pusillus* and *P. trichoides* to occur in a relatively limited range, which is contradicting to literature (Bouzillé et al., 2001; Spence et al., 1979, Table S4.). We argue that this is likely caused by the low sample size for these taxa in our field-dataset and other factors strongly influencing the distribution.

On the other hand, our analyses indicate that taxa such as *A. plantago-aquatica*, *E. canadensis, I. pseudacorus, J. effusus, M. aquatica, N. alba*, *P. pusillus, R. hydrolapathum,* and *S. erectum,* species generally considered freshwater taxa, indeed have a minimum and maximum salinity limit within the fresh (and oligohaline). Most of these taxa, even those with a limited sample size, occurred at salinities above 300 $mg\,l^{-1}$ Cl^-, which is considered the upper limit of freshwater (Moors et al., 1995; Oertli, 1964). The upper limit, therefore, seems too restrictive for most taxa considered as freshwater taxa (Barendregt et al., 1990; Lyon and Roelofs, 1986a; Moors et al., 1995). This indicates that the salinity classes, originally developed (Oertli, 1964) for organisms other than vascular plants may have to be reconsidered.

4.2. Methods applied determining responses

Currently, there is no consensus about how to quantify tolerance. Here we used a salinity distribution proxy (MX^2) and CPA to determine the salinity class at which the largest increase or decrease in occurrence takes place. The accurateness of the outcome of these analyses strongly depends on the number of samples and the class width. We argue, however, that the combined analysis of the MX^2 value and the CP is a useful approach to obtain insight in tolerance ranges. Certainly more data allowing the use of smaller class sizes or exclusion of classes altogether would further improve the assessment of salinity tolerance. Further, one could argue that the classification of fresh water taxa – having an species optimum and CP within the 0–199 $mg\,l^{-1}$ – is already relatively high, and that any of these taxa are not “true” freshwater taxa. However, most classifications agree on a range in between the 100–500 $mg\,l^{-1}$ (Remane and Schlieper, 1972); and the Dutch derivation of the Water Framework Directive uses the 300 $mg\,l^{-1}$ as the border between fresh and brackish systems.

Our literature overview shows that maximum values found based on our field-data are often in the same range as observed in previous studies. We stress, however, that maximum tolerance salinities derived based on experiments may overestimate maximum concentrations at which taxa can occur under natural conditions, particularly when experimental growth conditions are optimal and there is no interspecific competition. These experimental salinity ranges therefore have less practical application for management goals, which often focus on ecosystems under multiple pressures. Against this background, we used these literature-derived values more as a basis for validation of our field-data analysis than as absolute values.

Distribution of macrophyte species is not driven by salinity alone, but by other (potentially correlating) variables as well. Well known variables influencing macrophyte species occurrence are herbicides, NH_3, de-rooting by fish or birds and mechanical removal (Bajer et al., 2009), turbidity and sulphide (Geurts et al., 2009; Lamers et al., 2013). Also, salinity tolerance can be greatly influenced by fluctuations in salinity (Hinojosa-Garro et al., 2008; Remane and Schlieper, 1972), which – due to the low temporal resolution - could not be accurately inferred from our data. Thus, the results only give a partial view under which circumstances these taxa can occur and should be interpreted carefully.

4.3. Results in the light of the Water Framework Directive

Overall, the salinity maxima of most of the taxa in our data analyses fall consistently within the range reported in literature. According to the Dutch assessment systems under the European Water Framework Directive, a certain macrophyte coverage is necessary for achieving Good Ecological Status (GES) or Good Ecological Potential (GEP), with certain species having a higher weight in the evaluation than others (Altenburg et al., 2012). When it comes to evaluating the status of brackish waters, however, some species that we identified as being able to occur in brackish environments are not included in the species assessment and therefore do not count towards the total score of the water body. The presence of *M. spicatum* or *R. aquatilis* in brackish lakes (Dutch Lake type M30), for instance, is not taken into account when assessing the biological water quality (Altenburg et al., 2012). According to our analysis, *M. spicatum* and *R. aquatilis* can occur under concentrations of 1200 and 480 $mg\,l^{-1}$ Cl^-, respectively. This oligohaline character is also supported by other literature (Hinojosa-Garro et al., 2008; Orth, 1994) which was apparently disregarded when developing the assessment method. We argue, based on field-data and the literature overview, that these taxa should also be included into the list of species indicative for the biological quality of brackish waters. We furthermore suggest to review the entire species lists for brackish systems based on the newest data available including this study.

Despite the limitations, our results indicate that current estimates of the upper limit of salinity ranges are too low for several taxa, and literature data on salinity ranges are especially scarce for oligohaline taxa. Our literature search, furthermore, pointed out that information on salinity tolerance is scattered with many papers dealing with single species. Further, a range of different units for salinity are used which cannot always exactly be converted (see also Herbert et al., 2015). Our field-data analyses combined with literature data points out that important advances can still be made regarding the assessment of salinity tolerance ranges of macrophytes. Correct assessments are needed to properly evaluate the ecological quality of surface waters in the entire salinity spectrum.

Acknowledgements

We gratefully acknowledge Waterschap Scheldestromen and persons responsible for acquiring and delivering the used monitoring data: Yvonne van Scheppingen, Ron Brand and Cynthia de Kruijff.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aquabot.2019.103129.

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