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Factors determining the timing of swarming of European flat oyster (*Ostrea edulis L.*) larvae in the Dutch Delta area: Implications for flat oyster restoration

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ABSTRACT

Flat oyster (*Ostrea edulis L.*) beds were once a dominant habitat type in the Dutch Delta and North Sea, but overharvesting resulted in soft sediment habitats devoid of oysters. Natural recovery of oyster beds will be slow if the natural substrate is lost and therefore, many oyster restoration projects have been set up worldwide. One way to enhance the success rate of restoring flat oyster beds is adding substrate at the moment that larvae are ready to settle. For this, more insight into the drivers of the timing of larval release is needed, which was the aim of this study. Generalized Additive Models (GAMs) were created based on historical data form the Oosterschelde and Lake Grevelingen of the abundance of swarming flat oyster larvae. It was shown that the temperature explains major part of the variation of larval occurrence. The addition of the temperature sum gave best results in the Oosterschelde. It was shown that the first peak in number of oyster larvae was predicted at a temperature sum of 576 degreedays. In Lake Grevelingen daily temperature yielded higher deviance explained values. Furthermore, the lunar cycle also contributed to the timing of larval release in the Oosterschelde, but not in the Lake Grevelingen, most likely since tides are absent in this waterbody. Chlorophyll-a partly explained larval occurrence in Lake Grevelingen, suggesting food abundance is another driving factor in the timing of gametogenetic processes of flat oysters. Furthermore, day-in-year and mean temperature also contribute significantly to the timing of larval swarming in both water bodies. When validated, this information can be used to predict the optimal time window of deployment of substrate for spat settlement in order to increase the success rate of oyster bed restoration.

1. Introduction

Shellfish reefs were once a dominant ecological structure in estuaries around the globe, but currently it is estimated that 85% of the historical oyster reefs are lost (Beck et al., 2011). Moreover, the remaining native oyster reefs are often in a poor condition, especially the oyster reefs in Europe (Beck et al., 2011). Therefore, flat oysters (*Ostrea edulis* Linnaeus, 1758) and flat oyster beds are listed as 'threatened' in the OSPAR convention (OSPAR commission, 2008). Oyster beds are considered as an ecologically important habitat, as oysters are ecosystem engineers and these beds provide multiple ecosystem services, such as water filtration, shoreline protection, provision of habitat and food for many other species and thereby enhancing biodiversity and fishery conditions (Beck et al., 2011; Coen et al., 2007).

In the North Sea and Dutch Delta, extensive beds of *O. edulis* have existed, which was then among the most commercially important marine resource of this area (Olsen, 1883; Houziaux et al., 2011; Sawusdee et al., 2015; Smaal et al., 2015). The widespread and intensive harvesting of oysters is considered the main cause for the decline of oyster beds (Korringa, 1946; Houziaux et al., 2011). As early as in the 1860s richly colonized biogenic oyster beds were destroyed by fishing activities such as bottom trawling, resulting in the soft sediment habitats without oysters present today (Houziaux et al., 2011).

The natural geographic range of *O. edulis* extends from the western Mediterranean and the Black Sea, along the coast of western Europe and the British Isles until 65 degrees north in Norway (Bromley et al.,

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2016a), where it is generally found in estuarine areas on sheltered, hard substrate (OSPAR, 2008). *O. edulis* is a larviparous and protandrous hermaphroditic species, so it first reproduces as male and thereafter alternates between the sexes (Ó Foighil and Taylor, 2000). In the months June to August gamete release (i.e. the spawning act) takes place and sperm, organized in spermatozeugmata (Foighil, 1989), is released into the water column and drawn into the female mantle cavity. Here the fertilization takes place and the young larvae are brooded for 6 to 10 days (Korringa, 1940; Ó Foighil and Taylor, 2000). Subsequently, the oyster larvae, now 170–180 µm in size, will be released into the water column, which is known as the swarming act (Korringa, 1940). Then, during the pelagic stage, the larvae grow until they reach a size of approximately 300 µm, taking 7 to 10 days depending especially on the water temperature (Korringa, 1940). Finally, the oyster larva, now called pediveliger, extends its foot, searches for suitable hard substratum to settle and cementation occurs; this process is called spatfall (Laing, 1995).

Existing oyster beds have a positive feedback on the success of oyster settlement and subsequent recruitment (Kennedy and Roberts, 2006), which implies that natural recovery of oyster beds will be slow if the natural substrate is lost (Smaal et al., 2015). Therefore, artificial oyster beds have been created worldwide to restore different oyster species and to meet conservation goals (La Peyre et al., 2014; Sawusdee et al., 2015). According to the European Marine Strategy Framework Directive, the degraded North Sea ecosystem must be returned to a 'Good Environmental Status' (Directive 2008/56/EC). Consequently, several *O. edulis* restoration feasibility studies have been carried out, for instance for South Wales (Woolmer et al., 2011), for the German Bight (Gercken and Schmidt, 2014) and for the Dutch part of the North Sea (Smaal et al., 2015; Kamermans et al., 2018). In addition, several pilot projects were started in Europe (see https://www.platteoester.nl/; https://noraeurope.eu/; Pogoda, 2019, Pogoda et al., 2019). However, at global scale, several oyster restoration projects failed to create rich biogenic oyster beds, for instance because of overrating the reproductive power of the flat oyster or difficulties of oyster translocations (Korringa, 1946; Bromley et al., 2016b). Hence, it is important to build a scientific basis for oyster restoration (La Peyre et al., 2014). Furthermore, since conservation projects aim at increasing the cover of the sea floor with oyster beds through creating self-sustaining beds, more research is needed to identify the critical success factors of oyster bed expansion.

Availability of suitable settlement substrate is considered one of the principal factors governing recruitment success of oyster populations (Korringa, 1946: Smyth et al., 2018). Therefore, one of the ways to expand an oyster bed is to provide settling substrate for oyster spat. To facilitate spat settlement, hard substrate must be provided at the moment that the larvae are ready to settle (Korringa, 1940). Although bacterial biofilms enhance settlement success (Tamburri et al., 2008; Rodriguez-Perez et al., 2019) extensive fouling and sedimentation must be prevented since these hamper settlement (Korringa, 1940; Kamermans et al., 2004). Therefore, the window of opportunity of optimal substrate conditions for spatfall is short, and it would be advantageous if the timing of the swarming of the larvae could be adequately predicted. Korringa (1940) stated that between 15 and 20% of the flat oyster population spawns synchronous, so there must be one or more controlling factor(s). Several studies aimed to gain insight in the environmental factors determining the timing of the different reproduction processes of the flat oyster. Korringa (1947) collected an impressive dataset on the daily variation in larval abundance in the Oosterschelde between the end of May and August to aid oyster farmers in deployment of empty mussel shells as spat collectors for *O. edulis*. He showed that during the reproductive season multiple peaks in larval abundance occurred, of which the first peak was usually the highest. Based on this dataset, Korringa (1947) hypothesized that the lunar cycle through its influence on the tide is an important factor determining the multiple peak in spawning and larval swarming, of which the first peak mostly occurs between June 26 and July 10, irrespective of actual water temperature. In later research he investigated the relation between the timing of swarming and the water temperature and showed that temperature requirements differ between distinct flat oyster populations (Korringa, 1957). Furthermore, temperature was suggested by other studies as a factor that controls gametogenesis of flat oysters (Cano et al., 1997; Joyce et al., 2013). Besides temperature, it is suggested that food conditions are important for the development of flat oyster larvae (Robert et al., 2017) and also for flat oyster gonad growth and larval timing (Ruiz et al., 1992). Although different factors have been suggested in literature, these have not yet been studied in combination with each other to determine which factors contribute significantly to the timing of the swarming of flat oyster larvae in order to build a predictive model. Previous research suggested that temperature sum is a better parameter than daily temperature and more appropriate for studying bivalve reproduction (Broell et al., 2017). The temperature sum (also known as growing degree days, heat units or thermal time) can be described as the accumulated temperature, when higher than a threshold temperature, over a period of time (McMaster and Wilhem, 1997; de Jong and van der Have, 2007).

The dispersal mechanisms of larval marine invertebrates are influenced by a variety of abiotic factors, including temperature, salinity, hydrodynamics, bathymetry, tidal phase, biotic factors such as food availability and larval release site and behavioural factors (swimming speed and depth preference; Shanks, 1995). This study tested which environmental factors contributed significantly to the timing of flat oyster larvae swarming. The factors that were examined as explanatory variables in present study were temperature sum, mean year temperature, daily temperature, day-in-year, tidal difference, lunar cycle and chlorophyll-a content. These factors were chosen based on suggestions from literature (e.g. Korringa, 1947; Ruiz et al., 1992; Mann, 1979; Joyce et al., 2013) and the availability of data. Other factors that potentially influence larval release (salinity – Rao, 1951; wind – van Woesik, 2009; insolation - van Woesik et al., 2006), were considered not to differ substantially during the season that larvae are released. Generalized additive modelling was used to analyse the influence of these environmental factors on the larvae density in the water, separately or in combination.

2. Materials and methods

2.1. Site descriptions

2.1.1. Oosterschelde

The Oosterschelde (Fig. 1) is a sea arm located in in the southwest of the Netherlands. This waterbody used to be an estuary of the river Scheldt with an open connection to the North Sea. In 1896 this estuary was disconnected from the Scheldt and in 1986 a half-open dam was placed near the outlet to the North Sea. The bottom configuration of the Oosterschelde is irregular with creeks, channels and intertidal areas. The average salinity is around 30‰, and the tidal range varies between 2.5 and 4 m, resulting in strong tidal currents and intensive mixing (Smaal et al., 2009). Furthermore, it has been calculated by Korringa (1940) that in this period, only 3.7% of the water in the basin was renewed each tidal cycle, which is favourable for flat oyster reproduction (Korringa, 1940). Up to 1963 the eastern basin in the Oosterschelde, especially the Yerseke bank, was an important production area for flat oyster farming. After a severe winter (1963) and the introduction of the parasite *Bonamia ostrae* in 1980 (Engelsma et al., 2010) the flat oyster population in the Oosterschelde became functionally extinct (Beck et al., 2011). Korringa (1947) estimated that in the period 1936 till 1946 on average 21,8 million mother flat oysters lived in the Oosterschelde, but with high annual variability (10 till 30 million mother oysters).

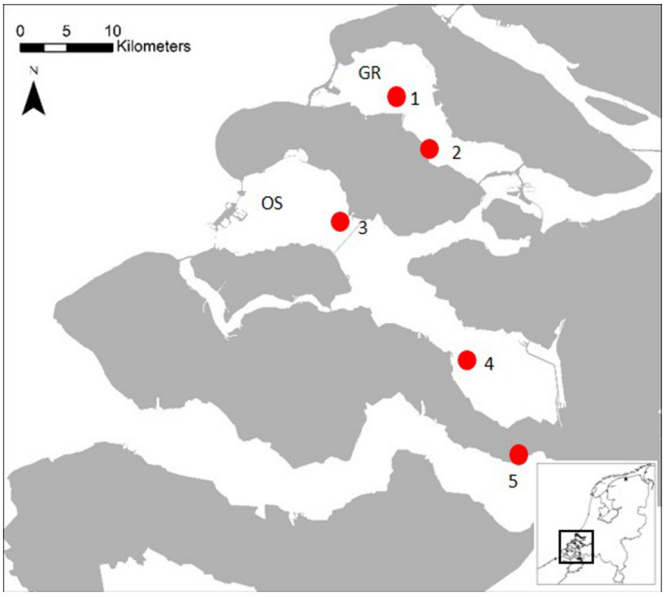

Fig. 1. Locations of sampling stations in Zeeland, a province located south-west in The Netherlands. OS is Oosterschelde, GR is Lake Grevelingen, 1 is Bommenede, 2 is Dreischor, 3 is Zierikzee, 4 is Yerseke Bank, 5 is Bath.

2.1.2. Lake Grevelingen

Lake Grevelingen (Fig. 1) is a relative shallow, enclosed basin with a salinity of 29–32‰ and a mean depth of 5.1 m. The construction of the Grevelingendam (in 1965) at landward side and the Brouwersdam (in 1971) at seaward side, led to freshening of the resulting lake, which resulted in water quality problems. Limited seawater exchange became possible in 1978 by constructing a sluice on the seaward side after which it became saline. Yet, given the lack of strong currents and a limited tidal range of 0.2 m (Rijkswaterstaat, 2013), seasonal stratification and bottom hypoxia in the deeper parts of the lake still exist (Hagens et al., 2015; Seitaj et al., 2017). In the early seventies of the last century flat oysters were discovered (H.W. Waardenburg, pers. comm.), which formed the basis for oyster farming in Lake Grevelingen. This lake is now well-known for the commercial production of flat oysters, cultured in combination with Pacific oysters using bottom cultures at 2 to 8 m depth (Engelsma et al., 2010). Flat oyster landings fluctuate and are presently around 5 to 7.5 million oysters per year (www.agrimatie.nl). Dijkema and Bol (1984), Kamermans et al. (2004) and van den Brink et al. (2013) monitored larval abundance in Lake Grevelingen for the timing of spat collector deployment for *O. edulis*.

2.2. Creating a dataset

A dataset was created by assembling the available historical data of *O. edulis* larvae counts in the Oosterschelde and the Lake Grevelingen, including 33 years of larvae counts. These numbers of flat oyster larvae in the water column were collected by sampling in the months May until August, as within this period flat oysters spawn and release their larvae in the Dutch Delta. Although this dataset is composed of data from different sources, the method of taking the larvae samples remained the same: every time 100 l of water was collected (from one location, or in case of a mixed sample from 5 different locations: 20 l each) using a plankton net with a mesh size of 100 µm, and the number of *O. edulis* larvae in a subsample was counted by microscope. Korringa (1947) took his samples 60 cm above the bottom (daily, except on Sundays and during stormy weather, in the summer period between 1935 and 1946), Dijkema and Bol (1984) took their samples at 1.5 m depth and the other samples were taken at the surface (weekly in the summer period in several years between 1979 and 2011). Simultaneous measurements at the surface and near the bottom indicate that the larvae distribution is uniform throughout the water column (Korringa, 1940; Dijkema and Bol, 1984; Kamermans et al., 2004). For the larvae counts collected by Korringa (1947) and Dijkema and Bol (1984) no raw data was available, so the program GetData Graph Digitizer version 2.26.0.20 was used to digitalize their graphs.

Table 1
Information about the sources or method used for collecting specific data included in the dataset of the present study.

Parameter	OS	GR	Location	Source
Daily water temperature	x	x	OS: Bath	Rijkswaterstaat
			GR: Bommenede	Rijkswaterstaat
Temperature sum	x	x	*Calculated from daily water temperature*	
Mean water temperature[a]	x	x	*Calculated from daily water temperature*	
Day-in-year	x	x	*Calendar data (1st of January = 0)*	
Day in lunar cycle	x	x	*Calendar data (full or new moon = 0)*	
Tidal range	x		Zierikzee	Rijkswaterstaat
Chlorophyll-a[b]		x	Dreischor	Rijkswaterstaat

[a] Mean value of the water temperatures at 08:00 h of all days between the 1st of April and the 31st of August.
[b] Interpolation was used to overcome missing values. Source 'Rijkswaterstaat' refers to http://waterinfo.rws.nl. OS = Oosterschelde and GR = Lake Grevelingen.

For every larvae sample data point (n = 971) the corresponding date, week number, day-in-year number (1 January = day 0), location, depth of sampling and number of days after full or new moon were noted (full or new moon = day 0) (Table 1). Furthermore, environmental data (chlorophyll-a content, tidal range and daily surface water temperature) was acquired from the databases of Rijkswaterstaat (RWS), a Dutch governmental body responsible for the management of the infrastructure in the Netherlands (http://waterinfo.rws.nl). Tidal data was only collected for the Oosterschelde, since the Lake Grevelingen lacks significant tides. Chlorophyll-a content data was only available for the Lake Grevelingen dataset, and data was interpolated because RWS measured these parameters only once every two to four weeks.

For all data points, the surface water temperature was collected daily from the nearest RWS measuring station (Fig. 1). The temperature at 08:00 h was used as the temperature of that day. For the Oosterschelde dataset no station was available with daily temperature measurements, but the temperatures of Bath, located in the Western Scheldt and closest location at that time, showed no bias when validated with the limited available temperatures of the Yerseke bank (taken from: Korringa, 1947). Based on the water temperature of the sampling day itself the temperature sum was calculated using this formula:

$$\text{Temperature Sum} = \sum_{1st\ of\ January}^{end\ \ date} (T_i - T_{th}) * \Delta d$$

The temperature sum is in degreedays (°C*d), T_i is the water temperature (°C) at day i, T_{th} is a predetermined threshold temperature for physiological activity and Δd is a set time step in days (Mann, 1979). For *O. edulis* the threshold temperature for gonad development is determined at approximately 6.75–7 °C (Joyce et al., 2013; Mann, 1979). In the present study T_{th} = 7 °C and Δd = 1 day were used to calculate the temperature sum for every data point, only 1945 was excluded because of missing temperature measurements. Negative day values were set at 0, i.e. if the water temperature for instance dropped to 6 °C, this day contributed 0 degreedays to the temperature sum instead of −1 degreedays. Furthermore, for each year separately, a general seasonal mean water temperature value was calculated for both water bodies. This was done by taking the average temperature value of all daily water temperatures from the 1st of April till 31st of August, since this is the season with temperatures above 7 °C affecting gonad development of flat oysters. For the creation of the models, the incomplete

sample points were excluded, which resulted in $n = 891$.

2.3. Data analysis

R studio, version 3.6.1, and R version 1.2.5001. were used to model the relation between flat oyster larvae densities (number of larvae per 100 l) and environmental variables over the period that larval counts were available (from end of May until August), independently for both areas. Available data were explored following the protocol by Zuur et al. (2010). The presence of outliers, multicollinearity, and relations between larval density and environmental and temporal variables was assessed using boxplots, Cleveland dotplots, pairplots, Pearson correlation coefficients, variance inflation factors, and multipanel scatterplots from the lattice package (Sarkar, 2008). For each area, an initial model was created and then compared to alternative models following the methodology described in Coolen et al. (2018). Given that the relations between larval density and the environmental variables were assumed to be non-linear, the models were created using the gam function from the mgcv package (Wood, 2011) and these terms were included as smoothers. Such generalized additive models (GAMs) are an extension of the widely used generalized linear models (GLMs) and this regression tool is able to handle non-linear relations (Guisan et al., 2002). The use of GAMs in ecological modelling can increase our understanding of ecological systems since these models are data-driven and thereby adequately represent underlying data (Guisan et al., 2002).

The Oosterschelde initial model (model 1) included temperature sum, day number in the lunar cycle and daily temperature:

$$\ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + \varepsilon_i$$

Where LD is the larval density for sample i and term $f()$ marks a smoothed function. The residuals ε_i were assumed to be normally distributed with a mean of 0 and variance of σ.

Alternative models were created to evaluate effect of the following:

- Model 2: Exchanging temperature sum for day-in-year number (0 = 1 January);
- Model 3: Exchanging day number in lunar cycle for tidal range;
- Model 4: Removing temperature sum from the model without replacement;
- Model 5: Removing day number in lunar cycle from the model without replacement;
- Model 6: Adding mean temperature of the sampling year;
- Model 7: Removing daily temperature from the model without replacement.

This resulted in the formulation of seven models with different combinations of variables (see Table 3 for all model formulas).

In addition to temperature sum, day in the lunar cycle, daily- and mean temperature, for Lake Grevelingen also chlorophyll-a concentrations were included in the model. Since Lake Grevelingen data originated from various sources and three different water depths, data source and sampling depths were included as random effects to allow predictions of generalized larval densities regardless of depth and source.

The initial model for Lake Grevelingen (model 8) took the following form:

$$\ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + f(\text{chlorophyll}-a_i) + \varepsilon_i$$

The residuals ε_i were assumed to be normally distributed with a mean of 0 and variance of σ.

Alternative models were created to evaluate effect of the following:

- Model 9: Exchanging temperature sum for day-in-year number;
- Model 10: Removing temperature sum from the model without replacement;
- Model 11: Removing chlorophyll-a from the model without replacement;
- Model 12: Removing day number in lunar cycle from the model without replacement;
- Model 13: Removing daily temperature from the model without replacement;
- Model 14: Adding mean temperature of the sampling year.

All variables were included as smoother, except for mean temperature as it was found during model evaluation that this resulted in overfitting and therefore mean temperature was included in the model as non-smoothed linear effect.

All models were initially created using the Poisson distribution with log link and compared using the Akaike Information Criterion (AIC; Akaike, 1973). The model with lowest AIC was assumed to have the best fit. As the model residuals showed to be overdispersed, models were then compared again using the quasi-Poisson distribution with log link. Significance of variables and deviance explained by the models, as presented in Table 3, are based on these quasi-Poisson models. Both selected models were validated to assess if underlying assumptions of homogeneity of variance and normality of the residuals were met. For these models, residuals were plotted against all variables in and outside the model as well as fitted values to assess model fit.

3. Results

3.1. High variability of timing larval peak within years

In the twelve years of larvae sampling in the Oosterschelde, the peak in number of larvae was on average 749 ± 415 flat oyster larvae per 100 l seawater, with a maximum of 1734 in 1939 (Table 2 and S1). The peak was observed between day-in-year 177–198 and between a 493–661 degreedays. For Lake Grevelingen, more variation was observed in the timing of the peak and the maximum number of larvae (Table 2 and S2-S4). On average 1235 ± 1325 larvae were observed as maximum number of *O. edulis* larvae per sample. Moreover, the peak was observed between day-in-year 165–247. So there is a high variation between the years, e.g. in 1979 the first peak was observed at a temperature sum of 313 degreedays, and in 1991, 1992 and 2011 the larvae peak occurred at an accumulated temperature above 1100 degreedays. The variability could partly be assigned to the different areas: the first peaks in the Oosterschelde all occurred at a lower temperature sum and in a smaller timeframe than in Lake Grevelingen.

The water temperatures in Lake Grevelingen are on average higher than in the Oosterschelde, which results in higher temperature sum values at the end of the season in this waterbody (Fig. 2). Yet, at the start of the reproductive season the temperature sum values of Lake Grevelingen and the Oosterschelde are still quite similar. Furthermore, the standard deviation of the temperature sum values in Lake Grevelingen is larger than the Oosterschelde, suggesting a higher annual variation in water temperature.

3.2. Generalized additive modelling to describe larval densities

The evaluation of the available models resulted in the selection of the Oosterschelde model that included temperature sum (tempsum), the number of days in the lunar cycle, the daily water temperature and mean water temperature (model 6). Except daily temperature ($p = .056$), all variables had a significant effect ($p < .001$) on larval density. The deviance explained by the model was 28% with an adjusted r^2 of 0.168. Model validation showed no unwanted patterns. A

Table 2
All years included in the dataset, described with number of samples, maximum number of larvae present in 100 l of seawater, the day-in-year and temperature sum of the sample containing the highest number of larvae of that season and the data source and location of sampling.

Year	Area	n	Max. # larvae	Peak: day-in-year	Peak: tempsum	Data source larvae counts	Location sampling
1935	OS	18	442	193	628	Korringa, 1947	Yerseke bank
1936	OS	38	209	180	498	Korringa, 1947	Yerseke bank
1937	OS	65	578	186	671	Korringa, 1947	Yerseke bank
1938	OS	65	566	190	561	Korringa, 1947	Yerseke bank
1939	OS	76	1734	177	503	Korringa, 1947	Yerseke bank
1940	OS	51	826	183	623	Korringa, 1947	Yerseke bank
1941	OS	66	451	186	493	Korringa, 1947	Yerseke bank
1942	OS	63	410	189	569	Korringa, 1947	Yerseke bank
1943	OS	59	598	179	561	Korringa, 1947	Yerseke bank
1944	OS	63	1068	186	535	Korringa, 1947	Yerseke bank
1945	OS	58	808	198	unknown	Korringa, 1947	Yerseke bank
1946	OS	61	1303	177	661	Korringa, 1947	Yerseke bank
1979	GR	20	224	165	314	Dijkema and Bol, 1984	Mixed sample, 5 GR oyster plots
1980	GR	27	66	210	740	Dijkema and Bol, 1984	Mixed sample, 5 GR oyster plots
1981	GR	31	202	191	633	Dijkema and Bol, 1984	Mixed sample, 5 GR oyster plots
1982	GR	32	308	214	920	Dijkema and Bol, 1984	Mixed sample, 5 GR oyster plots
1983	GR	25	802	193	584	Dijkema and Bol, 1984	Mixed sample, 5 GR oyster plots
1987	GR	21	2903	203	unknown	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
1988	*GR*	*5*	*3026*	*181**	*586**	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
1989	GR	12	5285	192	777	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
1990	GR	8	1527	182	711	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
1991	GR	13	715	247	1340	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
1992	GR	20	550	232	1344	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
1994	*GR*	*5*	*342*	*181**	*579**	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
1995	*GR*	*2*	*1057*	*177**	*527**	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
1999	*GR*	*2*	*278*	*180**	*706**	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
2001	GR	6	121	190	720	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
2002	GR	11	2054	210	1019	Kamermans et al., 2004	Veermansplaat, Hompelvoet & Vlieger
2003	GR	11	3260	202	1039	Kamermans et al., 2004	Veermansplaat, Hompelvoet & Vlieger
2004	GR	9	1452	210	1009	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
2005	GR	11	737	215	1118	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
2006	GR	6	371	206	1026	Unpublished data RIVO/IMARES	Mixed sample, different GR oyster plots
2011	GR	11	650	222	1231	van den Brink et al., 2013	Stampersplaat

* indicates that these numbers are not reliable since it is hard to define an accurate peak when $n \leq 5$.

full overview of all evaluated models is presented in (Table 3).

Removal of the tempsum from the model lowered the deviance explained by 10.8%. Furthermore, replacing tempsum for day-in-year lowered the deviance explained by 3.2% and resulted in an increase of ΔAIC by 3444, indicating that tempsum is a better variable to explain larval occurrence. The addition of days in lunar cycle or tidal difference improved the model with 9.6% and 9% respectively, showing that tides (directly or indirectly by the moon) influence flat oyster larval occurrence as well. Daily water temperature is significant in the models 1, 2, 3, and 4, but not in models 5 and 6. Yet, the removal of this variable resulted in a lowered deviance explained of 4,2%. Lastly, adding the mean water temperature increased the deviance explained with 4% and resulted in the model with lowest AIC-value.

For Lake Grevelingen, model 14 was found to have the best explanatory value, with a deviance explained of 63.2% and adjusted r^2 of 0.54. This model included the variables tempsum ($p = .06$), lunar cycle ($p = .44$), daily water temperature ($p = .11$), chlorophyll-a content ($p < .001$) and mean water temperature ($p < .001$). The addition of the variable chlorophyll-a content resulted in an increase of deviance explained of 9.1% and this variable was significant in all models, indicating its importance. On the contrary, the addition of lunar cycle did not result in significant *p*-values. Moreover, the addition of tempsum only yielded a significant p-value in model 13. Removal of daily temperature and mean temperature lowered the deviance explained with 6.1% and 0.7% respectively.

Predictions, based on model 6 describing the Oosterschelde, of number of larvae over an increasing temperature sum (Fig. 3B) show that the first and highest peak in numbers of larvae can be expected at a temperature sum of 576 degreedays and a second but smaller peak is shown at 1039 degreedays. On day 2, which corresponds with springtide in the Netherlands, relatively low concentrations of larvae are predicted. In contrast, on day 12, which is a few days after neap tide, relatively high concentrations of larvae are predicted. This is shown in more detail in Fig. 3D, from day 4 until day 12 it is predicted that larvae counts increase, with a peak around day 12. Fig. 3F shows that the predicted number of larvae is increasing after the water temperatures reached 17 °C with two small peaks at 18.7 °C and 20.3 °C. Furthermore, higher mean water temperatures of spring and summer result in higher predicted numbers of larvae (Fig. 3H). The addition of this variable does optimize the model and showed highest deviance explained, making it the best model to describe larval densities in the Oosterschelde. However, since the mean temperature can only be calculated at the end of the year, it cannot be used for making predictions of the present season. If this is the target of using a model to predict larval release, model 1 would be most useful.

The figures depicting the GAMs describing Lake Grevelingen shsow different shapes. Fig. 3A, based on model 14, shows an increasing curve until 903 degreedays and thereafter a slow decrease in predicted number of larvae. Furthermore, this model reveals a minor influence of the lunar cycle, since the two lines almost overlap. This can be explained by Fig. 3C, where the number of larvae against the different days in the lunar cycle result in an almost horizontal line. Fig. 3E shows that the predicted number of larvae increases till 18.8 °C, subsequently slightly decreases and thereafter increases again. Moreover, higher mean water temperatures of spring and summer result in higher predicted numbers of larvae (Fig. 3G), similar to the pattern in the Oosterschelde. Lastly, the predicted number of larvae against the chlorophyll-a content shows a negative trend. However, the chlorophyll-a data were interpolated, which limits the accuracy. Therefore, this graph must be interpreted with care.

Fig. 4 shows the predicted number of larvae on the different day numbers in the reproductive season. In both areas, the addition of day-

Table 3
GAM results of 14 different models describing OS (Oosterschelde) and GR (Lake Grevelingen). In model 8–14 depth and data source were added as random factors.

Model	Model formula	Significance	Area	n	ΔAIC	Deviance explained
1	$ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + \varepsilon_i$	Tempsum: *** Lunar cycle: *** Daily temp: **	OS	625	4814.64	24%
2	$\ln(LD) = \alpha + f(Day\text{-}in\text{-}year_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + \varepsilon_i$	Day-in-year: *** Lunar cycle: *** Daily temp: **	OS	625	8257.64	20.8%
3	$ln(LD) = \alpha + f(Temperature\ sum_i) + f(Tidal\ range_i) + f(Daily\ temperature_i) + \varepsilon_i$	Tempsum: *** Tidal range: *** Daily temp: *	OS	625	5656.51	23.4%
4	$ln(LD) = \alpha + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + \varepsilon_i$	Lunar cycle: *** Daily temp: *	OS	625	16,964.92	13.2%
5	$\ln(LD) = \alpha + f(Temperature\ sum_i) + f(Daily\ temperature_i) + \varepsilon_i$	Tempsum: *** Daily temp: .052	OS	625	16,030.98	14.4%
6	$\ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + Mean\ temperature_i + \varepsilon_i$	Tempsum: *** Lunar cycle: *** Daily temp: .056 Mean temp: ***	OS	625	0	28%
7	$ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + \varepsilon_i$	Tempsum: *** Lunar cycle: ***	OS	625	9669.71	19.8%
8	$\ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + f(\text{chlorophyll-a}_i) + \varepsilon_i$	Tempsum: .18 Lunar cycle: .31 Daily temp: ** Chl-a: ***	GR	266	14,915.03	62.5%
9	$ln(LD) = \alpha + f(Day\text{-}in\text{-}year_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + f(\text{chlorophyll-a}_i) + \varepsilon_i$	Day-in-year: ** Lunar cycle: .64 Daily temp: ** Chl-a: ***	GR	266	13,578.82	61.7%
10	$ln(LD) = \alpha + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + f(\text{chlorophyll-a}_i) + \varepsilon_i$	Lunar cycle: .12 Chl-a: *** Daily temp: ***	GR	266	17,706.71	61.5%
11	$ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + \varepsilon_i$	Tempsum: .10 Lunar cycle: .27 Daily temp: *	GR	266	30,176.35	53.4%
12	$ln(LD) = \alpha + f(Temperature\ sum_i) + f(Daily\ temperature_i) + f(\text{chlorophyll-a}_i) + \varepsilon_i$	Tempsum: .07 Chl-a: *** Daily temp: **	GR	266	18,717.00	60.3%
13	$ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + f(\text{chlorophyll-a}_i) + \varepsilon_i$	Temp sum: ** Lunar cycle: .81 Chl-a: ***	GR	266	22,539.07	56.4%
14	$ln(LD) = \alpha + f(Temperature\ sum_i) + f(Days\ in\ lunar\ cycle_i) + f(Daily\ temperature_i) + Mean\ temperature_i + f(\text{chlorophyll-a}_i) + \varepsilon_i$	Temp sum: .06 Lunar cycle: .44 Daily temp: .11 Mean temp: *** Chl-a: ***	GR	266	0	63.2%

'***' indicates significance with a p-value < .001, '**' indicates p-value < .01 and '*' indicates p-value < .05, if the *p* value is > .05, the exact value is given.

in-year resulted in significant *p*-values ($p < .01$). In the Oosterschelde, based on model 2, the peak is predicted at day number 181 and in the Grevelingen, based on model 9, this peak is predicted at day number 193.

4. Discussion

The present study confirmed that the water temperature (i.e. temperature sum, mean- and daily temperature), lunar cycle or tidal range, day-in-year number and chlorophyll-a content can influence the timing of flat oyster larval swarming. It is depending on the waterbody which environmental factors explain the occurrence of *O. edulis* larvae best.

The temperature sum could explain most of the variation in the occurrence of larvae in the Oosterschelde, and the GAM predicted a peak in larval occurrence at an accumulated temperature of 576 degreedays. Furthermore, in this sea arm, the lunar cycle, daily temperature and mean temperature explained part of the variation in the swarming of oyster larvae. Korringa (1947, 1957) already suggested that lunar cycle and temperature influence the swarming of oyster larvae and this could be confirmed for this waterbody. On the other hand, tempsum could explain a relatively minor part of the variation in Lake Grevelingen and the addition of the factor lunar cycle did not result in significant *p*-values. Yet, chlorophyll-a, day-in-year, daily water temperature and mean temperature explained part of the variation in larval occurrence in Lake Grevelingen. So, temperature, described by three different variables in this study, influences flat oyster larval occurrence. Although all variables express a different aspect of temperature, there is also some overlap and therefore, models in which a combination of these temperature factors are included have to be interpreted with care.

The reason for different results between Oosterschelde and Lake Grevelingen is probably a mix of different biological and physical explanations. Firstly, these waterbodies are not similar: the Oosterschelde is a sea arm and Lake Grevelingen is an enclosed saltwater lake and therefore lacks tides. Furthermore, it is known that food abundance in addition to temperature has a substantial effect on larval development rates in molluscs (e.g. Filgueira et al., 2015). This could indicate that the lower tempsum value of the Oosterschelde can be explained by a higher chlorophyll-a content. However, since there was no data available on food abundance in the Oosterschelde, it was not possible to test this statistically. Moreover, since the data originates from two different time periods it was impossible to compare the same weather conditions on the two distinct waterbodies.

Previous research into the temperature sum of the flat oyster

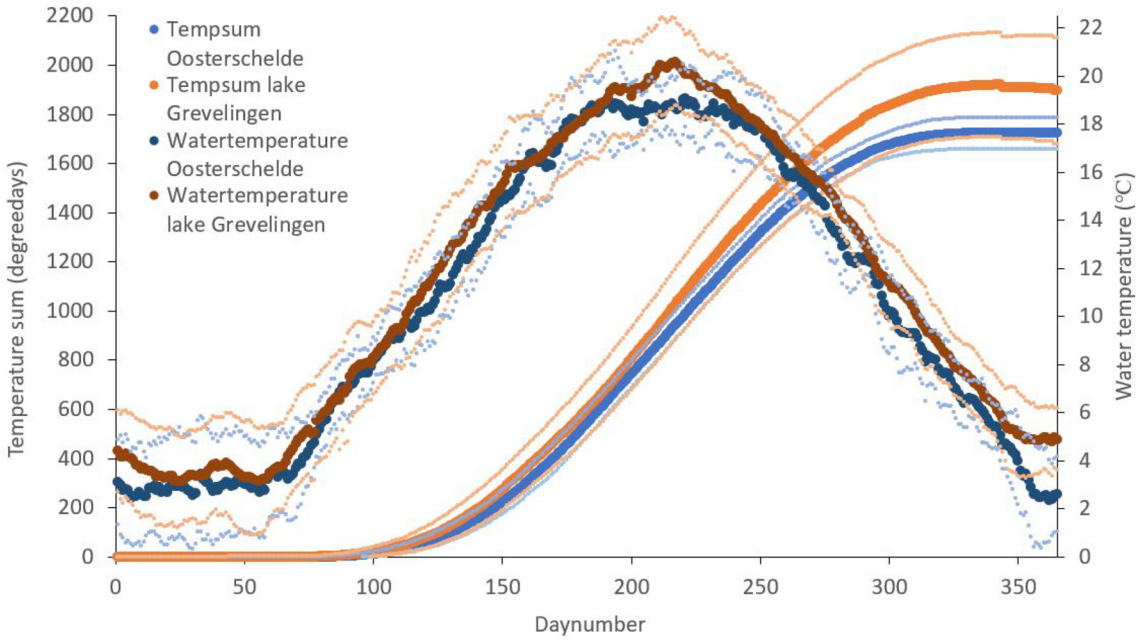

Fig. 2. Annual development of the temperature sum (solid lines) and water temperature (dotted lines) of de Oosterschelde (1935–1946, blue lines and dots) and Lake Grevelingen (21 years in the period 1979–2011, orange lines and dots). Small and light-coloured dots represent the standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

spawning event resulted in degreeday values of 404 and 554 for a lab and a field study respectively (Mann, 1979; Wilson and Simons, 1985). To be comparable with this study, which describes larval release instead of spawning, a value of about 155 degreedays must be added since this is the approximate time between the spawning and swarming event (Korringa, 1940; Ó Foighil and Taylor, 2000), converted into degreedays. This yields comparable results with the degreeday value in the Oosterschelde. On the other hand, in Lake Grevelingen a value of 903 degreedays was found. Although this variable was not significant, it can indicate that there is not one general value that is able to describe the right moment in all systems accurately.

Yet, a fact that complicates the use of the temperature sum is determining the start of the reproductive season. In general, the accumulation of the temperature starts when the temperature passes the threshold temperature, which is often after a winter period. This study included the data from the 1st of January onwards. However, previous research suggests that the temperatures prior to a dormant winter period can be important as well (Joyce et al., 2013). Oysters can pass part of the gametogenic cycle before resting during winter, which gives them a head-start at the moment the threshold temperature is reached, resulting in a lower temperature sum. Furthermore, genetic differences between different flat oyster populations exist (Vera et al., 2016) and oysters from different geographic locations may differ in the temperature they need for gonad development (Korringa, 1957; Ruiz et al., 1992). In the past 200 years, the Netherlands imported flat oysters from at least 10 different source populations (Bromley et al., 2016b).

The larvae counts in the Oosterschelde revealed two peaks in number of larvae in the water column of which the first peak showed higher numbers of larvae than the second peak. This phenomenon could be explained by the decreasing number of oysters functioning as female oysters at the end of the reproductive season (Joyce et al., 2013). Joyce et al. (2013) relate this to the depletion of metabolic reserves throughout the reproduction: the production of oocytes is costlier than the production of spermatozoa.

A property of this dataset is that specific day numbers and calculated temperature sums were assigned to the days that larval abundance was sampled. But since oyster larval occurrence is highly irregular, as shown in the daily samples of Korringa (1947), it is likely that some important larvae peaks have been missed. This is especially the case for the Lake Grevelingen dataset, where samples were only taken weekly. Furthermore, sample data was collected from at least five different sources and different locations. It is important to consider the bias that may have been caused by the different locations, a location on top of an oyster culture plot (e.g. Yerseke Bank in the Oosterschelde) directly notes an increase of larvae after swarming, while other locations further away will probably encounter the larvae several days later. Furthermore, since the created dataset dates back to the 1930's not all environmental factors were available for both water bodies, resulting in a bias when comparing the influence of the different factors between these two areas. A next important step should be the validation of the models. Preliminary tests with datasets obtained from the Dutch North Sea show that the larval peak could be roughly predicted in 2 out of 3 years in a nearshore area and that larvae were present at 2 off-shore locations in the period that the peak abundance was predicted (Didderen et al., 2018, 2019a, 2019b). However, tests with more detailed datasets (e.g. from France) are needed.

The present study showed that including chlorophyll-a as environmental factor improved the Lake Grevelingen model, indicating that food abundance is an additional driving factor in larval release. This is in accordance with previous research, which concluded that well-fed flat oysters spawn earlier in the season (Ruiz et al., 1992). Moreover, Ruiz et al. (1992) concluded that the spawning of flat oysters coincided with the phytoplankton bloom, to assure recovery. For *Crassostrea virginica*, it is even suggested that chlorophyll-a content influences the adopted reproduction strategy of the oysters: opportunistic (continuous and asynchronous) when the food is irregular and conservative (seasonal and synchronous) in case of regular food supply (Aranda et al., 2014). However, the chlorophyll-a content values used in this study can only be seen as an indication of the real chlorophyll-a content, since daily measurements are lacking and interpolation was used to estimate missing values. Still, significant results were obtained, demonstrating the possible driving force of food abundance on the timing of reproduction of flat oysters.

For many fish and some bivalves it is known that photoperiod can be a driver of gametogenetic processes (Joyce et al., 2013), this is for instance known for the Pacific oyster (Fabioux et al., 2005). However,

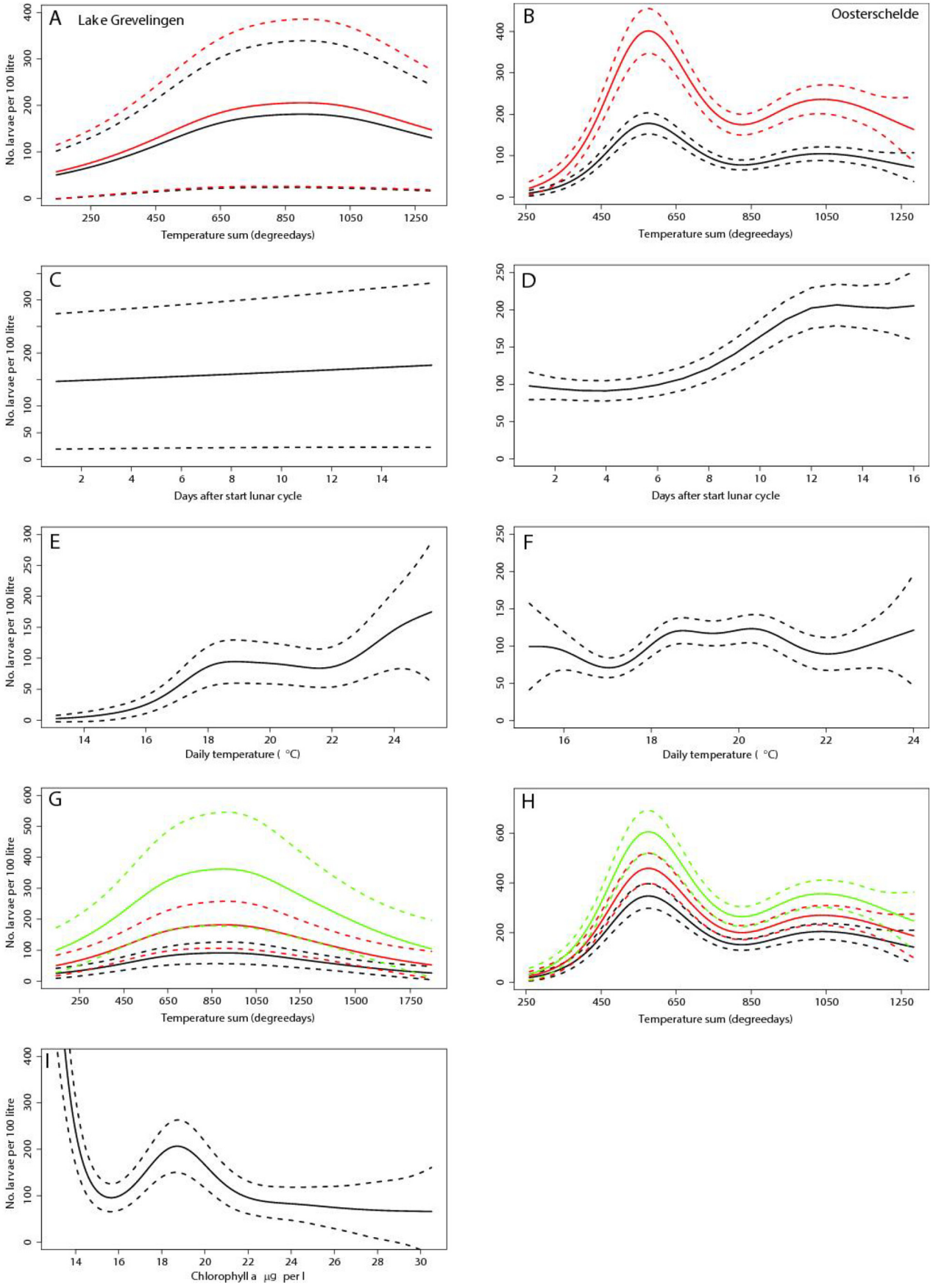

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Fig. 3. Prediction of number of larvae per 100 l in Lake Grevelingen (left) and Oosterschelde (right), based on GAMs 14 and 6 respectively. (A and B) prediction of number of larvae on the 2nd (black) and 12th day (red) after the start of the lunar cycle, (C and D) show the influence of the lunar cycle at the peak temperature sum on larval concentration, (E and F) show the influence of daily temperature on larval concentration, (G and H) show the influence of the mean temperature (black = 15 °C, red = 16 °C and green = 17 °C) on larval concentration and (I) shows the influence of chlorophyll-a content (in µg/l) on larval concentration. The dotted lines indicate the standard error of the prediction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

previous research by Korringa (1946) and Joyce et al. (2013) showed that this is not true for *O. edulis*. The present study showed that the lunar cycle did influence the timing of swarming of flat oyster larvae in the Oosterschelde. The fact that the addition of the lunar cycle to the model of the Lake Grevelingen (which lacks tides) did not yield better results, suggests that tidal difference is a possible mechanism behind this observation. Yet, tidal difference explained 0.6% less than lunar cycle. This small difference can be due to the fact that tidal differences are also affected by wind speeds and directions, making them less consistent than day number in lunar cycle.

In the Oosterschelde, the larval swarming event occurs mostly around 9 to 12 days after the start of the lunar cycle, corresponding to the period of neap tide plus a few days. Presuming that the brooding of the fertilized eggs takes 8 days (Korringa, 1940; Ó Foighil and Taylor, 2000), the spawning event of releasing the spermatozeugmata to the water column must have occurred around spring tide. A possible explanation for this timing may be that high current speeds experienced during spring tide favour dispersal of spermatozeugmata and lower the chance of fertilization of close-by relatives, while low current speeds experienced during neap tide, favour retention of larvae and keep the larvae longer near the oyster bed where the conditions for oysters are favourable for settlement, survival and growth. It is known for more marine species that current speeds affect the timing of the release of gametes or larvae. For instance, many species of crab release their larvae at the high current speeds experienced during spring tide, probably to facilitate avoidance of predators (Morgan and Christy, 1995; Stevens, 2003). Furthermore, previous research showed that Pacific oysters also release their gametes at the early phase of the water current peak (Bernard et al. 2016). On the contrary, rock-boring date mussels, *Lithophaga lithophaga,* (Žuljević et al. 2018) and *Calyptogena* spp. deep sea clams (Fujikura et al. 2007), release gametes when the current speeds are low and the water is calm. It is suggested that this is related to the coordination of the reproduction by chemical cues (Fujikura et al. 2007; Žuljević et al. 2018).

5. Conclusions

With the formation of this large historical dataset and the creation of 14 different models, insight into the timing of reproduction of *O. edulis* is obtained. This study showed that the temperature sum, mean water temperature of the reproductive season, daily water temperature, chlorophyll-a content, day-in-year number, lunar cycle and tidal difference influence the larval occurrence, depending on the oyster population and local environmental conditions. Although validation of the models is still needed, in particular with respect to food abundance, a rough prediction of the first major larval peak is possible by using the accumulated temperature of 576 degreedays for an area similar to the Oosterschelde. For Lake Grevelingen chlorophyll-a, day-in-year and daily water temperature appeared to be better predictors than the temperature sum. Furthermore, in areas with tidal influence, it is predicted that this larval peak coincides with the end of the lunar cycle. The deviation between this predicted day and the observed day of the larval peak could be assigned to the influence of the food availability, but due to a limited chlorophyll-a dataset no simple prediction rules can yet be given for this specific factor.

Korringa (1946) already showed that the chances of flat oyster larval survival are small, i.e. under the favourable conditions of the Oosterschelde about 5% of the larvae reach metamorphosis at water temperatures of 20 °C. The deployment of clean shells (cultch) at the time that most flat oyster larvae are present will improve settlement success and consequently contribute to the success rate of restoration projects in the North Sea area, which aim to recover the once abundant oyster beds.

Contributors

M.A.M.M. carried out the data analyses and wrote the manuscript as part of her MSc thesis, J.W.P.C. guided the data analyses and edited the manuscript, T.M.v.d.H. generated the idea for the degreeday analysis, provided background information and edited the manuscript, P.K. generated the idea for the analysis of environmental parameters and timing of swarming of oyster larvae and edited the manuscript.

The authors declare that they have no conflict of interest.

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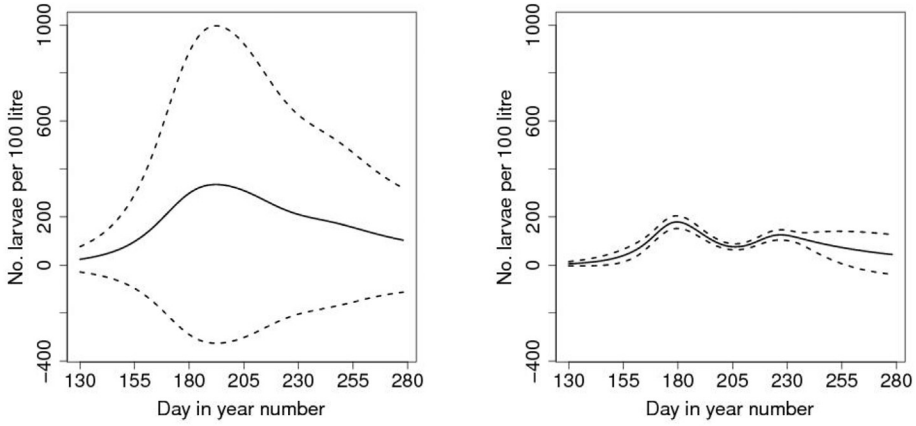

Fig. 4. Prediction of number of larvae per 100 l in Lake Grevelingen (left) and Oosterschelde (right), based on GAMs 9 and 2 respectively. The dotted lines indicate the standard error of the prediction.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seares.2019.101828.

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