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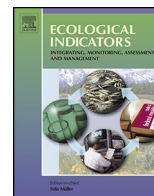
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Determination of tipping points for aquatic plants and water quality parameters in fish pond systems: A multi-year approach



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ABSTRACT

High levels of nutrients in fish ponds by fish farming may cause significant eutrophication leading to a loss in species richness and a decrease of cover of aquatic plants to phytoplankton dominance. This shift can be represented by a tipping point where a significant change in the state of the ecosystem is observed such as a change from high to low aquatic plants species richness and cover. A total of 100 fish ponds were studied during five years in the Dombes region, France, to determine tipping points in aquatic plant richness and cover using chlorophyll α (CHL), water transparency, Total N (TN) and Total P (TP) gradients with two statistical methods. The relationships between tipping points, nutrient loads and yearly variations in weather conditions were also evaluated. Looking at the five years data, tipping points were observed in aquatic plant richness at 6 and 60 $\mu\text{g/L}$ for CHL, and at 3.90 mg/L for TN concentration; as well as at 70 cm for water transparency, but no tipping point was found with TP. For aquatic plant cover, tipping points were observed at 11 $\mu\text{g/L}$ for CHL, 2.42 mg/L for TN, 0.05 mg/L for TP, and at 62 cm for water transparency. These tipping points showed a significant decrease of aquatic plant species richness and cover, linked to the nutrient concentrations which drive the competition between the primary producers phytoplankton and aquatic plants. However, tipping points could vary significantly between years. The inter-annual variability may be due to an early occurrence of phytoplankton blooms in some ponds in a year preventing the establishment of aquatic plants, and thus influencing the value of tipping points. Weather conditions influence the competition between primary producers by impacting chlorophyll α and nutrients concentrations. When weather conditions supported increased nutrient concentrations, the development of phytoplankton and aquatic plants was facilitated and tipping points in aquatic plant richness and cover occurred with relatively high values. Thus, a significant decrease of plant cover and richness occurred at higher level of nutrients compared to the other years. In these cases, aquatic plants dominated over phytoplankton for the spring period, and also often during summer. In conclusion, tipping points observed are mainly linked to the competition between aquatic plants and phytoplankton. In shallow and eutrophic systems like fish ponds where nutrients are not a limiting resource, weather conditions act temporarily during spring as the main regulator of this competition.

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1. Introduction

Freshwater waterbodies such as lakes and ponds are very numerous covering more than 3% of the earth's surface (Downing et al., 2006). Most of ponds have small catchment areas, have their own physical-chemical characteristics and are physically heterogeneous habitats (Williams et al., 2003), making them highly different

from one to another. Thus, ponds may vary considerably in species richness of the different communities, allowing a high diversity at the regional scale (Davies et al., 2008; Oertli et al., 2002; Rosset et al., 2014; Wezel et al., 2014; Williams et al., 2003).

In fish ponds, all aquatic organisms in the food web are influenced by nutrient levels (Declerck et al., 2005; Moss et al., 2003; Søndergaard et al., 2005a) and by fish farming practices (Broyer and Curtet, 2011; Horvath et al., 2002; Lemmens et al., 2013; Oertli et al., 2013). Weather conditions can also influence the state of fish ponds (Scheffer and van Nes, 2007) by particularly promoting phytoplankton biomass with high temperatures (Jacobsen and Simonsen, 1993; Li et al., 2015; Yang et al., 2013). Primary producers such as phytoplankton and aquatic plants, are crucial for the structuration

Abbreviations: CHL, chlorophyll α ; TP, total phosphorus; TN, total nitrogen; TMEAN, threshold by mean method; SEGMENTED, segmented method.

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of the food web in fish ponds (Korinek et al., 1987; Pálffy et al., 2013; Scheffer, 2004; van Donk and van de Bund, 2002). Phytoplankton is the main source of primary production and works as a precursor for community-level processes (Korinek et al., 1987; Pálffy et al., 2013). Aquatic plants can promote species diversity at the ecosystem level, especially invertebrates diversity (Declerck et al., 2005; Oertli et al., 2002), decrease nutrient levels and reduce the turbidity of the water column (Scheffer et al., 1993; van Donk and van de Bund, 2002). These two groups respond greatly to changes in the environment and are useful to predict ecosystem change (Beck et al., 2014; Korinek et al., 1987; Pálffy et al., 2013).

Fish farmers commonly use several practices like supplementary feeding and water fertilization to optimize the global productivity of the aquatic ecosystem and specifically to increase fish biomass (Broyer and Curtet, 2012; Horvath et al., 2002; Korinek et al., 1987; Wezel et al., 2013b). However, the excessive input of nutrients can cause a regime shift from high coverage of aquatic plants (clear water state) to phytoplankton dominance (turbid water state or algae blooms) (Jeppesen et al., 1997; Pálffy et al., 2013; Rinke et al., 2009; Scheffer et al., 2009, 2001, 1993). This regime shift may be based on three processes: (1) the turbidity increases with increased nutrient levels causing phytoplankton growth, (2) aquatic plants decrease turbidity by storing nutrients and competing for light with phytoplankton, and (3) aquatic plants disappear when the turbidity exceed a critical tipping point (Scheffer and van Nes, 2007).

A tipping point may be defined by the threshold where a significant change in the state of an ecosystem is observed (Lenton et al., 2008; May, 1977; Wall, 2007). In this study, a tipping point is defined as a significant change from high to low species diversity and cover in aquatic plants. In fact, a decrease of aquatic plant richness can lead to a decrease of the global productivity of the fish pond system (Scheffer, 2004) due to the fact that aquatic plants promote the diversity of other species such as macro-invertebrates (Declerck et al., 2005).

The aim of this study is first to determine tipping points where significant changes occur in aquatic plant richness and cover using chlorophyll α (CHL), water transparency, Total N (TN) and Total P (TP) gradients, and to evaluate variations over different years with the fish ponds analyzed. Secondly, we evaluate the relationship between tipping points, nutrient loads and yearly variations in weather conditions.

2. Materials and methods

2.1. Study area and site selection

The study was carried out in the Dombes region in south eastern France which is characterized by about 1100 man-made fish ponds and 11,500 ha of water surface organized in connected networks. Fish are stocked in spring and are harvested during autumn or winter when ponds are emptied. After a few days or weeks, the ponds are refilled with water from either upstream fish ponds or from rainfall in the pond catchment. The dominating fish species raised in fish ponds is the common carp with more than 60% on the total fish yield, followed by roach and rudd, and a lower quantity of tench, pike or pikeperch (Wezel et al., 2013b).

The pond surface area varies considerably from 1.8 ha to more than 79 ha and the surface of fish ponds in our data averaged 14.8 ± 13.3 ha. The average of depth of ponds is 0.7 ± 0.2 m with a minimum of 0.3 and maximum of 1.2 m. The average net fish production in the studied fish ponds is 209 ± 142 kg ha⁻¹, ranging from a negative production of -1.4 (less fish harvested at the end than fish placed in the pond at the beginning of the breeding) to a maximum of 720 kg ha⁻¹.

In this study, a total of 100 fish ponds were studied during five years in the Dombes region, France, including 26 ponds which were monitored several years (two or three years). A multiannual study scale was chosen in order to analyze the variability of tipping points over years and to study the seasonal effect due to variations of weather conditions. As fish ponds are emptied every year for fish harvest, the inter-annual variability of the 26 multiannual ponds is significantly different in term of CHL and plant cover (p -value of Levene test < 0.001). Thus, a multiannual sampled pond is considered as different form one year to another and the statistical analyses will consider these different years as independent data. The number of ponds sampled per year was: $n = 24$ (2008), $n = 25$ (2009), $n = 17$ (2012), $n = 21$ (2013) and $n = 13$ (2014).

2.2. Aquatic plants

Aquatic plants were determined at the beginning of summer, following the protocol described in Arthaud et al. (2013) and Vanacker et al. (2015). The number of sampled quadrats increased with the surface area of the fish pond and varied from 18 to 140. For each quadrat, submerged and floating aquatic plants were identified to species level and their abundance was recorded using cover-abundance estimates of Braun-Blanquet (1932). The richness of aquatic plants was determined by the Jackknife first order which is the best diversity index to determine tipping points in this taxonomic group (Vanacker et al., 2015). Jackknife first order was developed by Burnham and Overton (1979) and reduces the underestimation of the number of species in an assemblage based on the number represented in the sample. Plant cover of each fish pond was determined by averaging the cover values of each species in each quadrat. A total of 87 aquatic plant species were recorded in the Dombes area during the five years of sampling.

2.3. Water samples

In order to decrease the effect of strong temporal variability that occurs in eutrophic waterbodies (Jeppesen et al., 1997), we collected between 8 and 6 samples in spring depending of the year. For each sample, CHL and nutrient concentrations were determined and the water transparency measured. The water quality was evaluated in spring during all the aquatic plant development until the maturity of plants. Water samples were collected with a Van Dorn sampler (Uwitech, Austria) in one point near the outlet of each pond, the deepest part of the pond.

2.4. Chlorophyll α

CHL was extracted for 24 h in a 90% acetone solution and was then measured with a Shimadzu UV/VIS spectrophotometer UV-2101 (Schimadzu Corporation, Kyoto, Japan) in 2008 and for the following years with a spectrophotometer JASCO UV/VIS spectrophotometer V-530 (JASCO, Japan) at 630, 645, 663 and 750 nm. The CHL concentration per unit volume was then calculated using the Parsons and Strickland formula (Parsons and Strickland, 1963). The median

2.5. Water transparency

The transparency of the water was evaluated since 2009 with a Secchi disc in each sampling date. No transparency data was recorded in 2008. However, these missing values were determinate by modelling the transparency from CHL values. In each sampling date in spring 2008, we determinate the transparency by the relationship of the other years $y = 142.43 x^{-0.329}$ ($R^2 = 0.609$, $n = 610$) where y is the transparency (cm) and x is the CHL.

2.6. Nutrient concentrations

TN was measured using nitrogen persulfate reagent powder for digestion method and measured at 410 nm wavelength (HACH method 10072). TP was analyzed by method of PhosVer® 3 with acid persulfate digestion (HACH method 8190).

2.7. Weather parameters

Weather parameters such as solar radiation, rainfall and air temperature are commonly used in several fields and are determinant in the evapotranspiration of an ecosystem (Valipour, 2015, 2014; Valipour and Eslamian, 2014). We used daily means of rainfall and air temperature from the Marlieux weather station in central Dombes area. For solar radiation, we used data from two weather stations equidistantly located from Marlieux (Lyon and Macon weather stations) as this data was not available for the Marlieux weather station. Daily mean of weather parameters during spring, from the 21st of March to the 21st of June, was used for comparison between years. Mean daily temperature was calculated by taking into account daily values of temperature. Weather conditions affect the equilibrium of ponds under extremes conditions leading to a shift in which the system subsequently remains for a long time (Scheffer et al., 2001; Scheffer and van Nes, 2007). A long-term period value of four weeks before the respective sampling date was chosen in order to analyze the effects of weather conditions on water quality parameters and tipping point values, and to evaluate the response of the pond system to general weather condition, and not to extreme situations such as storms. Thus, in order to have the totality of solar radiation, degree days and rainfall which influenced the pond before the sampling date, daily rainfall, daily solar radiation and daily temperature were summed up during the four weeks before the sampling date.

2.8. Data analysis

The spring median of nutrients, CHL and transparency were calculated for each fish pond and for each year. As extreme values can often occur in shallow ponds (Scheffer, 2004), the median was selected rather than the mean to have a better representation of these parameters in pond systems.

A Kruskal-Wallis one way analysis and a Wilcoxon post hoc for non-normal distribution, or an ANOVA one-way analysis with a Tukey HSD test for normal distribution were performed to show significant differences between water quality, aquatic plant cover and richness, or weather parameters between sampling date.

Correlations between aquatic plant biodiversity and water quality parameters, as well as between weather parameters and water quality parameters, were studied by Spearman correlations. All statistical tests were carried out with the statistical program R (R Development Core Team, 2013).

Tipping points were determined using two statistical methods according to the study of Vanacker et al. (2015). TMEAN (Threshold by Mean) determines tipping points as a threshold with a statistical significant difference in the mean. A tipping point is determined by an ANOVA model as the value with the minimum residual mean square. To test if tipping points are relevant with the TMEAN model we applied a z test which analyzed the differences in mean and standard deviation between the total group and groups before and after a tipping point. The second method, SEGMENTED, determines tipping points by integrating linear regression with one pivotal point. The tipping point found by SEGMENTED is the mean with its standard error of potential tipping points observed in a range of the parameters. The SEGMENTED method was developed in the 'Segmented' R package (Muggeo, 2008). To evaluate if the tipping

point is relevant, we applied a Davies test (Davies, 1977) to check a change of the linear regression slope.

3. Results

3.1. Aquatic plant diversity and water quality parameters throughout the years

The aquatic plant cover and the plant richness were not significantly different between the years (p -value of 0.329 and 0.121 respectively) (Fig. 1). According to the whole 5-year dataset, the average was 14 species per pond, and the plant cover reached 37%. Although the highest values of CHL were recorded in 2008 (65 $\mu\text{g/L}$) and the lowest for 2013 (16 $\mu\text{g/L}$), no significant differences were observed for CHL between the years (p -value of 0.181). However, we observed important differences between years for the other water quality parameters. The annual values of water transparency were significantly different (p -value of 0.001) with the lowest values in 2008 (56 cm) and 2009 (59 cm), and the highest values in 2013 (99 cm). TN and TP also varied strongly between the years (p -values <0.001). The lowest concentrations of TN were observed in 2013 (1.13 mg/L) and 2014 (1.44 mg/L) while the values for the other three sampled years were not significantly different from each other. For the TP concentrations, significantly higher values were found for 2008 and 2012 (0.28 ± 0.08 mg/L and 0.33 ± 0.20 mg/L respectively) and the lowest values in 2013 at 0.12 mg/L.

Aquatic plant richness and plant cover were highly correlated (Table 1). Both were negatively correlated with the CHL and positively correlated with water transparency. In term of nutrients, only one negative relationship was observed between aquatic plant cover and the concentration of TP. As expected, CHL was positively correlated with nutrients and negatively with water transparency.

3.2. Aggregate tipping points and variations from year to year

Using the whole dataset of five years two tipping points were observed at 6 $\mu\text{g/L}$ and 60 $\mu\text{g/L}$ of CHL for aquatic plant richness and with TMEAN method (Fig. 2, Table 2). Below 6 $\mu\text{g/L}$ the average of aquatic plant richness was above 20 species, decreased to 12 species when CHL was between 6 and 60 $\mu\text{g/L}$, and diminished to 9 species when CHL was above 60 $\mu\text{g/L}$. With the SEGMENTED method, tipping points were observed at 12 ± 4 $\mu\text{g/L}$ of CHL in aquatic plant richness. However, tipping points obtained with CHL for plant richness varied through the five years. No tipping points were observed in certain years: 2009 for SEGMENTED, 2012 and 2013 for both statistical methods. With TMEAN, all tipping points observed for plant richness in the different years were below 10 $\mu\text{g/L}$ of CHL, except the second tipping point. Two tipping points were recorded in 2008 where a significant decrease of plant richness from a mean of 30 to 20 aquatic plant species was recorded above 5 $\mu\text{g/L}$, and a second step from a mean of 20 to 9 species was observed above 39 $\mu\text{g/L}$ of CHL. Another low value of a tipping point at 5 $\mu\text{g/L}$ of CHL was also found for 2014. With SEGMENTED, tipping points were observed in 2008 and in 2014. A significant decrease was also observed above 71 ± 27 $\mu\text{g/L}$ in 2008 when the aquatic plant richness diminished to 10 species in average, but with a slow decrease of plant richness. In 2014, a tipping point found with SEMENTED was at 16 ± 8 $\mu\text{g/L}$ of CHL.

In relation to the nutrient concentrations and water transparency, tipping points were found in some cases strongly dependent on years (Table 2 and Annexes). No tipping point was observed with the SEGMENTED method in water transparency. Looking at the whole 5-year dataset, aquatic plant richness significantly increased from a mean of 10 species to 20 species with a

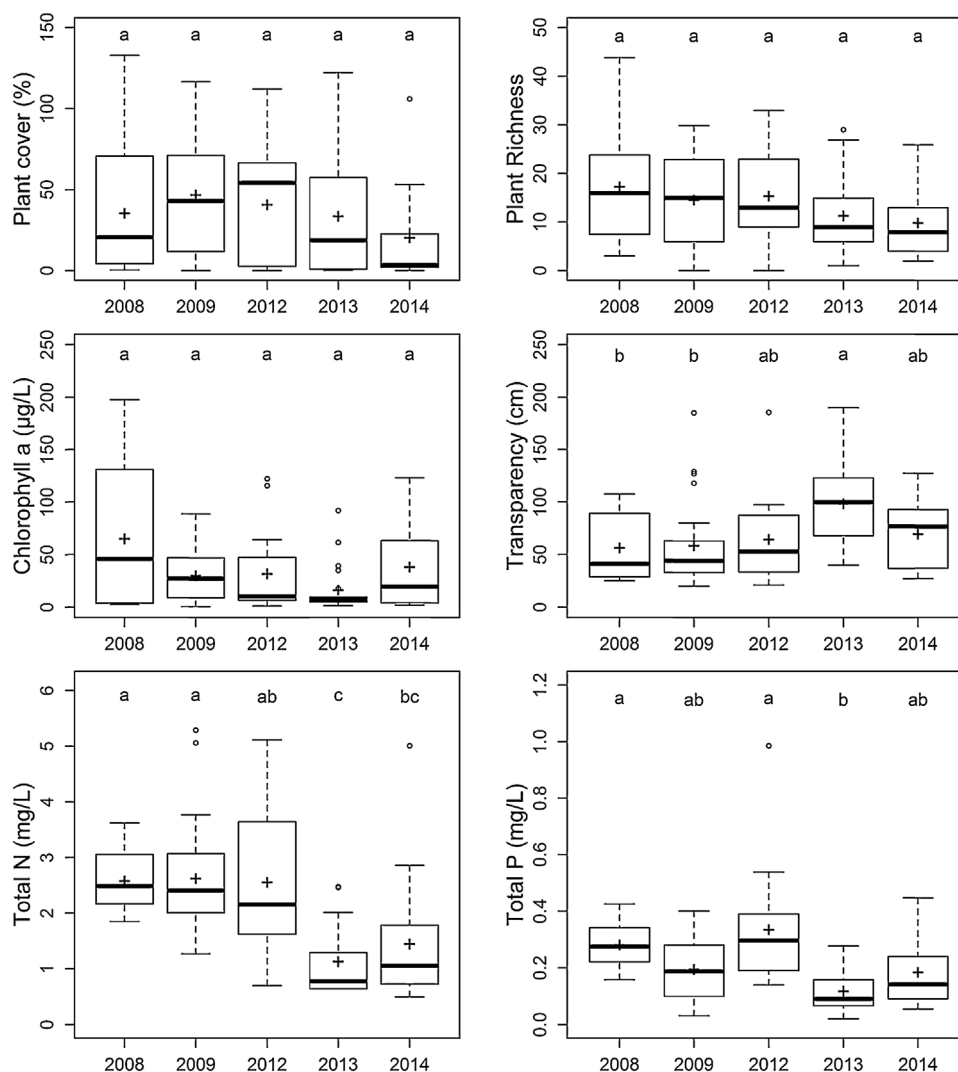


Fig. 1. Boxplots of aquatic plant richness and cover, and median of spring water quality parameters in fish ponds of the Dombes study area, France. Different letters above the bars denote significantly different values between years for the respective parameters ($p < 0.05$). + corresponds to the mean of the different years. Whereas plant species richness, plant cover and chlorophyll α were not significantly different between the years, this was found for the other water quality parameters for certain years.

Table 1
Spearman's rank correlations between aquatic plant richness, plant cover and water quality parameters in fish ponds of the Dombes study area, France ($n = 100$). Significance levels are: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: no significant relationship. High correlations were found between plant cover and plant richness, CHL and transparency, as well as for TP and TN.

	Plant Richness	Plant cover	CHL	Transp.	TN	TP
Plant Richness	1					
Plant cover	0.735***	1				
CHL	-0.466***	-0.510***	1			
Transparency	0.317**	0.361***	-0.885***	1		
TN	NS	NS	0.507***	-0.561***	1	
TP	NS	-0.277**	0.601***	-0.687***	0.734***	1

water transparency deeper than 70 cm, with the TMEAN method. In 2008, two tipping points were also observed at 39 cm and 75 cm for aquatic plant richness with TMEAN.

For TN, tipping points were observed at 3.9 mg/L for aquatic plant richness with the TMEAN method looking at the whole dataset of five years. However, tipping points for specific years were only observed with TMEAN in 2008 at 2.43 mg/L and 2012 at 3.91 mg/L, and for 2008 with SEGMENTED at 3.18 mg/L. For TP, tipping points were observed below 0.10 mg/L in 2013 and 2014 with TMEAN and for SEGMENTED only in 2014. In 2012, tipping point was observed for a value of 0.38 mg/L.

In plant cover, one tipping point was observed for the five-year dataset at 11 $\mu\text{g/L}$ with the TMEAN method where we observed a plant cover mean near to 60% for a concentration of CHL below 11 $\mu\text{g/L}$, and which decreased to 20% above this threshold. With SEGMENTED the tipping point was at $15 \pm 6 \mu\text{g/L}$ of CHL. Tipping points were observed with TMEAN for all years with a highest value in 2008, where a decrease from 60% to less than 10% of plant cover was recorded at 39 $\mu\text{g/L}$ of CHL, but globally, the tipping points for other years varied slightly between 4 and 10 $\mu\text{g/L}$ of CHL. As in plant richness, the lowest value of tipping points was found for 2014 at 4 $\mu\text{g/L}$ of CHL.

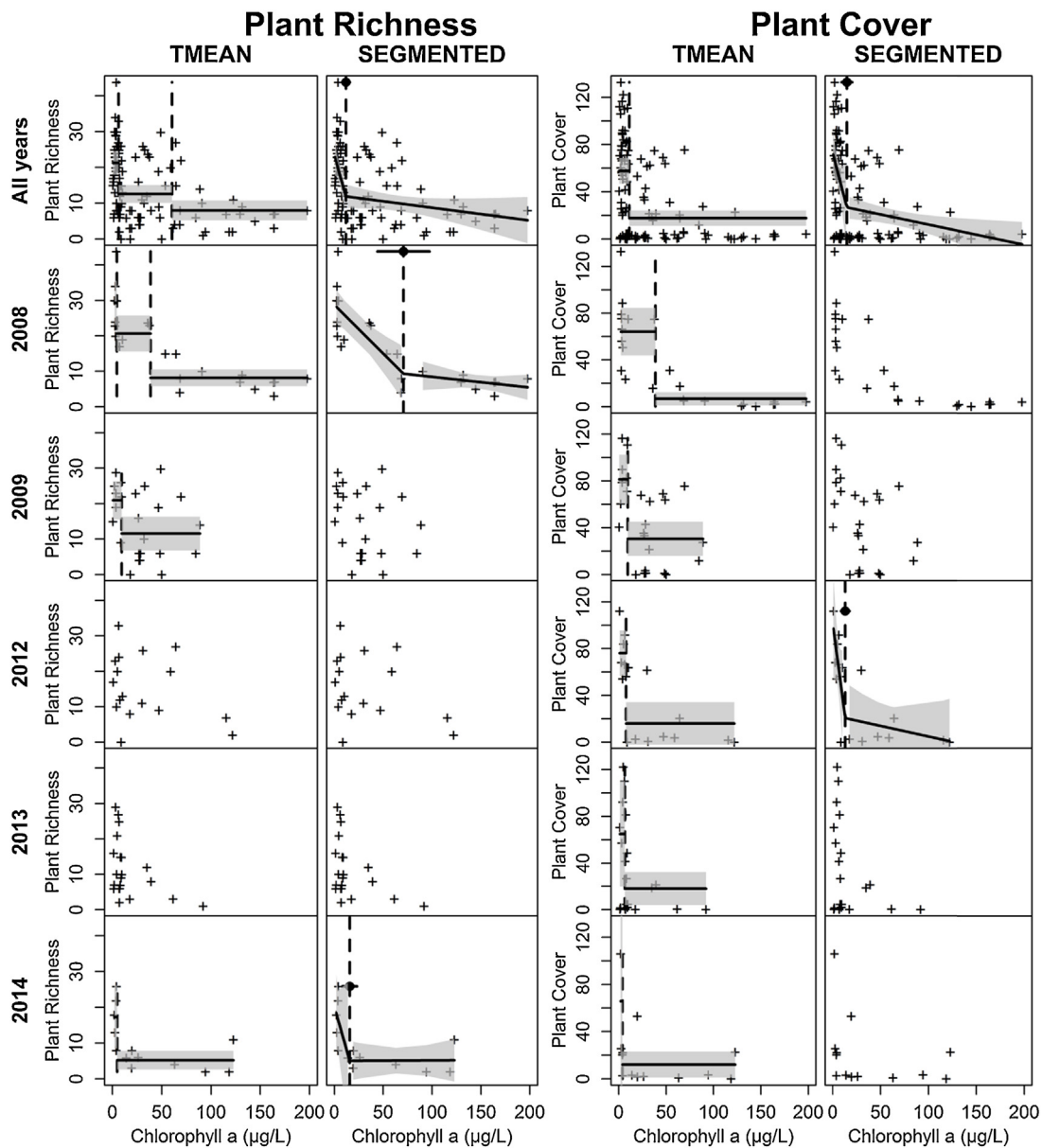


Fig. 2. Tipping point analyses between aquatic plant richness (left side) and aquatic plant cover (right side) for chlorophyll α in fish ponds in the Dombes study area, France, in relation to different study years and two statistical methods applied (TMEAN and SEGMENTED). Tipping points varied between certain years and for both methods. High values of tipping points were recorded in 2008, while in the other years near to 10 $\mu\text{g/L}$ of chlorophyll α . For some years no tipping points were detected.

In relation to nutrient concentrations and water transparency, tipping points in plant cover were found in some cases strongly dependent on years, similar to plant richness. Aquatic plant cover increased from a mean of 20% to 55% of aquatic plant cover above a tipping point at 62 cm of water transparency with the TMEAN method and the 5-year dataset. No tipping point was observed with SEGMENTED.

For TN, two tipping points were observed at 2.4 and 3.9 mg/L for plant cover looking at the whole dataset of five years. As in plant richness, tipping point was observed with TMEAN only in 2008 at the same value at 2.43 mg/L and in 2012 at 2.49 mg/L of TN. No tipping point was observed with SEGMENTED. Furthermore, in TP, two tipping points were observed at 0.05 mg/L and 0.24 mg/L with TMEAN method looking at the whole dataset of five years. Except in 2014, where no tipping point was observed, tipping points for TP were found in most cases in different years between 0.05 mg/L and 0.35 mg/L, and up to 0.44 mg/L of TN with SEGMENTED in 2012.

3.3. Weather conditions

All characteristics of each sampled year and their tipping points in aquatic plant richness and cover were recorded in the Table 3. Spring rainfall and spring solar radiation were significantly different between years (p -value of 0.001 in rainfall and <0.001 in solar radiation, Fig. 3). In 2014 the maximum mean value of solar radiation was recorded with 1956 W/m^2 (Table 3). Maximum values for rainfall occurred in 2008 and 2013 (3.1 mm and 3.2 mm, respectively), and the lowest in 2014 with 1.8 mm. Although temperature was not significantly different between years, the maximum value of daily temperature was recorded in 2009 with 14.5 °C while the lowest value was observed in 2013 at 11.9 °C.

CHL, water transparency, and TN were strongly influenced by weather conditions during the four weeks before the sampling dates (Table 4). Significant positive correlations were observed between CHL and TN with temperature and solar radiation. In contrast, water transparency was significantly negatively correlated

Table 2
Tipping point analyses in water quality parameters for aquatic plant richness and cover in fish ponds in the Dombes study area, France, in relation to sampling years and two statistical methods applied (TMEAN and SEGMENTED). Tipping points differed between years for plant richness and plant cover, and depending on the water quality parameters. The TMEAN method detected more tipping points than SEGMENTED.

		Aquatic plant richness		Aquatic plant cover	
		TMEAN	SEGMENTED	TMEAN	SEGMENTED
CHL ($\mu\text{g/L}$)	All years	6/60	12 \pm 4	11	15 \pm 6
	2008	5/39	71 \pm 27	39	–
	2009	9	–	10	–
	2012	–	–	8	13 \pm 5
	2013	–	–	6	–
	2014	5	16 \pm 8	4	–
Water transparency (cm)	All years	70	–	62	–
	2008	39/75	–	43	–
	2009	–	–	54	–
	2012	–	–	70	–
	2013	–	–	–	–
	2014	79	–	–	–
TN (mg/L)	All years	3.90	–	2.42	–
	2008	2.43	3.18 \pm 0.12	2.43	–
	2009	–	–	–	–
	2012	3.91	–	2.49	–
	2013	–	–	–	–
	2014	–	–	–	–
TP (mg/L)	All years	–	–	0.05	–
	2008	–	–	0.24	–
	2009	–	–	0.09	–
	2012	0.38	–	0.35	0.44 \pm 0.10
	2013	0.06	–	0.05	0.07 \pm 0.01
	2014	0.08	0.09 \pm 0.01	–	–

with temperature and solar radiation. Furthermore, TP was only significantly correlated with the solar radiation.

4. Discussion

4.1. Eutrophication gradient and tipping points for plant richness and cover

In general, the diversity of aquatic plants in fish ponds in the Dombes area is very high for eutrophic shallow water bodies (Robin et al., 2013; Wezel et al., 2014) and no significant difference was observed between years in fish ponds sampled. As often stated in literature (Arthaud et al., 2012b; Declerck et al., 2006, 2005; Robin et al., 2013; Scheffer et al., 2001, 1993; Søndergaard and Moss, 1998), we expected that plant cover and also richness would be negatively correlated with increased nutrient concentrations in fish ponds. Often, aquatic plants disappeared in shallow lakes when nutrients were too abundant leading to a high phytoplankton biomass (Søndergaard and Moss, 1998). This finding was confirmed by our tipping points which were found in all tested parameters of water quality, except for aquatic plant richness and TP. But with the tipping points we could show that this is not a typical linear decrease along a certain gradient as often stated in literature. For example, a significant drop of richness or cover was observed at specific concentrations of nutrients. Transitions in an ecosystem may have negative impacts by gradually changing the environment or by discrete perturbations when a critical threshold is passed (Bestelmeyer et al., 2011). Such tipping points should not be understood as thresholds above or below which we will have a certain number of species or cover, but as a point from which a regime significantly shifts.

A significantly higher aquatic plant diversity above 20 species was observed in fish ponds where the concentration of TN was below 3.90 mg/L with the TMEAN method and below 2.42 mg/L of TN and 0.05 mg/L of TP for a high aquatic cover above 60%. These tipping points were observed while no direct negative correlation with

TN and both aquatic plant richness and cover was found. In contrast to our results, a negative correlation was observed between the aquatic plant cover and TN in Dutch shallow lakes (Scheffer, 2004). However, aquatic plants were more susceptible to be impacted by TP than by TN in many studies (Arthaud et al., 2012b; Declerck et al., 2006; Scheffer et al., 2001, 1993) where aquatic plants were negatively correlated with increasing TP. Similarly to these results, a significant negative correlation was observed in our data between plant cover and TP. However, for TP, no direct correlation or tipping point for plant richness were observed looking at the whole five year dataset, suggesting that the diversity of aquatic plants in all years was not significantly influenced by TP. Though, Arthaud et al. (2012b) showed a significant decrease of functional richness and functional dispersion of aquatic plants in fish ponds along the TP gradient.

Tipping points found in our study for nutrient concentrations could vary between years, which might be explained by variation of nutrients in some years, but also by weather conditions. The latter can play an important role in the state of water bodies and also for a regime shift from clear to turbid water states (Scheffer and van Nes, 2007). The years 2008 and 2012 showed high nutrient concentrations and higher tipping points were observed in TN and TP. These high tipping points found for 2008 and 2012 indicate a change from “Poor” to “Bad” status according to the classification of Moss et al. (2003) and Søndergaard et al. (2005b). Thus, a higher diversity and cover of aquatic plants might be even observed in a “Poor” state of water in our fish ponds in certain years before a significant drop of diversity or cover appeared thereafter with increased nutrient concentrations. Although 2008 and 2012 presented not extreme weather conditions during spring, our data showed that high values of temperature and solar radiation were positively correlated with increased TN, and solar radiation positively correlated to TP. Rainfall did not have any direct effect on the water quality of the fish ponds in our study, although Wezel et al. (2013a) found a significant transfer of nutrients from water catchments to the fish pond in certain years with higher and irregular rainfall in spring. Also,

Table 3

Water quality parameters and their tipping points recorded with TMEAN in aquatic plant richness and cover, and weather parameters in the five years of sampling of the Dombes fish ponds, France.

	2008	2009	2012	2013	2014
Water quality parameters					
CHL ($\mu\text{g/L}$)	55 \pm 61	24 \pm 24	26 \pm 31	20 \pm 27	31 \pm 38
Transparency (cm)	56 \pm 27	69 \pm 45	73 \pm 41	95 \pm 38	72 \pm 32
TN (mg/L)	2.43 \pm 0.50	2.49 \pm 0.96	2.11 \pm 1.17	1.53 \pm 1.40	1.67 \pm 1.34
TP (mg/L)	0.281 \pm 0.103	0.177 \pm 0.102	0.349 \pm 0.190	0.156 \pm 0.110	0.258 \pm 0.173
Aquatic plant cover (%)	35 \pm 37	47 \pm 36	41 \pm 38	34 \pm 40	20 \pm 30
Aquatic plant richness	17 \pm 11	15 \pm 9	15 \pm 9	11 \pm 8	10 \pm 8
Tipping points in aquatic plant richness					
CHL ($\mu\text{g/L}$)	5/39	9			5
Transparency (cm)	39/75		31		79
TN (mg/L)	2.43		3.91		
TP (mg/L)			0.38	0.06	0.09
Tipping points in aquatic plant cover					
CHL ($\mu\text{g/L}$)	39	10	8	6	4
Transparency (cm)	43	54	70		
TN (mg/L)	2.43		2.49		
TP (mg/L)	0.24	0.09	0.35	0.05	
Daily weather condition during the spring period					
Rainfall (mm)	3.1 \pm 5.0	2.1 \pm 4.5	2.7 \pm 5.1	3.2 \pm 6.2	1.8 \pm 4.4
Temperature ($^{\circ}\text{C}$)	12.6 \pm 4.7	14.5 \pm 4.6	13.7 \pm 4.4	11.9 \pm 5.0	13.1 \pm 4.1
Solar radiation (W/m^2)	1627 \pm 727	1874 \pm 658	1724 \pm 695	1526 \pm 775	1956 \pm 687
Weather characteristics 4 weeks before the sampling date					
Sum of rainfall (mm)	83 \pm 30	53 \pm 20	85 \pm 28	99 \pm 49	50 \pm 15
Sum of temperature ($^{\circ}\text{C}$)	414 \pm 106	462 \pm 104	397 \pm 100	311 \pm 86	366 \pm 49
Sum of solar radiation (W/m^2)	51223 \pm 7384	58,559 \pm 9916	51,632 \pm 7245	42,242 \pm 6983	57,909 \pm 9700

Kato et al. (2009) found that the concentrations of TP were higher during rainy days, while the concentrations of TN were lower. Furthermore, in 2013 and 2014 where extremer weather conditions occurred, tipping points in nutrients were found at lower concentrations, below 0.09 mg/L of TP, and no tipping point in TN was observed. According to Søndergaard et al. (2005b), a concentration below 0.10 mg/L of TP corresponds to a “Good” ecological status. Soranno et al. (2008) also evaluated tipping points in TP for the aquatic plant cover in US lakes, but they observed significant thresholds between 0.008 and 0.028 mg/L of TP that were much lower than the tipping points found in our study. However, the majority of fish ponds were eutrophic, and even if nutrient levels in 2013 and 2014 were low for fish pond systems, aquatic plant richness and plant cover were similar to other years. Indeed, 2014 was the year where the highest value of the solar radiation was recorded, leading to a high penetration of light in the system promoting the photosynthesis of vegetation (Sand-Jensen and Borum, 1991). As for nutrients, tipping points in water transparency varied from 39 cm to 79 cm for aquatic plant richness and cover depending on the year, but were below the thresholds of Søndergaard et al. (2005b) and Moss et al. (2003) with 80 or 90 cm in shallow lakes indicating a change from “Poor” to “Bad” status. However, the mean depth of our fish ponds samples was only 71 cm indicating that aquatic plants could probably develop in some ponds on the whole surface of the respective ponds because of a sufficient penetration of light into the water (Robin et al., 2013). Thus, in some

pond studied here, the values of tipping points for transparency do not necessarily indicate a regime shift.

4.2. Competition between aquatic plants and phytoplankton

The competition of light is one the most important factor between phytoplankton and aquatic plants as the lack of penetration of light into the water limits the development of phytoplankton and aquatic plants (Sand-Jensen and Borum, 1991). If phytoplankton strongly develops, the turbidity of the water increases leading to a drop of the light penetration available for aquatic plants. With high turbidity levels, phytoplankton was able to dominate over aquatic plants. Thus, to have potentially high aquatic plant diversity above 20 species and a plant cover above 60%, according to the five years of sampling in fish ponds, the concentration of CHL generally should be below 6 $\mu\text{g/L}$ and 11 $\mu\text{g/L}$, respectively. In addition to the 6 $\mu\text{g/L}$ CHL tipping point for aquatic plant richness, a second one was also observed at 60 $\mu\text{g/L}$ CHL with the TMEAN statistical method. These both tipping points may indicate the disappearance of different aquatic species at different CHL levels. As found in Arthaud et al. (2012b), small caulescent aquatic plant species disappeared at already low level of CHL in fish ponds, e.g. 6 $\mu\text{g/L}$. They also observed the disappearance of tall caulescent aquatic plant species which might be related to the tipping point found at 60 $\mu\text{g/L}$, and only tall or free floating plant species may survive at higher levels of CHL in fish ponds. Furthermore, Robin et al.

Table 4

Spearman's rank correlation in the sampling dates between weather parameters and water quality parameters in fish ponds in the Dombes area, France. The total of sampling dates in the five years study is 32. Significance levels are: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS = no significant relationship. Values of weather parameters were accumulated values from four weeks before the respective sampling date. Significant relationships were observed for both CHL, water transparency and TN with temperature and solar radiation, as well as for TP and solar radiation.

	Temperature	Rainfall	Solar radiation
Chlorophyll α (CHL)	0.475**	NS	0.366*
Water transparency	-0.693***	NS	-0.544***
Total nitrogen (TN)	0.519**	NS	0.511**
Total phosphorous (TP)	NS	NS	0.388*

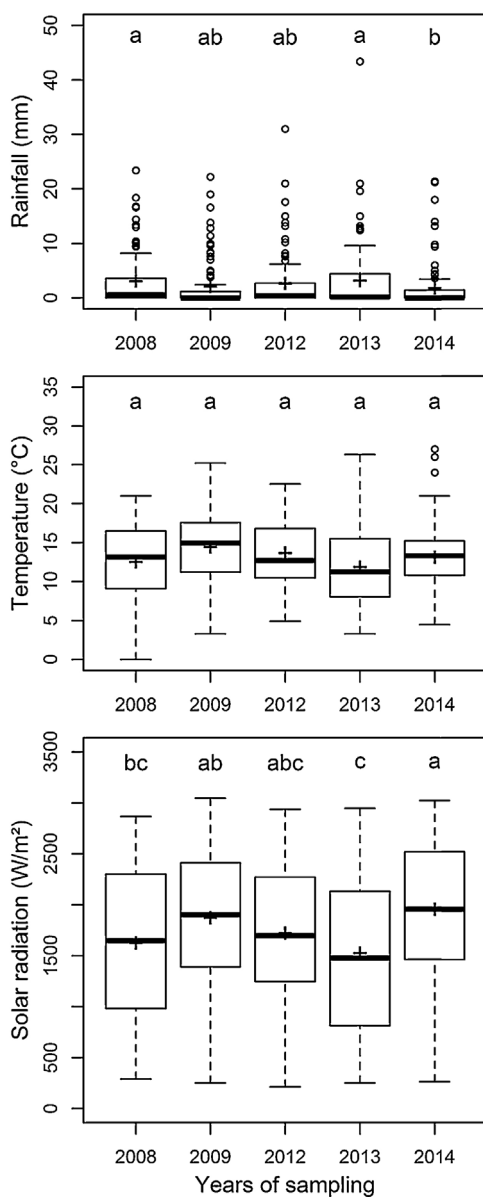


Fig. 3. Boxplots of mean daily weather parameters during spring in each sampling year for the Dombes study area, France. Different letters above the bars denote significantly different values between years for the respective parameters ($p < 0.05$). + corresponds to the mean of the different years. Significant differences were observed between certain years for the rainfall and the solar radiation.

(2013) also found tipping points of 50–60 $\mu\text{g/L}$ of CHL indicating a significant decrease of aquatic plant species, macro-invertebrate families and dragonfly species in fish ponds with higher concentrations of CHL. In addition, tipping points were observed at 70 and 62 cm of water transparency in aquatic plant richness and cover, respectively, indicating a low value of water transparency. This was mainly due to phytoplankton biomass development facilitated by increased nutrient concentrations and the turbidity of the water (Buiteveld, 1995; Mazumder and Havens, 1998) allowing only the presence of floating species to develop in this environment (Arthaud et al., 2012a). Indeed, the high phytoplankton biomass causes a breakdown in aquatic plant species richness to become very species-poor fish ponds with only occurrence of *Polygonum amphibium* Linnaeus 1753, *Spirodela polyrhiza* (L.) Scheid. 1839, *Lemna minor* Linnaeus 1753, and *Utricularia australis* R.Br. 1810 (Arthaud et al., 2012a). These floating species are very good indicators of eutrophication.

As for nutrients, tipping points in CHL varied between years and the majority of tipping points were found for a range from 4 to 16 $\mu\text{g/L}$ for plant richness or plant cover corresponding to a change in ecological state from “High” to “Good” ecological status (Moss et al., 2003; Søndergaard et al., 2005b). In our fish ponds, the highest value of tipping points in CHL was recorded in 2008 which corresponds to a year where nutrient concentrations were very high. Similarly to 2008, the year 2012 also showed high nutrient concentrations, but only a tipping point between CHL and aquatic plant cover was found, but not for richness. For these both years also, higher tipping points were observed in TN and TP leading to a decrease from about 20 to 10 aquatic plant species. As the drop of aquatic plants richness and cover will be found in higher value of CHL, TN and TP, this indicated that the development of aquatic plants was possible in spite of higher nutrient concentrations, suggesting that in these years, aquatic plants were able to better withstand the competition with phytoplankton than in other years. Consequently, in spring when nutrient concentrations were very high, tipping points in aquatic plant richness occurred with relatively high values, and were observed in all parameters of water quality tested at 39 $\mu\text{g/L}$ for CHL, at 3.9 for TN, 0.20 mg/L for TP, and at 70 cm for water transparency.

During the five years of the study, high values of temperature and solar radiation were positively correlated with increased CHL. Although we studied only air temperature, we looked at the accumulation of degree days during four weeks before sampling date, which therefore impacts water temperature. Thus, our results seem to be consistent with other studies (Jacobsen and Simonsen, 1993; Li et al., 2015; Yang et al., 2013) which showed positive relationship between the water temperature and phytoplankton biomass. Indeed, the temperature is one of the most important variables which influence primary productivity, and so the development of phytoplankton communities (Jacoby et al., 2000; Li et al., 2015). If temperatures were significantly high during spring, CHL affects the water transparency and limits aquatic plant cover and richness leading to a phytoplankton dominated pond during all the year. According to model predictions of climate change and taking into account the impact of the temperature on competition between primary producers, the probability that lakes switches from a clear to turbid state will be higher in the coming years (Mooij et al., 2007). The increase of temperature promotes the development of nutrient-tolerant species and especially the development of cyanobacteria (Chen et al., 2013; Elliott, 2012; Li et al., 2015; Schabhüttl et al., 2013). Furthermore, the light availability is essential for the growth of all phototrophic organisms (Sand-Jensen and Borum, 1991), and the solar radiation is very important for the development of vegetation in aquatic ecosystem. As the lack of penetration of light into the water limits the development of phytoplankton and aquatic plants, in particular for rooted aquatic plants that do not reach the water surface and that are greatly dependent on light availability, but less often limited in nutrient (Sand-Jensen and Borum, 1991). In 2013, the lowest values of temperature and solar radiation and the highest rainfall values were recorded correlating to fish ponds with low concentrations of nutrients which limited the usual development of phytoplankton and aquatic plants. Conversely, in 2014, the highest value of solar radiation combined with the lowest value of rainfall, also led to low concentration of nutrients. With extreme weather conditions and low nutrient concentrations, tipping points were recorded below 10 $\mu\text{g/L}$ for CHL, below 0.10 mg/L for TP, and no tipping point was recorded for TN and in certain years for water transparency. Thus, tipping points were linked to the nutrient concentrations and turbidity which drive the competition between both primary producers, phytoplankton and aquatic plants. However, these switches mainly depend on short term weather conditions and nutrient concentrations available in the system and vary from year to year.

4.3. Using different statistical methods in temporal analyses of tipping points

Both statistical methods used to calculate tipping points in this study were based on linear regression: an ANOVA model for TMEAN and a model based on a 'difference-in-slope' coefficient in two linear predictors for SEGMENTED (Vanacker et al., 2015). With TMEAN, more tipping points could be found than with SEGMENTED, although we initially favored SEGMENTED in a methodological study including a sub-data of the fish ponds studied here (Vanacker et al., 2015). In fact, TMEAN is a simplistic method but the advantage of this method lies in the evaluation of a threshold by modeling the distinguished groups by their means. TMEAN showed a big step for the regression line at the tipping points and the differences before and after the tipping point are specified in the mean in the aquatic plant richness and a variation in the confidence interval (Vanacker et al., 2015). In this case, species richness or cover can be expected to reach a certain average number of species above and below the tipping point. Furthermore, TMEAN revealed in some cases two tipping points unlike SEGMENTED as in the case of CHL. Contrary to TMEAN, SEGMENTED indicated small decrease or a stabilization of aquatic plant richness or plant cover before and after the tipping point, and seems to be a more appropriate method to show a gradual change of water quality parameters (Vanacker et al., 2015). Although it was also important to take into account other properties such as a gradual change of concentrations (Carstensen and Weydmann, 2012), changes in the mean seemed to be more pertinent to determine tipping points in this study in looking at a multiple year dataset. Aquatic plant richness and cover revealed clearer differences in the mean of different groups, than showing gradual changes along different gradients.

5. Conclusion

The analyses of tipping points for a multiple year dataset showed high aquatic plant diversity above 20 species in fish ponds with concentrations of CHL generally below 6 µg/L, TN below 3.90 mg/L and the water transparency higher than 70 cm. Similarly to aquatic plant richness, a high aquatic plant cover above 60% was observed in fish ponds when the concentration of CHL was below 11 µg/L, the water transparency higher than 62 cm, and TN and TP below 2.42 and 0.05 mg/L, respectively. Nevertheless, tipping points should not be understood as thresholds above or below which we will have a certain number of species or cover, but as a point from which a regime significantly shifts. Based on our results, generally it could be recommended managing fish ponds in a way that CHL, TN and TP concentrations are below the found tipping points, and for transparency above to favor conditions allowing significant higher aquatic plant species richness and cover in fish ponds. If some shallow lakes, the removal of planktivorous fish and reintroduction of piscivorous fish is also a method to achieve to a better recolonization of aquatic plants and a decrease of phytoplankton levels after the conservation of a certain quantity of nutrients (Sand-Jensen and Borum, 1991).

However, tipping points were not stable over years and could vary significantly between years. This may be explained by weather conditions which influence the water quality. In particular, temperature and solar radiation directly influenced tipping points in CHL, TN and water transparency, and by rainfall which influenced indirectly. This means that fish management need to adapt to this variation in weather condition to optimize management of phytoplankton and aquatic plants. During years with extreme weather conditions, where nutrients can be relatively low for fish ponds, a significant decrease of plant species richness and cover can already occur at very low tipping point values.

The variation of tipping point may be well explained by difference in weather conditions over years and a long-term study of several decades should be an option to confirm the trend of these results. It should not be forgotten that fish pond management such as fertilization, may also strongly influence the eutrophication of the pond systems. Thus, this would be needed to be taken into account in further studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2015.12.033>.

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