

IMPACTS OF GREEN TIDES ON ICHTHYOFAUNA IN ESTUARINE ECOSYSTEMS

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1 Introduction

1.1 Coastal systems – productivities, richness and functions

Coastal ecosystems represent a continuum between the land and the ocean where freshwater and seawater are mixed together (fig.1). Freshwater, characterized by huge concentration of nutrient (i.e. N, P and Si) derived from land drainage, contributes to enhance the primary production of coastal ecosystems and makes estuary the highest productive system on earth (Costanza et al., 1997; Underwood and Kromkamp, 1999). The strong primary production located on the coastal area contributes to a high concentration of food available for secondary consumers (Largier, 1993; Holbrook et al., 2000).



Figure 1: Estuarine system features by Statham in 2012.

This high secondary production is one of the reason why the coastal systems provide major ecological services to the fish community (Seitz et al., 2014). These services take various forms: the major service performed by estuarine and coastal systems to ichthyofauna is the nursery function. A nursery is defined as a restricted area where juvenile's growth is stimulated (Gibson, 1994). After the larval transport, larvae metamorphose in marine juveniles and settle in estuarine and coastal systems during Spring. The success of settlement depends of the larval input and on the quality of the nursery (Pihl et al., 2005). The growth of juvenile fish is enhanced by the presence of favorable conditions such as foods, temperature and shelter. Quality of the coastal habitat plays a key role for the marine fish species renewal, and in this way is essential for the fishery (Seitz et al., 2014). In fact, the recruitment success (*i.e.* when juvenile join the reproductive population) depends on the growth which occurs in the nursery zone (Gibson, 1994). This nursery function represents the most important function for fishery. In fact 30% of ICES species in Northeast Atlantic rely on this nursery function of coastal and estuarine systems (Seitz et al., 2014).

The availability of food attracts not only juveniles but adults. Estuaries and coast represent also feeding grounds for numerous fish. In 2014, Seitz et al revealed that 20% of the 59 Northeast Atlantic fish species evaluated by the ICES foraged in estuarine and coastal systems at adult

stage. Some fish are subservient to this type of ecosystem. Estuarine areas are thus a permanent habitat for many resident species.

Finally, estuarine and coastal systems constitute crucial area for reproduction. Some fish use these ecosystems as migration corridors. While anadromous fish migrate through estuaries from sea to fresh water for reproduction, catadromous fish have opposite migration. The spawning function depends on estuarine and coastal ecosystems for 10 % of the ICES species and migration routes for 8 % (Seitz et al., 2014).

The different ecological services (*i.e.* nursery, food area, reproductive area and migration corridor) are thus essential for the fishery. Indeed 77 % of the cumulative landing of commercial fish in Northeast Atlantic are dependent from estuarine and coastal habitats (Seitz et al., 2014). These species represented 6 556 411 tons of landings in 2010. In addition to this economical interest and value of estuaries, these ecosystems are also essential for non-commercial fish and organism which contributes to a healthy ocean environment.

1.2 Green tides – an anthropogenic change of estuarine systems

Estuarine and coastal areas are subject to a high human activity. 60% of the human population lives near to the coasts and along estuaries. This population is expected to increase in the next decades (Beck and Airoldi, 2007). The attractiveness of coastal shore is explained by the ecological services dispensed by this ecosystem for the human population (Costanza et al., 1997). The growing human activity leads to degradations of the costal and estuarine ecosystems.

1.2.1 Description of green tides

Organic and inorganic nutrients (*i.e.* phosphorus, nitrogen, silicium) are one of the source of degradation caused by urbanization, industry and agriculture (Diaz and Rosenberg, 2008; Liu et al., 2013; Lyons et al., 2014). The increase of nutrient concentration coupled with low residual hydrodynamic conditions (*i.e.* after removing cyclic tidal circulation) within estuarine areas, leads to eutrophication (Nixon, 1995; Valiela et al., 1997). Eutrophication leads to two main disturbances: excessive proliferation of green macroalgae (called green tides) and anoxia episodes.

In the last decades, green tides events have increased in intensity and frequency (Valiela et al., 1997) (fig. 2). In temperate latitudes, these green tides (named hereafter GT), are controlled by the input of nitrogen (N). If the enrichment in nitrogen is beyond the level of the self-regulatory capacity of the estuary, massive proliferations of opportunistic macroalgae could be observed (Valiela et al., 1997). Temperature, hydrodynamic condition and light are the other factors controlling the green tides events (Merceron et al., 2007).



Figure 2: World map of green tides events according to Ye et al (2010). The stars represent the most impacted areas during the last three decades.

The composition of green tides is characterized by fast growing species, with high tolerance to salinity range (Zhou et al., 2015). Three taxa of opportunistic algae represent the majority of algae who composed these blooms: *Caetomorpha spp.*, *Cladophora spp.* and *Ulva spp.* (Valiela et al., 1997; Anderson et al., 2015).

1.2.2 Ecological effects of green tides

If economic costs are known (*i.e.* tourism, risk for the human health), there are few studies developed to assess the consequences of green tides on animal community (Lyons et al., 2014). The impacts induced by green tides depend on the estuary features and the organism studied. The duration and concentration of opportunistic algae are also critical factors controlling the level of disruption (Baden, 1990). Green tides lead to positive or negative impacts on fauna, with regards to their intensity (Hull, 1987).

The most obvious effect of GT is the shift on floral composition (Pihl et al., 1994). The autochthonous primary producers (*i.e.* seagrass and benthic microalgae) are replaced by mats of opportunist green macroalgae (Sundbäck et al., 1996). This change in algae composition induces a degradation of the habitat quality (Pihl et al., 2005). This modification of the bottom habitat by GT affects notably macrobenthic communities (Quillien et al., 2015).

Lyons et al (2014) revealed that fish community are negatively impacted by green tides. However, the consequences on this biologic compartment are still poorly studied. We describe below some results that revealed the negative effects of green tides on fish community.

For instance, in Swedish bay and in Baltic sea, a massive change on fish composition was induced by GT and a decrease of predatory fish was observed (Pihl et al., 2005; Österblom et al., 2007). This phenomenon was explained by a decrease of foraging efficiency of predatory fish. Mats of green macroalgae procure a shelter for small grazing fish and benthic macrofauna

(Pihl et al., 1995). Small fish take advantage of proliferation of green macroalgae. Their biomass increases with the decrease of larger predatory fish (Eriksson et al., 2009). Massive green tides reduce fish density (Le Luherne et al., 2016) and species richness (Pihl et al., 1994). The response of fish community depends notably on the vertical distribution of species (Le Luherne et al., 2016). Benthic fish is the most sensitive biological compartment, due to the degradation of their habitat by GT (Pihl et al., 1994, 2005; Wennhage and Pihl, 2007; Le Luherne et al., 2016).

The nursery function performed by estuaries is especially compromised by green tides. Mats affect the success of recruitment of larvae and marine juveniles (Baden, 1990; Pihl et al., 2005). Marine juveniles seem to be the most endangered states of life during GT (Pihl et al., 2005; Le Luherne et al., 2016). In addition to decrease in density, individual performances of juvenile fish (i.e. lipid storage and growth) are perturbed by GT (Le Luherne et al., submitted). Nursery is a key process that affected the population size (Pihl et al., 2005). If this essential service is impacted by GT (Stoner et al., 2001), then the productivity of a numerous North temperate commercial fish could be lower (Peterson et al., 2000; Stoner et al., 2001; Seitz et al., 2014).

Massive fish mortality events were also observed during green tides (Le Luherne et al., 2016). Two causes of mortality were revealed. First, huge concentration of ammonium (NH_4^+) produced by bacteria. These events were unusual because the toxic level of NH_4^+ is rarely reached. Anoxia is the second factor provoking massive mortality. Anoxia caused by GT was responsible for a massive mortality on fish. The fishery was mostly impacted by this event. The capture per unit effort for lobster in the Baltic sea, highly valuable commercial species, decrease from 30 kg.m² to 3.5 kg.m² in 6 years. The CPUE for other commercial fish, cod and plaice, decrease to 90 % during the same period (Baden, 1990).

1.3 Study aims

During the last decade, the number of impacted sites and the intensity of GT have been increasing on the Brittany coast (Ménesguen and Piriou, 1995), with high proliferation in spring season. The rise of green tides in Brittany raise concerns about the ecological impacts caused by this phenomenon.

The economical costs of GT are important in Brittany. In 2015, the cost of algal removal campaigns reached 998 000 euros for 15 townships (source CEVA). This cost is associated to the tourist economy. However, the loss of other ecological services is still not well evaluated.

This study aims to provide a quantitative evaluation of the potential impacts of GT on fish community in Brittany. This analysis was conducted in 13 estuaries with contrasted levels of GT. Potential effects of GT on ichthyofauna were examined across changes in fish density (total and by functional groups), species richness and indices of functional diversity.

2 Materials and methods

2.1 Study areas and beam trawl data

From 2007 to 2014, fish were sampled in 13 estuaries located in Brittany (fig. 3), as part of the EU Water Framework Directive.



Figure 3: Location of the 13 estuarine zones sampled in this study.

Standardized sampling campaigns were realized 2 times in a year, in Autumn and Spring, for each site. The 13 sites studied were sampled with a beam trawl with an opening of 1.5 m wide and 0.5 m high, 8 mm stretched mesh in the cod end. The beam trawl hauls were realized against the current during 15 min, and the mean surface trawled by each haul was about 1 100 m². Surveys in each estuary were designed to cover the various potential estuarine habitat. For this purpose, a minimum of 8 beam trawls was carried out in each range of salinity within each estuary (oligohaline, mesohaline, polyhaline). Salinity and depth were recorded for each beam trawl, this information allowed us to take account of environmental gradients which influence fish composition. 90 campaigns were carried out, with 1348 beam trawl hauls realized in the 13 estuarine zones. Since 2007, 97 fish species were captured, and 79125 individuals were trawled.

These 13 estuarine areas are characterized by a gradient level of green tides intensity. Certain estuaries (*e.g.* Odet) are not impacted by GT while others (*e.g.* Pont l'Abbé) are largely impacted.

2.2 General approach

In order to quantify the response of the fish community under the stress provoked by GT, we used fish metrics and descriptors of green tide intensity. The statistical approach consists to model the variability of fish community due to the proliferation of green macroalgae, accounting for the environmental function which control the fish assemblage (fig. 4).



Figure 4: General approach used in order to provide a quantitative evaluation of the potential impacts of green tides on fish community in Brittany.

2.3 Metrics of fish community

Fish diversity was estimated from species richness. Species richness was defined as the number of species captured for each haul. The total density was defined as the number of individuals captured per haul. The total density is well known as an indicator of the habitat quality. Density of fish and species richness are supposed to decline with the intensification of perturbations, like GT (Delpech et al., 2010).

Functional diversity was also considered in this study. The 97 species caught were classed into 5 functional guilds and the density was calculated for each guild. Functional guilds are defined as a group of species exploiting the same resource in similar ways (Elliott and Dewailly, 1995). In this study, 3 guild of position and 2 guild of life traits were considered (table 1). The vertical distribution guild (i.e. pelagic, demersal and benthic) is related to spatial occupation in the water column and illustrates the dependence of organism to the bottom sediment. Ecological guilds (*i.e.* resident and marine juvenile) describe the use of estuaries during the life cycle (for

example, marine juvenile describe the nursery function performed by estuaries). These five ecological guilds allowed to evaluate which compartment is affected by green tides.

Criterion	Guild	Definition
Vertical distribution Pelagic		Species living in the water column
	Demersal	Species living in the water layer just above the bottom
	Benthic	Species living on the substratum
Ecology	Marine juvenile	Species using the shallow coastal waters and estuaries primarily as nursery ground
	Resident	Species spending their entire lives in shallow coastal waters and estuaries

 Table 1: Ecological and vertical distribution guilds by (Elliott and Dewailly, 1995)

In addition, we used 19 morphological traits to specify ecological functions and offer a representation of the functional structure (Paumier, 2015). These morphological measures were carried out at species scale (fig. 5). The species caught in the 13 estuaries were associated to 19 morphological traits. These 19 traits were chosen to assess three ecological function: the locomotion capacity, the foraging behavior and the use of the habitat. The practical details relating to the 19 morphological traits and their measures are present in annex I.



Figure 5: The 19 morphological traits measured for the 97 species caught in the 13 sampled estuaries. The hatched area represents the measure of the fin area.

Two indices of functional diversity were calculated using the morphological trait, using the FDpackage (Laliberté and Legendre, 2010). These indices of functional diversity, the functional richness and the functional divergence, were measured by quantifying the distribution of species in a multivariate functional trait space (Mason et al., 2005; Villeger et al., 2008). These indices describe the distribution of the species and their abundances in the functional trait space, where each morphological trait represents a coordinate of this space. The functional richness is defined as the area of the functional trait space occupied by the community. The functional divergence describes the distribution of the abundance within the functional space (Villeger et al., 2008). The calculation of these two indices is explained with a simplified example in fig. 6.



Figure 6: Estimation of the two independent indices of functional diversity (Villeger et al., 2008), illustrated on a simplified virtual community with nine species and with only two morphological traits. (a) One species is represented by one point in the functional space according to his trait values. The diameters of the points depend on the species abundances. (b) The functional richness corresponds to the convex hull volume. (c) The functional divergence is calculated by the deviation of the distance from the mean distance of center of gravity (large circle; center of gravity: Gv) corresponding to the length of the black lines linking each species to the circle.

2.4 Environmental drivers of fish community

The natural features controlling the fish community are described below.

2.4.1 Season

The season is a major factor controlling the distribution of species within estuaries (Courrat et al., 2009). In fact, the settlement and migration are seasonal events. Ecological functions of estuaries (i.e. nursery, reproductive area and migration corridor) are performed seasonally (Elliott and Quintino, 2007). As the fish communities change dramatically between Spring and Autumn, analyze were developed on two sub-data set, composed of the spring campaigns and the autumn campaigns separately.

2.4.2 Ecoregion

A biogeographic classification based on the Marine Ecoregions of the World (Spalding et al., 2007), was used to separate the 13 estuaries between the site localized in the North and in the South (fig. 7).

Ecoregion was defined by Spalding et al as an "area of relatively homogeneous species composition clearly distinct from adjacent system". Five estuaries belong to the ecoregion "Celtic sea" (named after "North Brittany" in our study) and 8 estuaries belong to the ecoregion "South European Atlantic Shelf" (named after "South Brittany") (fig. 7).



Figure 7: Boundary of the two ecoregion on the Brittany's coast , according to Spalding et al (2007): (a) Celtic Sea; (b) South European Atlantic Shelf.

2.4.3 Salinity and depth

Salinity and depth are crucial factors structuring the fish community in estuaries (Le Pape et al., 2003; Courrat et al., 2009). These parameters were available for each trawl haul on survey data.

2.5 Descriptors of green tides at different scales

In order to quantify the intensity of green tides, we used data provided by the CEVA (Center for Study and Promotion of Algae), and data collected during the trawl survey in 2013 and 2014.

2.5.1 Descriptors of green tides at estuarine area scale

The CEVA evaluate GT in Brittany since 1997. In this study, we use data collected between 2008 and 2014 (*i.e.* trawl survey period). The data were obtained using a standardized protocol. Flights were carried with a CESSNA plane, during the high coefficient of tide. During the flight, a photographer takes pictures of the impacted sites. Pictures were then spatialized using their

coordinates. Field controls were carried out to determine the constituent algae beaching observed when flying.

The pictures were used to calculate a ratio of surface colonized by *Ulva* spp. From these ratios, the CEVA proposed three descriptors of proliferation. The first proxy is the maximum percentage of the potential area covered by *Ulva* spp. The second proxy is the gross area affected by green tides, in hectare. For each proxy, thresholds were defined by experts, who take account of historical data. The Ecological Quality Ratios (EQR) was formed by the mean of the score of these 2 proxies (table 2). This indicator is a validated proxy of estuarine quality used by the Water Framework Directive, for each year one value of EQR corresponding to an ecological status was available and was used as a proxy of proliferation in our models (table 3).

Proxy 1	Proxy 2	EQR	ecological status
0-5	0-10	1-0.8	High
5-15	10-50	0.8-0.6	Good
15-25	50-100	0.6-0.4	Moderate
25-75	100-250	0.4-0.2	Poor
75-100	250-6000	0.2-0	Bad

 Table 2: Thresholds reflecting the ecological status for each proxies, and the Ecological Quality Ratios (EQR).

2.5.2 Descriptors of green tides at local scale of proliferation

As a complement to the mean green tides distribution procured by the EQR, we used two different sources of descriptors providing information on GT at the scale of each estuary: the surface ratios (used by the CEVA to create the EQR) and the weight of algae caught per haul. These two descriptors allowed to integrate a spatial component of proliferation in our models (table 3).

For each estuary, the surface ratios provided by the CEVA are an average value of the surface covered by *Ulva* spp. from 2008 to 2015. Preliminary analysis allowed to validate the interannual study of this distribution. The surface ratios were available in the form of a shapefile. We used this shapefile in order to map the mats for each estuary. We plotted the positions of beam trawl hauls and collected the surface ratios associated with each haul to model the effect of GT in fish community.

The weight of algae per beam trawl was obtain from an additional protocol added to the WFD trawl survey in 2013 and 2014. These measures of biomass allowed to obtain an information concerning the environment where the trawls were carried out. These data were available for 227 beam trawl hauls. The density of *Ulva* spp. was used as a proxy of proliferation, integrating a spatio-temporal component (table 3).

Scale of the metrics of green tides proliferation	Beam trawl data	Metrics of green tides proliferation	Variable
estuarine area scale	2008-2014	Ecological Quality status	Factor
local scale of proliferation	2008-2014	Surface Ratio (percentage)	Continuous
local scale of proliferation	2013-2014	Biomass of ulva per beam trawl (kg.m ⁻²)	Continuous

Table 3: The three different metrics for the green tides proliferation

2.6 Preliminary data analyses

We tested by different methods the effects of environmental features (i.e. ecoregion, salinity and depth) before testing the effects of GT metrics (fig.4).

2.6.1 Ecoregion

The ecoregion effect was investigated with a multivariate analysis based on the fish composition found in each biogeographical unit. We used a non-metric multidimensional scaling (NMDS) to represent the position of fish community in multidimensional space. We selected species with a percentage of occurrence by trawl superior to 5 %. These species are susceptible to have a too high influence on the measure of rank dissimilarity. In order to performed this analysis, we formed a matrix of dissimilarities using the Bray-Curtis dissimilarity calculation. The quality of the representation was validated with a stress under 0,2. We used a sufficient number of iterations until an appreciable stress is reached.

2.6.2 Depth and salinity

Generalized additive models (GAM) were carried out to analyze the effects of depth and salinity on fish metrics. Too few data were available for this method. Consequently, results of GAM were analyzed as preliminary analyses only. This approach was not used to analyze the impacts of GT on ichthyofauna.

This exploratory analysis proceeded in three phases (fig. 8). We developed generalized additive models to explain fish densities according to two natural factors (step I).

Fish density ~ factor (salinity) + factor (depth)

Statistical significance of each factors on fish densities was tested (step II). Then, depending to the significance of the factors, depth and salinity were used as predictors of density through the models. Predictions of fish densities were graphically analyzed in order to detect linear trends (step III). If a linear trend was observed between the fish density and a natural effect (e.g. depth), this environmental effect was integrated as linear factors in our models. In particular cases, the data-set was reduced (i.e. few data were excluded, less than 5 %, close to maximal or minimal values of salinity and depth) in order to get a linear effect for the main range of a factor. If non-linear trends were observed, we attempted to integrate the factor as a class-variable.



Figure 8: General approach used to integrate natural factors (i.e. salinity and depth). This decision tree illustrates the different choices depending on the trends observed between the fish metrics (total fish density and density by functional groups) and the natural factors. In brackets, steps of the procedure.

2.7 Statistical models

The statistical significances of the environmental effects (i.e. ecoregion, depth and salinity) and of the GT effects were evaluated in our different models (see 2.7.1-2.7.3) with an analysis of deviance (level of statistical significance: 5%). The models were validated when the hypothesis of independence and normality of the residuals deviance were fulfilled. R software was used to developed these models.

2.7.1 Delta models for fish density

We observed a large number of zero values for fish metrics based on fish density (fig. 9). According to this "0 inflated" distribution of the fish density by species, we choose to use deltadistribution models (Aitchison and Brown, 1957). This approach is appropriate to analyze fish survey data, highly zero inflated (Stefansson, 1996). The modeling approach of delta-distribution consists to fit two independent models, a model on zeros values and a model on positive values (Aitchison, 1955; Pennington, 1983). These two independent sub-models formed were then coupled in order to provide prediction.



Figure 9: Distribution of the total density of fish (a) and the log-transformed density of fish (b) in spring campaigns.

Binomial models

Binomials models were carried out for the presence of the fish. The link function used for these models was a logistic function (table 4). The area under the curve (ROC) was used as a criterion to evaluate the performance of these models (Manel et al., 2002).

Y (1/0) ~ factors (environment) + factor (ulva*) + ε

Y $_{(1/0)}$ represents the presence or the absence of fish caught (1 or 0). The factor named "ulva*" corresponds to the three GT metrics. The environmental factors (*i.e.* ecoregion, salinity and depth) were accounted for incorporate the "ulva*" effect.

Table 4: Variance and Link F	Family for the binomial me	odel
------------------------------	----------------------------	------

Data	Distribution	Link
Presence-absence	Binomial	$logit(u) = \frac{1}{1 + e^{-u}}$

• Models for positive densities

After preliminaries test, we selected log-transformed densities to analyze positive value. This transformation allowed to improve linearity and homogeneity of variance for these sub-models (fig. 9; annex III).

$$Log(Y_{(>0)}) \sim factors(environment) + factor(ulva*) + \varepsilon$$

Y (>0) is the density of fish (number of individuals per bean trawl haul) when fish were caught.

Table 5: Variance and Link Family for the density positive model

Data	Distribution	Link
Log-transformed positive density	Gaussian	Identity $\eta = g(E(Y_i)) = E(Y_i)$

• Predictions of densities

The two sub-models were coupled in order to estimate fish density (Stefansson, 1996; Le Pape et al., 2003). This prediction (noted \hat{Y}) was obtained by the multiplication of the probability of presence with the positive density. A correction (Laurent, 1963) was calculated on the densities positives models. This translation method allowed to obtain an estimation from a GLM model with log-transformed density.

$$\hat{Y} = Y_{(1/0)} * e^{\ln(Y_{(>0)})} * e^{\frac{\alpha^2(\ln(Y_{(>0)})}{2})}$$

The uncertainty of delta-distribution model is complex, because of the impossibility to realize an analytical combination of the errors of the two models. In order to quantify the uncertainty of parameter estimates, we used a random sampling approach. We predicted the presence of fish with 5 000 binomial models, and log-transformed densities on 5 000 sub-samples generated with the gaussian model on positive values. We calculate the 10 %, 50 % and 90 % quantiles of the 5 000 predictions to take account of the uncertainty on fish metrics estimates according to GT effects.

Density of pelagic species was zero inflated with few and low density. We developed a binomial model for the presence of this guild. All the other fish metrics were modelled using delta distribution for fish density.

2.7.2 Generalized Linear Models for Species Richness

The species richness was modelled with generalized linear model using a Poisson regression (table 6). We assume that the Poisson errors is the most adapted distribution for analyzing count data for fish species in trawl surveys (Courrat et al., 2009).

SR ~ factors (environment) + factor (ulva*) +
$$\varepsilon$$

Table 6: Variance and Link Family for species richness

Data	Distribution	Link
Species richness (SR)	Poisson	log

2.7.3 Linear Models for the two indices of functional diversity

The two independent indices of functional diversity (i.e. functional richness, functional divergence) was modelled with linear models.

Functional indices ~ factor (environment) + factor (EQR) + ε

3 Results

3.1 Fish metrics

We calculated the proportion of each guild on trawl data (fig. 10). The majority of fish caught belonged to the demersal guild (75 %), followed by the benthic guild (25 %). The pelagic guild was under-represented (over 5 %). The resident species were the most caught species (about 80 %), the marine juveniles represented a quarter of the density.



Figure 10: Proportion of fish species found in the different guilds for a haul in Spring: (a) guilds of vertical position; (b) ecological guilds.

3.2 Preliminary analyses

3.2.1 Effects of ecoregion on descriptors of ichthyofauna

There is a clear distinction between the two region based on fish species distribution (fig.11). Ecoregion turned out to be an important factor structuring the density of the benthic guild and the species richness. Ecoregion was introduced as a factor in the different models.



Figure 11: Two-dimensional ordination of the different estuaries sampled from non-metric multidimensional scaling. The two polygons symbolize the two ecoregion (i.e. North and South Brittany). The numbers represent the different species.

3.2.2 Effects of salinity and depth on descriptors of ichthyofauna

For the spring campaigns, the bathymetry (*i.e.* where the beam trawls were carried out) ranged between 0 and 23 meters and salinity between 2,5 and 35,1. The range of salinity, for the autumn campaigns, fluctuated between 5,2 and 35,1 and the depth between 0 and 20 meters.

Density of fish clearly increase for depth superiors to 16 meters (fig. 12), a poorly sampled depth range. In order to obtain linear effect of the depth on fish metrics, we decided to cut back the data (table. 7). The trawl realized over a depth equals to 16 meters were excluded. The loss of information with this cut represent less than 1 % of the survey data.



 Table 7: Description of the environmental data and their utilization in our models.

Environmental factors	Raw data	Transformed data	
Depth	0 -23 meters	0,75 - 16 meters	
Salinity	5,20 - 35,10	5,20 - 35,10	

Preliminary gam was used on the selected data (table. 7) to detect effect of salinity and depth on the metrics describing the fish community (annex II). As an example, salinity appeared to have a linear effect on positive densities and presence-absence of the benthic guild in Spring (fig. 13). Salinity was integrated as continuous variables for the two-sub models (table 8).

For the majority of fish metrics (except for the pelagic species) the salinity was introduced as a continuous variable. For the pelagic fish, the salinity appeared to have a non-linear effect (annex II) and thus two class of salinity were formed, mesohaline (5.20-18) and polyhaline (18-35.10), and integrated in the model describing the variability of pelagic fish (table 8).

The depth was introduced as a continuous variable for the totality of fish metrics (table 8). This environmental factor appeared to have a negative effect on fish density.



Figure 13: Density and probability of presence and positive densities of benthic organism according to the range of salinity and depth using GAM in Spring.

 Table 8: Models and environmental factors selected for the two seasons. The statistical significance of each natural factor is given in brackets. *p<5%; **<1%; ***<0.1%</th>

Metrics of fish community	Models	Spring	Autumn
Density	Pres-abs	Depth (***) + Ecoregion (***)	Depth (***)
	>0	Depth (***) + Ecoregion (**)	Depth (***)
Species richness (S)		Depth (***) + Ecoregion (**)	Ecoregion (***) + Depth (*)
Density of benthic guild	Pres-abs	Ecoregion (***) + Salinity (***)	Ecoregion (***)
	>0	Ecoregion (***) + Salinity (***)	Ecoregion (***)
Density of demersal guild	Pres-abs	None	Depth (***)
	>0	None	Depth (***)
Density of pelagic guild	Pres-abs	Class of salinity (***) + depth (**)	Depth
Density of Resident guild	Pres-abs	Depth (***)	Depth (***)
	>0	Depth (***)	Depth (***)
Density of Marine juvenile guild	Pres-abs	Salinity (***) + Ecoregion (**)	None
	>0	Ecoregion (***) + Salinity (***)	None

3.3 Effect of green tides at estuarine area scale

Ecological quality ratios were introduced in addition to the selected environmental descriptors in the different seasonal models for fish density. None of the 13 estuaries presented a "bad ecological status" according to the score of the EQR (table 2) for the period studied. Low variabilities of EQR were observed during 2008 to 2014, except for the Aber Wrach, the Blavet and the Pont l'Abbé estuaries (fig. 14).



Figure 14: Ecological Quality Ratios from 2008 to 2015

Table 9 summarizes the results of the models carried out with the ecological quality ratios. Significant effects were detected for many fish metrics. Despite this statistical significance of the EQR, models were selected on 2 criteria: the percentage of deviance explained by the model and the need of a monotonous relationship between the fish metric and the EQR (fig. 15). For the second criterion, the different models were simulated for the 4 conditions (*i.e.* poor, moderate, good and high). If an increasing density gradient is observed from poor status to high status, the condition of a monotonous relationship between the fish metric and the EQR is verified and the fish metric is selected.



Figure 15: Selection of the models where statistical significance of the EQR were found, based on two criteria

Following these criteria selection, only 2 models were valid: the one explaining the benthic fish density and the one describing the functional divergence. For the other descriptor, the relation between EQR and fish metrics were unclear, and the deviances explained by the model were low (inferior to 10 %). The deviance explained by the density of benthic fish was the highest.

The density of benthic fish and the functional divergence appeared to be negatively impacted by GT for both season (table 9).

For each ecoregion, the benthic fish density model was applied for bad conditions (high GT, the "poor" value of EQR), and for the best conditions (low GT, the "high" score of EQR). As demonstrated in the preliminary analysis, benthic fish densities are higher in South Brittany and decrease with high salinity within estuarine ecosystem (fig. 16). The density is higher for a "high" ecological status than for the "poor". Furthermore, the quantile for each fitted value do not overlap, the difference of density between the two ecological status is distinguishable.

The functional divergence associated with the "poor" ecological status is lower than those associated with the higher ecological status (fig. 17). The differences between the "poor" and "high" ecological status were well marked.

Table 9: Results of the models carried out: The positive densities and the probability of presence in spring and august according to the ecological quality ratios. Statistical significance (Sign), the direction of the relation between EQR and the fish metrics (Direction) and the percentage of the deviance explained by the model are given. NS, non-significant; NA, non-applicable. *p<5%; **<1%; ***<0.1%.

Metrics of fish community	Models	Sign. (Spring)	Sign. (Autumn)	% deviance	Direction
Density	Pres-abs	*	NS	>10 %	NA
	>0	*	***	>10 %	NA
Species richness (S)		***	***	>10 %	NA
Density of benthic guild	Pres-abs	***	*	32 %	-
	>0	**	***	26 %	-
Density of demersal guild	Pres-abs	***	NS	>10 %	NA
	>0	***	***	>10 %	NA
Density of pelagic guild	Pres-abs	***	***	>10 %	NA
Density of Resident guild	Pres-abs	***	**	>10 %	NA
	>0	***	***	>10 %	NA
Density of Marine juvenile guild	Pres-abs	***	**	>10 %	NA
	>0	**	***	>10 %	NA
Functional richness		NS	NS	>10 %	NA
Functional divergence		**	**	23 %	-



Figure 16: Estimation of benthic species for a virtual estuary located in South Brittany and North Brittany, sampled in spring with a beam trawl



Figure 17: Value of the functional divergence according to the ecological quality ratios

3.4 Effect of green tides at local scale of proliferation

3.4.1 Descriptor of proliferation: surface ratios

Spatial Preliminary analyses was carried out to assess for each haul a ratio of surface covered by green macroalgae (*i.e.* surface ratios). Few beam trawls were realized within the macroalgae mat (fig. 18). In Spring, 54 trawl hauls were realized in green algae mats (8 %) and 71 in August (10 %). The 125 hauls realized within the mats represent a very low part of the 1348 beam trawl hauls conducted (9 %).



Figure 18: Map of the four of the 13 studied estuaries (a: Elorn, b: Aulne, c: Morlaix and d: Blavet). Points symbolized the average position of beam trawl realized within each site. The hatched grid represented the green macroalgae mats.

In addition, the surface ratios corresponding to the 125 beam trawl hauls were very low. The maximal surface ratio where hauls were realized, is 36.2 % for the spring campaigns (table 10; fig. 19).

Table 10: Maximum and mean of the surface ratios where the beam trawls were realized.

Distribution of data	Spring campaign (57 hauls)	Autumn campaign (71 hauls)
Mean of surface ratios	0,3 %	0.4 %
Maximum of surface ratios	36 %	47 %

The effects of surface ratios on the fish metrics for the 21 models were not statistically significant for the spring and the autumn campaigns.



Figure 19: Frequency of beam trawl associated with the percentage of the area covered by mats of *Ulva* spp. (a); frequency of beam trawl realized in the mats of *Ulva* spp.

3.4.2 Descriptor of proliferation: *Ulva* spp. density per trawl haul

Biomass of *Ulva* spp. caught per beam trawl haul were available for the 2013 and 2014 campaigns only. In spring, 8 estuaries presented data of density of green macroalgae (Aber Wrach, Aven, Belon, Blavet, Elorn, Morlaix, Pont l'abbé, Trieux) for a total of 135 hauls. In autumn, 7 estuaries had records of the density of Ulva spp. (Aber Wrach, Belon Blavet, Elorn, Morlaix, Scorff, Trieux) for a total of 73 hauls.

The densities of *Ulva* spp. caught during the spring and autumn campaigns were very low (fig. 20). The mean values of density were equal to 0.0025 kg/m^2 for the spring campaigns and 0.0045 kg/m^2 for the autumn campaigns (table. 11). The average surface trawled was equal to 1.087 m^2 . The WTF campaigns were not realized within the mats formed by green macroalgae.

Distribution of data	Spring campaign (135 hauls)	Autumn campaign (73 hauls)
Mean of biomass	0.0025 kg/m ²	0.0045 kg/m ²
Maximum of biomass	0.05 kg/m ²	0.18 kg/m ²

Table 11: Maximum and mean of the density of *Ulva* spp. per beam trawl haul.



Figure 20: Ulva density (kg/m²) caught per beam trawl in 2013 and 2014 in spring (a) and in autumn (b).

The effects of *Ulva* density on the fish metrics for the 21 models were not statistically significant for the spring and the autumn campaigns.

4 Discussion

4.1 A preliminary description of fish community in estuarine systems

4.1.1 Beam trawl data

The Water Framework Directive campaigns were realized with a beam trawl. This gear is well adapted to sample the benthic and demersal community, but not for the pelagic species (Courrat et al., 2009; Delpech et al., 2010). The use of this benthic gear explains the low proportion of pelagic species. We compared the composition of the communities in the 13 estuaries to the community defined in a meta-analysis that aiming to describe the fish assemblage of 17 European estuaries (Elliott and Dewailly, 1995). In this community, 50% of the community is composed of benthic fish and 25 % each of the demersal and pelagic species (Elliott and Dewailly, 1995). In our data, the proportion of demersal fish is higher (about 70 %) and the number of pelagic fish is very low (about 5 %). This comparison revealed that the fish community is not well represented in our data, and suggested a sampling bias. Nevertheless, GT mainly disturb benthic and demersal species (Le Luherne et al., 2016), thus this sampling bias does not compromise the validity of the approach.

4.1.2 Fish metrics

The 10 fish metrics selected for this study were chosen to assess the ecological functions performed by estuarine systems for fish. We used these fish descriptors because they appeared

complementary, accounting for both specific and functional diversity of fish (Mason et al., 2005; Villeger et al., 2008; Delpech et al., 2010).

4.2 Environmental drivers of fish community

By their structure, estuaries are exposed to strong environmental gradients (*i.e.* salinity, oxygen concentration and temperature) in the water column. These dynamic systems are very stressful for fish. Fish inhabiting estuaries are composed of species adapted to these natural constraints. This adaptation of communities in estuaries was described as "environmental homeostasis" in the "estuarine quality paradox" theory (Elliott and Dewailly, 1995; Dauvin, 2007; Dauvin and Ruellet, 2009). In the light of this theory, it was crucial to take into account environmental parameters. Several environmental parameters were used in this study to describe fish community, before providing an evaluable assessment of GT consequences.

4.2.1 Season

The season was considered as an important natural factor controlling the fish community (Courrat et al., 2009). This control was revealed in our study. For instance, the proportion of marine juvenile was lower during the autumn campaigns than in Spring. This trend revealed a high mortality or migration phenomenon between these two seasons. In fact, it is well known that juvenile suffer of high mortality rates during their settlement (Le Pape and Bonhommeau, 2015).

4.2.2 Ecoregion

A preliminary analysis revealed that the density was higher in South Brittany than in North Brittany, with significant differences in the species composition. This result suggests that estuaries localized in South Brittany present a much sustainable habitat than estuaries located in North Brittany. Indeed, the density is a well know indicator which evaluate the quality of habitat (Delpech et al., 2010). The delimitation of the two ecosystems (i.e. South and North Brittany) was already proposed by Spalding et al (2007). Our result confirmed this biogeographical classification.

4.2.3 Depth and salinity

These two environmental factors had significant statistical effects on the 8 fish metrics, and are important factors that structured the fish community in estuarine areas (Courrat et al., 2009). However, we know that there are many other factors which could influence fish community, as the sediment granulometry, water temperature of the water, or oxygen concentration. The addition of these data, would allowed improving the modelling of factors that control the fish community. However, adding variables could complicate the model. According to the parsimony theory, the addition of variables must lead to a higher explanatory power, if not the variable should be excluding. Furthermore, these kind of descriptors were not available in our data set.

4.3 Impacts of green tides on fish community at different scales

Our study aimed to provide a global appreciation of the impact of green tides on the fish community at different spatial scale, from a global evaluation of GT effects to a local scale assessment of these effects (i.e. from EQR to algal coverage).

4.3.1 Spatio-temporal and spatial scale

Two approach were used to analyze the local effect of GT on fish community: the surface ratios and the biomass of *Ulva* spp. per beam trawl haul.

None of the 10 metrics present a significant response for these two descriptors of GT intensity. These results (i.e. for the density of *Ulva* spp. per beam trawl haul and the surface ratios) were not expected, these indicators of local intensity of GT were expected to have the most significant effect of fish community.

The lack of response of the fish community to green tides can be explained by the low surface covered by *Ulva* spp. on trawling areas. We observed that the trawl hauls were realized beside of the mats of green macroalgae by the mapping of trawl hauls points. The low values of surface ratios associated to the beam trawl hauls explain the non-significances of these factors in our models. Le Luherne et al (2016) revealed the impacts of GT on fish community and observed a threshold density of *Ulva* spp. from which the fish community was significantly impacted. This threshold was equal to 0.3 kg/m². In our data, the maximum of density of ulva recorded was equal to 0.05 kg/m² in spring and 0.18 kg/m² in autumn. The absence of significant effects on fish metrics seems to be coherent according to Le Luherne et al (2016). Indeed, during WFD beam trawl survey, trawl hauls avoid the algal mats and do not appear appropriate to study their impacts.

4.3.2 Some patterns at estuarine global scale

During a green tide event, the main perturbations induced by GT occur at a local scale. These perturbations could be divided into three types: physical, chemical and trophic perturbations, both affecting the fish community within a small area. We supposed that the principal effects of GT were geographically restricted to the mats of *Ulva* spp. More precisely, the effects of GT seem to operate within the mats of *Ulva* spp., so fish species which live outside of the mats would not be affect by GT. During a green tide event, the main perturbations induced by GT occur at a local scale. For example, a decrease in fish foraging efficiency intervenes within the mats of *Ulva* spp. The degradation of the physical structure of the habitat is also a very local effect of GT. We can also take the example of anoxia, these conditions are only associated with sediment located below the mats of *Ulva* spp. (Baden, 1990; Sundbäck et al., 1996).

However, at estuarine scale, the negative effect of green tides was revealed on the benthic fish. Benthic fish are the most sensitive guild to green macroalgae proliferation (Bowen and Valiela, 2001; Bricker et al., 2008; Le Luherne et al., 2016). Indeed, GT impact at first the estuarine floor (*e.g.* change in complexity of the sediment). Benthic fish are therefore the most impacted species due to the degradation of their habitat. The GT could be responsible of a shift from a benthic to a pelagic community (Bowen and Valiela, 2001). Our study also reveals the negative

impact of GT at estuarine scale on fish community using the functional divergence. A threshold was found between the "poor" and the "moderate" ecological status. The decrease of the functional divergence could indicate that the most abundant species had their morphological traits closed to the center of the functional trait space. Patterns of functional divergence indicate that there is a low degree of niche differentiation when the estuaries are significantly affected by GT. The proliferation of opportunistic green macroalgae impacts ecosystem functioning. Mason et al (2005) assigned the decline of functional divergence to a less efficient resource use. Under this assumption, the green tides seem to have a negative impact on benthic fish. This impact was already demonstrated in many studies (Pihl et al., 1995; Österblom et al., 2007; Eriksson et al., 2009).

The analysis at global scale by the use of Ecological Quality Ratios reveals that the impacts of green tides depend on the vertical distribution of fish species. The most impacted guild seems to be the benthic fish according to our study. However, this result should be analyzed with caution.

4.4 Perspective

We assume that the different descriptors of the fish community were sufficiently diversified (i.e. density, density of vertical and ecological guilds and indices of functional diversity) to get a good representation of the community. Similarly, the descriptors of GT procured by the CEVA and the WFD (i.e. EQR, surface ratios and density of algae per beam trawl) were appropriate. These descriptors had the advantages of evaluate the intensity of green tides throughout different spatial and temporal scale. The major difficulty encountered in our study was the sampling protocol. The reason of the incapacity to provide a clear-cut answer on our problematic is caused by the avoidance of the mat of ulva during the WFD campaigns. Fish communities sampled within each estuary do not represent the community in the mats of Ulva spp. As a consequence, the WFD campaign do not appear appropriate for this problematic. GT impacts should be analyzed with a dedicated protocol. A diversity of protocol can be design, like before-after control impact (BACI) with controlled (Le Luherne et al., 2016) and impacted sites or a multi-scale sampling of impacted site (Quillien et al., 2015). The crucial characteristic of the sampling protocol is to sampled the fish community within the mat of ulva. Specific survey integrating mats in their spatial design should been developed, but the clogging of trawl by green algae during GT is a main problem to design appropriate sampling protocol (Le Luherne et al., 2016).

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6 Annex

6.1 Annex I – Measures of the 19 morphological traits

measures	traits	codes
1	standard length	SL
2	Body Height	BdD
3	Height of the caudal fin	CD
4	Caudal peduncle height	CpD
5	Fork length	Cs
6	Length of the caudal fin	CL
7	caudal peduncle length	CPL
8	Length of pectoral fin	PL
9	Length of the pelvic fin	PelL
10	Height of the head (eyes)	HD
11	Eye	HeD
12	Head length	HL
13	Eye	EH
14	Width of eyes	EW
15	Body height to the pectoral fin	BdpD
16	Body height (by pec fin)	BdDp
17	Position of mouth	Mp
18	Number of barbs	Bar
19	Surface of the caudal fin	Sef

components	functional traits	measures	interpretations
Habitat	Eye position	Hed/HD	Position in the water column and prey position
Habitat	Position in the water column	factor	Position in the water column
swim	Restrictor peduncle	CD/Cpd	Endurance and acceleration
swim	relative length of the caudal	CL/SL	propulsion capability
swim	Aspect of the caudal fin	Cd ² /Cs	Endurance and maneuverability
swim	Relative height of the body	BdD/SL	Maneuverability
swim	Position of the pectoral fin	BdpD/BdDp	Maneuverability and stability
swim	relative length of pectoral fin	PL/SL	Maneuverability and stability
Detection	Size of the eyes	EL-EW/HL	visual acuity
Detection	Number of barbs	Bar	Foraging in sediment
Capture	Position of mouth	Mp	Food intake
Capture	Head size	HL/HS	Maximum fishing capacity
Capture	maximum size	Lmax	Maximum fishing capacity
trophic category	Diet	factor	trophic guild

6.2 Annex II – Results of the GAM for Autumn and Spring

6.2.1 Autumn





• Total fish density

• Species richness



• Density of benthic fish





• Density of demersal fish



démersaux_(log) ~s(profondeur)+s(salinité)



38



• Density of marine juvenile



Guilde démersale ~s(profondeur)+s(salinité)



Guilde juvénile ~s(profondeur)+s(salinité)

Guilde juvénile ~s(profondeur)+s(salinité)



• Density of resident fish



Guilde résident ~s(profondeur)+s(salinité)

Guilde résident ~s(profondeur)+s(salinité)



• Presence of pelagic fish



6.2.2 Spring

Total density of fish •



5 4 3 Densité 2 35 30 25 20 5811118 5 Profondeur 15 10 15

Presence_dens ~s(profondeur)+s(salinite)

• Species richness



Richesse spécifique ~s(profondeur)+s(salinité)

Richesse spécifique ~s(profondeur)+s(salinité)

• Density of benthic fish





• Density of demersal fish





• Density of marine juvenile





• Density of resident fish





• Presence of pelagic fish



Présence pélagique ~ s(salinité) + s(profondeur)

6.3 Annex III – Validation of the models for positive densities

6.3.1 Autumn

• Total fish density



• Species richness





• Density of demersal fish



• Density of marine juvenile



• Density of resident fish



6.3.2 Spring

• Total density of fish



• Species richness



Obs. number

• Density of benthic fish



• Density of demersal fish



• Density of marine juvenile



Obs. number

• Density of resident fish



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Titre français : Impacts des marées vertes sur l'ichtyofaune des secteurs estuariens

Titre anglais : Impacts of green tides on ichthyofauna in estuarine ecosystems

Résumé : Les écosystèmes côtiers et estuariens font partie des plus productifs au monde. Ces systèmes remplissent diverses fonctions écosystémiques essentielles au maintien et au renouvellement des ressources halieutiques (*i.e.* nourriceries, étapes migratoires, lieux de reproduction et d'alimentation). L'accumulation d'activités anthropiques dans ces régions entrainent diverses perturbations parmi lesquelles des proliférations de macroalgues vertes (marées vertes). En Bretagne, les marées vertes s'intensifient et soulèvent des inquiétudes concernant les potentiels impacts écologique sur l'ichtyofaune. Cette étude vise à fournir un diagnostic quantitatif sur les impacts des marées vertes en intégrant plusieurs échelles spatiales. Des modèles statistiques ont été développés afin d'analyser la réponse de métriques décrivant l'abondance, la diversité spécifique et fonctionnelle de l'ichtyofaune aux proliférations de macroalgues vertes. Les réponses de la communauté ichtyologique face aux marées vertes dépendent notamment de la distribution verticale des espèces. Les espèces benthiques semblent être les plus affectées par cette perturbation.

Abstract: Coastal and estuarine systems are essential areas for the fish community. These systems provide unique ecological services for fisheries resources (*i.e.* nursery, food area, reproductive area and migration corridor). The growing human activity in these regions leads to numerous perturbations among which proliferations of opportunistic green macroalgae, commonly known as 'green tides' During the last decade, the number of impacted sites and the intensity of green tides has increased on the Brittany coasts. If economic costs are known (*i.e.* tourism, risk for the human health), only few studies were performed to assess the consequences of green tides on fish community. This study aims to provide a quantitative evaluation of the potential impacts of green tides on fish community in Brittany. These potential impacts were evaluated through several spatial scales. The analysis at a global scale reveals that the impacts of green tides depend on the vertical distribution of fish species. The most impacted guild seems to be the benthic fish according to our study.

Key Words: Fish community - diversity - green tides - Ulva spp. - estuarine systems - Brittany