A complete life cycle perspective on habitat requirements that control species distribution; a case study of *Psammechinus miliaris* in the Eastern Scheldt, Grevelingen and North Sea

Name	Kees te Velde
Student nr.	930428862090
Course nr.	YBI-85812
Supervisors	Mischa Streekstra and Tinka Murk
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Research Group	Marine Animal Ecology, Wageningen University and Research

Abstract

The distribution and abundance of an animal is largely dependent on its habitat requirements and these requirements often change throughout its life-cycle. So to know the true impact of (a)biotic factors on a species distribution and abundance, the whole life cycle should be considered. This study aims to illustrate a fast and relatively simple approach to gain knowledge on the importance of certain habitat requirements in controlling species abundance and distribution, by comparing habitat requirements of a species' life stages with the (a)biotic values found in nature. This is illustrated through a detailed case study of the sea urchin species, *Psammechinus miliaris*. Nine biotic and abiotic factors were studied, namely diet, food quantity, hydrodynamics, oxygen, hydrostatic pressure, salinity, bottom type / substrate, pH and temperature. For each of these abiotic and biotic factors, the minimum, maximum and average values were determined in the Grevelingen, North Sea and Eastern Scheldt through a literature study. The minimum and maximum habitat requirements for every abiotic and biotic factor were then determined through a literature study for each of the six *P. miliaris* life stages: gametes, embryo, larvae, metamorphosis, juveniles and adults. The literature search was extended by comparing data of *P. miliaris* observations and water temperatures in the Eastern Scheldt and Grevelingen Lake to explore the relationship between the yearly differences in water temperature and *P. miliaris* abundance over a 22 year period. The results show that hydrodynamics, temperature and bottom type are important limiting factors for *P. miliaris*, whilst hydrostatic pressure and salinity are of little to no importance. This study shows that there is a lack of research towards hydrodynamics and bottom type and it provides evidence that seawater temperatures below 1 °C negatively affect *P. miliaris* populations. This case study illustrates a relatively easy way to get a broad understanding of the habitat requirements of an organism, to identify knowledge gaps in the research area and to prioritise further research efforts.

Introduction

Many livelihoods depend on the abundance and distribution of specific animal and plant species, both directly and indirectly. Directly as food or a source of income. Indirectly, through ecosystem engineers like mangrove trees, that provide habitats to many important fish species and prevent erosion and sediment loss of coastal areas, thereby preventing land-loss and reducing the chance of floods (1). Because of this importance, the collapse of a species population can have huge economic- and societal consequences. To be able to anticipate and prevent collapses, or increase the abundance of a desired species, we need to understand what controls species abundance and distribution.

Species distribution and abundance depend largely on the habitat requirements of an animal. These requirements are the drivers of species abundance and distribution. For clarity, in this study “habitat requirements” is defined as: “(A)biotic features of the environment that are necessary for the persistence of individuals or populations.” (2). The range of an abiotic or biotic factor in which an organism is able to survive. (ie. “Corals require salinities between 30 and 40 parts per thousand”). All habitat requirements combined determine the boundaries of an organism's habitat. And these requirements often change throughout the life-cycle of an animal.

Abiotic and biotic factors have always been dynamic in nature, across different timescales. Temperature being one of the most obvious examples of how dynamic these factors can be. It can vary over timescales of 100.000s of years in the form of glacials and interglacials, to the smaller Milankovitch cycles of 1000s of years, to the timescales of around 10 years between El Niños and El Niñas, to monthly seasonal differences all the way down to differences in the day-night cycle (3). Although sometimes less obvious than temperature, many more (a)biotic factors have also shown great variation throughout history, including salinity, wind, pH and solar radiation (4,5).

These changes and fluctuations in (a)biotic factors can have great consequences for an animal's abundance and distribution. In recent

years and the years to come, many of these (a)biotic factors change faster than ever due to human impact. If burning of fossil fuels persists, global warming will exceed a 2C increase above pre-industrial average surface temperature (6) and pH of surface waters is predicted to reduce by 0.3-0.5 units by the year 2100 through ocean acidification (7). Environmental pollution can lead to big changes in coastal nutrient concentrations through discharges from wastewater treatment plants, rivers and coastal run-off (8). Natural habitats are disturbed by human activities like bottom trawling, that change the structure and composition of the seabed (9). To be able to make predictions on how the abundance and distribution of a species will change and protect the people that dependent on them, it is important to identify which (a)biotic factors have an important role in controlling abundance and distribution.

Because habitat requirements are complex and time consuming to research, most researchers focus on only a small part of an organisms life cycle. Embryo and larvae are generally considered the most vulnerable life stages to altered environmental conditions (10). However, many organisms and especially marine invertebrates, undergo several ontogenetic and ecological transitions throughout their life cycle. Hence, each stage can differ in sensitivity to environmental stressors (11). Also, when experiments are focussed on a single life stage, processes like acclimation, selection and carryover between successive life stages are ignored. So to know the true impact of (a)biotic factors on a species distribution and abundance, the whole life cycle should be considered.

The main aim of this project is to illustrate a fast and relatively simple approach to gain knowledge on the importance of certain habitat requirements in controlling species abundance and distribution, by comparing habitat requirements of a species' life stages with the (a)biotic values found in nature. This is illustrated through a detailed case study of the sea urchin species, *Psammechinus miliaris*.

I then display the kind of conclusions and knowledge gaps that can be discovered, when this approach is used. Also, I aim to identify the strong- and weak points of this approach.

The case study

Sea urchins are free spawning organisms that send their gametes freely into the water column. They have a free swimming planktonic larval stage and a benthic adult life stage. In this case study, 6 important life cycle stages are separated: gametes, embryo, larvae, metamorphosis, juveniles and adults.

The green sea urchin, *Psammechinus miliaris* is a common sea urchin in the coastal areas of the Netherlands. Removal experiments by Hughes et al. (2006) have shown that *P. miliaris*' grazing activity has a major impact on the structure and distribution of benthic marine organisms ((12) as cited by (13)). Therefore it is likely to be a keystone species in the coastal areas of the Netherlands. In the past years, due to overfishing and depletion of sea urchin stocks, interest in the aquaculture of *P. miliaris* has grown (14–16). *P. miliaris* is one of the more palatable sea urchins and its reproductive stages are relatively easy to acquire and keep in the laboratory (13,17). *P. miliaris* has also been reported to clean undesirable fouling organisms on mussel rope cultures (18). Therefore, *P. miliaris* has great potential for aquaculture. And due to the well-established reproductive and larval rearing methodology it is a good model organism for experimental studies (19).

P. miliaris observations has varied greatly over the years (20,21). This makes it an interesting organism for studying the (a)biotic factors and habitat requirements that are responsible for these fluctuations. To be able to make predictions for both (sea-based) aquaculture and the management and preservation of North Sea ecology, it is important to understand which habitat requirements are most likely to affect *P. miliaris* abundance and distribution. Therefore the following **main research question** was formulated: which *P. miliaris* habitat requirements are most likely to be limiting factors in the Eastern Scheldt, Grevelingen and the North sea? I hypothesise that temperature, substrate/bottom type, predation and food availability play the biggest roles in determining the distribution and abundance of *P. miliaris* in the Eastern Scheldt, Grevelingen and North Sea. To be able to answer the main research

question, the following two **subquestions** first require to be answered: first, what are *P. miliaris*' habitat requirements for each of its life stages? And second, what are the relevant abiotic factors (or habitat characteristics) in the Eastern Scheldt, Grevelingen and North sea?

The **aims** of the case study are to identify *P. miliaris*' (a)biotic requirements for diet, food quantity, hydrodynamics, oxygen, pH, hydrostatic pressure, salinity, substrate and temperature. These requirements will be related to the (a)biotic conditions reported for the Eastern Scheldt, Grevelingen and North Sea. This is done in order to evaluate which habitat requirements are most likely limiting factors for *P. miliaris* and thus influencing its abundance and distribution in the study area. These results may explain fluctuations in *P. miliaris* observations in the Grevelingen and Eastern Scheldt. Knowledge gaps in habitat requirements of the complete *P. miliaris* and other sea urchin life cycles will also become clear.

Methods

Stepwise description of the approach

Step 1: Problem definition, two options

- Start with a natural system with an important ecology and then pick a specific species that is important to the ecological functioning of the system, or;
- Start with an important species and then select a study area based on the distribution of the species and the societal and economic importance of the species in the area.

Step 2: Determine which (a)biotic factors should be studied based on the importance of these factors in conspecifics.

Step 3: Attain a profound understanding of the species' life cycle and determine all important life cycle stages.

Step 4: Determine the minimum, maximum and average values of the selected (a)biotic factors in the study area. This can be done through literature studies, if the information is available, and otherwise they can be determined through measurements.

Step 5: Determine the minimum, maximum and optimum habitat requirements of each of the

species' life stages, for all selected (a)biotic factors. This can be done through literature studies, if the information is available, and otherwise they can be determined through laboratory experiments.

Step 6: Compare the minima and maxima of habitat requirements with minima and maxima of the (a)biotic factors in the study area to determine the importance of (a)biotic factors in controlling abundance and distribution of the species:

- a. If minimum habitat requirements are all much lower than minimum (a)biotic factors and if maximum habitat requirements are all much higher than maximum (a)biotic factors the conclusion is: The specific factor is likely not a limiting factor for species abundance and distribution
- b. If minimum habitat requirements are all much higher than minimum (a)biotic factors or if maximum habitat requirements are all much lower than maximum (a)biotic factors the conclusion is: The specific factor is likely to be a limiting factor for species abundance and distribution
- c. If the minima and maxima of habitat requirements are close to the minima and maxima values of the study area then the current importance of the factor is debateable, but may be vulnerable to change.

Step 7: Knowledge gap analysis is carried out to explore the available data on conspecifics and how that relates to the conclusions

Methods of the case study

Step 1: Firstly, *Psammechinus miliaris* was selected because a) it has well characterised habitat requirements, b) it has potential for aquaculture, c) it can have profound effects on ecosystem structure and d) *P. miliaris* abundances have been shown to fluctuate over the years. Then the study area of the Eastern Scheldt, Grevelingen and North Sea (Figure 1) was selected as they are a) protected Natura 2000 areas, b) important to fisheries, shellfish farming and recreation, c) important feeding grounds to many protected bird species, and d) *P. miliaris* is one of the most common sea urchin

species in the area. Also, observational data

Figure 1; Study area (87).

from stichting ANEMOON and temperature data from Rijkswaterstaat were available to explore the relationship between the yearly differences in water temperature and *P. miliaris* abundance in the Eastern Scheldt and Grevelingen Lake.

Step 2: Nine biotic and abiotic factors were studied, namely diet, food quantity, hydrodynamics, oxygen, hydrostatic pressure, salinity, bottom type / substrate, pH and temperature. As these have all -at least to some extend- been considered important sea urchin habitat requirements (18,22–27).

Step 3: After studying the *P. miliaris* life cycle in literature, six distinct life cycle stages: gametes, embryo, larvae, metamorphosis, juveniles and adults were determined.

Step 4: For each of the (a)biotic factors, the minimum, maximum and average values were determined in the Grevelingen, North Sea and Eastern Scheldt through a literature study, using the search engines Scopus and the Wageningen University Library Global search. Google Scholar was used for snowballing and to look up primary references. The search was done in the period December 2016 - March 2017. Only papers in English and Dutch were included. The search words used were the names of the study areas; "Grevelingen", "North Sea" and "Eastern Scheldt" in combination with keywords for all of the habitat requirements that can be found in table 1.

Step 5: To determine the habitat requirements of each of the life stages, the same search engines and criteria were used as in step 3. Only now, the key word "*Psammechinus miliaris*" was used and gave about 200 search results. The articles that contained information on habitat requirements were selected for further reading. Snowballing was however the biggest

Table 1. Key words used for search result analysis for the main habitat requirements and the main sea urchin life stages in combination with the search terms: ("Sea urchin" OR "Sea urchins".) and "*Psammechinus miliaris*"

Habitat requirements	Life stages
"pH"	("Gamete" OR "Gametes" OR "Sperm" OR "Eggs OR "Egg")
"Temperature"	("Embryo" OR "Embryos")
"Hydrostatic pressure"	("Larvae" OR "Larva" OR "Larval")
"Salinity"	("Metamorphosis" OR "Settlement" OR "Recruitment")
("Hydrodynamics" OR "hydrodynamic" OR "flow speed")	("Juvenile" OR "Juveniles")
("Diet" OR "Diets")	("Adult" OR "Adult")
("Bottom type" OR "Bottom material" OR "Hard substrates" OR "Soft substrates" OR "hard substrate" OR "Soft substrate" OR "Bottom habitat")	
("Oxygen" OR "Hypoxic")	
"Food quantity"	

source of valuable articles on habitat requirements. Mendeley was used to manage references. The habitat requirement found in experimental studies were given priority, where minima and maxima are the first values at which 100% mortality occurs. The optimum values are the values at which growth and survival conditions are the highest. If no experimental data was found, the most extreme values at which *P. miliaris* had been observed in nature, were considered as the minimum and maximum habitat requirements. Observational data was mostly on adults.

Furthermore, I extended the literature search by comparing data of *P. miliaris* observations and water temperatures in the Eastern Scheldt and Grevelingen Lake to explore the relationship between the yearly differences in water temperature and *P. miliaris* abundance over a 22 year period.

Data on *P. miliaris* observations in the Eastern Scheldt and Grevelingen since 1994 was obtained from Stichting ANEMOON, a non-profit organisation that collects observations from Dutch recreational divers (28). To correct for the amount of observations and the differences in periodical observation intensity, the average encounter rate was calculated per year. Encounter rate is the percentage of all observations from one year that encountered at least one individual of *P. miliaris* during a dive. Water temperature data was collected by Rijkswaterstaat (Dutch government) from two measuring buoys in the Eastern Scheldt and one measuring buoy in the Grevelingen. These buoys measure water temperature every 10

minutes, and all data from 1994 up to 2016 was used (29). The average water temperature was then calculated per day. Organisms can generally survive short periods of extreme temperatures, but survival decreases when the occurrence of extreme temperatures increases. Therefore, the amount of days per winter that had an average temperature below a certain threshold, was determined, referred to as "cold days per winter". For instance the amount of days in the months November 2001 – February 2002 with an average water temperature below 1 °C. When data point were missing, values were corrected for the amount of days for which data was available, by calculation the percentage of cold days per winter (amount of days below 1 °C was divided by the amount of days that were available for that specific winter). Then, a Pearson correlation test was carried out between the percentage cold days per winter and the *P. miliaris* encounter rate.

Step 6: The minimum and maximum value of a reported habitat requirement were compared to minima and maxima of the (a)biotic factors in the study area to determine the importance of (a)biotic factors in controlling abundance and distribution of *P. miliaris*.

Step 7: Research focus comparison was carried out to explore the available data on conspecifics and how that relates to my conclusions. The Scopus search engine was used, on 21-01-2017. Selection criteria were: Published: 1970 – Present, Document type: Article or review, Searched in: Title, abstract and keywords. Table 1 shows all the key words that were used in the search.

Results

The *Psammechinus miliaris* life cycle

Sea urchins are free-spawning organisms that send their gametes freely into the water column. They have a free swimming planktonic larval stage and a benthic adult life stage. Six important life cycle stages are separated: Gametes, embryo, larvae, metamorphosis, juveniles and adults (Figure 3).

Figure 3; The *Psammechinus miliaris* life cycle

Spawning is likely to be stimulated by photoperiod and not temperature (30). As the days lengthen, *P. miliaris* starts spawning. Spawning period differs between locations but is generally somewhere between May and October (18). A female *P. miliaris* produces between 80.000 and 2.500.000 eggs in a single spawning event (31). *P. miliaris* only spawns during a single spawning period, there is no evidence of any secondary spawning events (17). The **Embryo stage** starts when the *P. miliaris* egg is fertilized, after about 48h when the stomach forms and the **planktonic larval stage** is initiated (26). Larvae are competent for settlement as soon as 20-21 days (26). When a suitable surface is found to settle on, the larvae starts its metamorphosis. In the laboratory, a surface covered with a mixture of algae and bacteria can induce settlement (32). When settled, the **metamorphosis** stage is initiated. After 5-7 days, the digestive tract is formed and the **juvenile** stage has begun (33). Figure 2 shows the complete metamorphosis of a

Lytechinus pictus sea urchin, from larvae to juvenile (32). Development is very similar in all echinoids. Individuals are considered **adult** as soon as they are sexually reproductive. *P. miliaris* individuals have been found to be sexually mature in its first year, ((34,35) as cited by (18)). Growth of adults and juveniles is maximal in early spring/summer months and growth rates slow over winter (36)(37). Most of

Figure 2; Development from fertilized egg to juvenile at different time intervals of the echinoid *Lytechinus pictus*. Adapted from Hinegardner (1969) (12).

the adult growth is completed at 3 years (38) and individuals may reach an age of more than 10 years (39).

Habitat and distribution

P. miliaris occurs in a diverse range of habitats (18) and is common throughout the North Sea (40), Grevelingen and Eastern Scheldt (21). It is also found around the British Isles, as far north as Scandinavia and as far south as Morocco but not in the Mediterranean ((41) as cited by (13)). In these areas, densities vary greatly with habitat type (13), with high densities mostly found in shallow or littoral locations (18). Even so, some *P. miliaris* populations can be found up to depths of 100 meters (41), while others are exposed to air at low spring tides (42)

Search result comparison:

The least researched habitat requirements for both sea urchins (Figure 4A) and *P. miliaris* (Figure 4B) are hydrostatic pressure, salinity,

hydrodynamics, bottom type and food quantity. For sea urchins the most researched are pH and temperature. This can be explained by the widely held assumption that calcifying organisms like echinoids are more vulnerable to ocean acidification than non-calcifying organisms (43). For *P. miliaris* diet is the most researched habitat requirement, mostly for aquaculture purposes. For both sea urchins and *P. miliaris*, the least researched life stages are the juvenile, metamorphosis and adult stage (Figure 4C,D). The early life stages probably gain much attention since sea urchins have been widely used as model organisms for embryo development (42).

Almost none of the researched habitat requirements had research conducted towards all *P. miliaris* life stages (Figure 5B). Only pH was mentioned at least once in combination with

each of *P. miliaris*' life stages. Hydrostatic pressure was only researched for the gamete and embryo stages and not for later sea urchin life stages (Figure 5A).

Although Figure 5 gives an idea of the available information, in practice some of the articles were not usable and some additional articles that contained valuable information were found through snowballing.

Habitat requirements and natural values

I will relate information that was found on each of the habitat requirements and life stages that were considered. As well as the information on the relevant natural (a)biotic factors in the study area.

Diet

In the laboratory, various microalgae are

Figure 4; Amount of search results for (A) Sea urchin habitat requirements, (B) *Psammechinus miliaris* habitat requirements, (C) Sea urchin life stages and (D) *Psammechinus miliaris* life stages

Figure 5; Habitat requirements search results per life stage for (A) Sea urchins and (B) *Psammechinus miliaris*

adequate food sources for *P. miliaris* larvae but the species *Dunaliella tertiolecta* is preferred for larger percentage of juveniles reach metamorphosis and larger juveniles are produced (26). Adults and juveniles are generalist, they graze on a huge variety of organisms. Analysis of gut content showed that *P. miliaris* feeds on hydroids, worms, echinoderms, crustaceans, diatoms, molluscs, bottom material, fresh plants, dead fragments of algae and animals on plants (13). *P. miliaris* individuals in aquaria were found to graze on a great variety of food, including barnacles, molluscs, bryozoans, ascidians, worms,

aquaculture and experimental studies as a

hydroids, small sponges, any kind of seaweed, dead crab and fish and faeces of crab and fish (44). Jensen (1969) reported better growth rates in *P. miliaris* feeding on *Laminaria saccharina* with epizoic animals than on clean *L. saccharina* (38).

High protein diets increase growth rates of adult and juvenile sea urchins significantly (16) (45). Additionally, when given the choice between the two macroalgae: *Palmaria palmata* and *Laminaria digitate*, *P. miliaris* adults showed a stronger preference for *P. palmata*. Which has

been credited to the high protein levels in *P. palmata* (45).

The Eastern Scheldt, Grevelingen and North Sea have a large variety of microalgae species available to *P. miliaris* larvae. The large range of organisms consumed by adults and juveniles are available throughout the North Sea, Eastern Scheldt and Grevelingen.

Food quantity

P. miliaris larvae fed a low amount of

microalgae *D. tertiolecta* (500 cells/ml) did not develop to metamorphosis. Larvae fed a high amount of microalgae (4000 cells/ml) could not maintain their position in the water column and fell to the bottom (26). The optimal diet depends on larval developmental stage (26,32,46). For the two, three and four armed larval stages, feeding rate was 1500, 4500 and 7500 cells/ml per day respectively (46). Larvae have been shown to survive periods of up to 11 days without food (46).

Figure 6; Minimum and maximum values of the *Psammechinus miliaris* habitat requirements for different life stages and the minimum and maximum values that occur in the North Sea, Eastern Scheldt and Grevelingen. For the abiotic factors: Hydrodynamics (A), pH (B), hydrostatic pressure (C), salinity (D) and temperature (E).

Unfortunately no studies were found to be concentrations or adult and juvenile food biomass in the Grevelingen, North Sea and Eastern Scheldt.

Hydrodynamics

In Scotland, *P. miliaris* typically occurs in dense populations in sheltered areas of sea lochs (18). This suggests it prefers sheltered, low flow speed areas. Still, in the English channel *P. miliaris* was found to be characterized by a relatively high near bed velocity of >0.4 m/s (23).

Figure 6A shows that North Sea and Eastern Scheldt the water currents can reach speeds that are much higher than the speed at which *P. miliaris* is generally observed. While water currents in the Grevelingen never exceed the maximum speed at which *P. miliaris* was observed.

Oxygen

Experiments by Spicer has shown that *P. miliaris* adults are unable to regulate oxygen uptake during progressive hypoxia (low oxygen concentrations). However, they do occupy environments exposed to strong hypoxic events, such as intertidal shores (24). As long as the hypoxic event is brief, it does not have severe negative effects on the sea urchins organs (24). Spicer does however not discuss the effect of longer exposure times to hypoxia. Large scale stratification has led to deoxygenation of the Grevelingen lake (47). During summer months, the water column is completely anoxic (< 2 mg/l O₂) below depths of 15 meters (48). So in the Grevelingen, *P. miliaris* can very well be exposed to anoxic water during long periods of time.

pH

P. miliaris sperm has been observed to have an improved swimming speed at reduced seawater pH (from 8.06 to 7.67) (49). Caldwell suggests that current seawater pH levels may be suboptimal for maximal sperm swimming speeds and may therefore even benefit certain aspects of the *P. miliaris* reproductive cycle.

conducted towards phytoplankton

Increased swimming speeds may however have negative effects on longevity.

In an experimental study by Miles et al. (2007), mortality occurred in adults of *P. miliaris* exposed to a pH of 6.63, but not until day 8 of exposure, while exposure to a pH of 6.16 resulted in changes to mobility and morphology prior to death. Most spines were lost and calcareous test was visible (50). Miles suggests that longer term exposure at a pH of 7.44, may have negative effects on adult survival. To avoid reduction in sea urchin adult survival, surface ocean pH lower than 7.5 should be avoided (50). An experimental study by Coleen et al. (2013) exposed *P. miliaris* adults and their offspring to a reduction in pH (from 7.98 to 7.7), from 42 days before spawning up to juvenile recruitment. The lower pH values negatively affected larval survival, but juvenile recruitment was the same across treatments (27).

The minimum pH in the Grevelingen, Eastern Scheldt and North Sea are between 7 and 7.7 while the minimum values adult *P. miliaris* individuals can survive are much lower than that (Figure 6; B).

Hydrostatic pressure

Both *P. miliaris* embryos and larvae can survive at greater pressures than the known adult depth limits. With larvae surviving up to 200 atm and embryos up to 100 atm, larvae showed a much greater potential for surviving at greater depths compared to embryos (22). Thus, both embryo and larvae can survive pressure conditions way beyond those encountered in the studied areas (Figure 6C).

Salinity

P. miliaris from deeper water show different salinity optima from those found in littoral locations ((25) as cited by (13)). Gezelius (1962) showed that eggs of the these two forms differed in the salinities over which fertilization was successful (for the deep-water forms 20-32, the littoral forms 26-38 ppt at 17-19 °C)((51) as cited by (13)). In 1932, *P. miliaris* was observed to the north of Wieringen in the former

Zuiderzee at a salinity of 15 ppt (40). While in the Grevelingen, Eastern Scheldt and North Sea, the salinity values are all within the range of 28 ppt to 35.5 ppt. Both *P. miliaris* adults and gametes can survive values much lower than 28 ppt. Gametes can tolerate salinities of 38 ppt which is higher but close to the maximum salinities found in the study area. And the maximum salinity tolerance of 32 ppt for the deeper water form is within the range observed in nature (Figure 6D).

Temperature

A temperature of 5 °C does not allow embryo development (22). In Denmark, *P. miliaris* is found at locations where winter temperatures are just above zero and it was found to reproduce in waters where the summer temperatures rarely exceed 11 °C (52). Littoral

populations are more tolerant to increased temperature and decreased humidity during aerial exposure (53). Also, an article from 1968 claimed that the severely cold winter of 1963 likely eradicated most of the *P. miliaris* population in the Netherlands (20).

In the Eastern Scheldt, Grevelingen and North Sea temperature varies from temperatures just below zero up to temperatures of around 25 °C, while the minimum habitat requirement for *P. miliaris* embryos is at about 5 °C (Figure 6E).

After examining the relationship between *P. miliaris* encounter rate and temperature, the amount of cold days per winter below 1 °C (figure 7E) was found to be correlated with the *P. miliaris* encounter rate in the Eastern Scheldt (figure 7C) [$r = -0.578$, $n = 18$, $p = 0.009$]. This was not apparent between these two variables in the Grevelingen (figure 7AB) [$r = -0.434$, $n =$

Figure 7; *Psammechinus miliaris* encounter rate (the ratio of recorded dives per year at which at least one individual was encountered) in the Grevelingen (A) and the Eastern Scheldt (C) and the ratio of cold days per winter (November-February) in the Grevelingen (B) and Eastern Scheldt (D,E). (data Stichting Anemoon/Rijkswaterstaat)

19, $p = 0.056$]. Also, no relationship was found between cold days below 2 °C (Figure 7D) and *P. miliaris* encounter rate in the Eastern Scheldt (figure 7C) [$r = -0.231$, $n = 18$, $p = 0.341$].

Bottom type

P. miliaris adults generally prefer hard substrates: As they are mostly found attached to the undersides of rocks and buried under gravel on the foreshore(18), they are abundant on oyster banks in the German and Danish Waddensea (18), in the English channel *P. miliaris* was found to be characterized by relatively high amounts of rock and shell components (23) and in Ireland, *P. miliaris* has been reported to be abundant on suspended rope cultures of mussels ((33) as cited by (18)). *P. miliaris* distribution has been found to coincide with *Laminaria saccharina*, on fronds and the rocky surfaces below them (18). Although *P. miliaris* has also been reported to prefers sandy grounds (40). The bottom type in the Grevelingen, North Sea and Eastern Scheldt mostly consists of soft, sandy substrates (54–57). The relatively small areas consisting of hard substrates include rocky reefs, oyster banks, shipwrecks and rocky shores from coastal protective dikes (58,59)

Predators

Many different planktonic invertebrate- and vertebrate predators, and benthic suspension feeders predate on echinoid larvae (42). Predation is likely to be one of the most important sources of mortality (60) and has even been suggested to be the main source of planktonic larval mortality (61). Mortality rates have been estimated from field studies to be in the range of 6–27% per day (60,62). Not much is known about predation on adult and juvenile *P. miliaris* in the North Sea. *P. miliaris* was however reported as an important food source for the velvet swimming crab *Necora puber* in Galicia, NW Spain (63). Additionally, in the Irish sea, it was also reported as one of the most important food sources for the seven armed sea star *Luidia ciliaris* (64). *Necora puber* is a common crab species in the Eastern Scheldt,

Grevelingen and North Sea (21,65). *Luidia ciliaris* does not occur in the Grevelingen, North Sea and Eastern Scheldt (66).

Discussion

Main conclusions

We found *Psammechinus miliaris*’ habitat requirements for each of its life stages?” and Most of the measurable (a)biotic factors (or natural values) in the North Sea, Grevelingen and Eastern Scheldt were found (figure 6 and appendix, table 4-6). Only food quantity values are missing for all study areas. Unfortunately none of the researched (a)biotic factors had information available on the habitat requirements of all six life stages. And of all the measurable (a)biotic factors, only oxygen concentration had no information available on *P. miliaris* habitat requirements (appendix, table 7).

Larval diet and food quantity

As many microalgae species are adequate food sources for *P. miliaris* larvae, the availability of specific algal species is probably not extremely important to *P. miliaris*. Although larval habitat requirements for food quantity are well researched, it is hard to draw conclusions on the importance of food quantity, as no information was found on food quantity in the study area. Although conclusions cannot be drawn for *P. miliaris* specifically, larval growth of several species of sea urchin and other invertebrates have been shown to be food limited in coastal waters, significantly extending the time for larvae to reach metamorphosis (67,68). Longer development times can have huge consequences for species abundance and distribution, because echinoid larval stages are generally associated with large mortality rates (in the range of 6-27% per day) (42,60,62). And one might argue that when larval development time is extended, this leaves them exposed to ocean currents for longer periods of time, allowing them to be carried over larger distances until they reach metamorphosis. However, to the authors knowledge, no information is available on the distribution *P.*

miliaris larvae but this might be an interesting area of research.

Adult and juvenile diet and food quantity

Food is probably unlikely to be limiting to adult and juvenile *P. miliaris* distribution due to the huge variety of organisms it is able to feed on. This is supported by Faller-Fritsch and Emson (1972) (from (69)), who concluded that *P. miliaris* distribution is more likely to be limited by physical factors than food availability.

It would however require some estimates of available food biomass in the study area, biomass requirements per *P. miliaris* individual and the amount of individuals present in the study area to be able to draw reliable conclusions on whether food availability is a limiting factor to *P. miliaris* abundance and distribution.

Hydrodynamics

Water currents are important to dispersal of echinoid larvae (42), and thus, high flow speeds may be advantageous to *P. miliaris* distribution. Too high flow speeds can however be unfavourable, when larvae are dispersed beyond the geographical region of their suitable habitats. Additionally, rapid currents can severely limit fertilization success due to dilution of gametes (42). Also, adults and juveniles may be exposed to much stress when currents are too strong for them to remain attached to the substratum.

As currents in the Eastern Scheldt and North Sea can reach much higher flow speeds than the speed at which *P. miliaris* is generally observed, I conclude that hydrodynamics is a very important factor in determining *P. miliaris* distribution in the Eastern Scheldt and North Sea. It would be interesting to conduct experiments to test for the effects of water currents on all life stages of *P. miliaris*, also it would be interesting to look for a relationship between *P. miliaris* observations and flow speeds.

Oxygen

Due to stratification in the Grevelingen lake, *P.*

miliaris individuals are likely to be exposed to anoxic conditions. *P. miliaris* adults are not affected by brief events of low oxygen concentrations, but the effect of longer exposure times is unknown. The effect of oxygen concentrations on other *P. miliaris* life stages is yet to be determined.

pH

This suggests that, although low pH values caused reduced survival in larvae, in the end it does not affect *P. miliaris* abundance.

Short term adult mortality due to low pH values occurs at a pH of 6.16, but negative effects do already occur at longer term exposure to a pH of 7.44. These values are mostly outside the pH ranges observed in the Grevelingen, Eastern Scheldt and North sea.

However, pH of worldwide ocean surface waters have been predicted to decrease with 0.4 units before 2100 and 0.7 units by 2250 due to ocean acidification (7). This reduction would decrease the mean pH in the study area from 8 now to 7.6 in 2100 and 7.3 in 2250 and from the minimum pH of about 7.6 now to 7.2 in 2100 and 6.9 in 2250. They would come close, but not exceed the *P. miliaris* minimum adult habitat requirements. However, it does exceed the pH value of 7.44 at which negative effects have already been observed. Thus, it could have negative effects on *P. miliaris* abundance and distribution.

So as of now, pH is unlikely to affect *P. miliaris* abundance and distribution. But that may change in years to come due to ocean acidification.

Hydrostatic pressure

Both embryo and larvae can survive pressure conditions way beyond those encountered in the Grevelingen, Eastern Scheldt and North Sea. Based on those results, it would be safe to conclude that hydrostatic pressure is not a limiting factor to *P. miliaris*. It does however raise the question why *P. miliaris* is not encountered below depths of 100m, if this is not explained by pressure conditions. To be able to say for certain that pressure is not a limiting

factor to *P. miliaris* in the study area, the hydrostatic pressure tolerance for the gamete, juvenile, metamorphosis and adult life stages should also be determined.

Salinity

The minimum salinity tolerance limits for both gametes and adults are way below the salinity values generally found in the study area. The maxima are only known for gametes, the deeper water (S) forms can fertilize over higher salinities than the maxima found in the study area. Although most results suggest that salinity is not a limiting factor, the eggs of littoral forms of *P. miliaris* do not fertilize at salinities above 32 ppt. While in the natural areas, 32 ppt is quite commonly exceeded.

Based on the results, it would be safe to say that relatively low salinities in the Grevelingen, North Sea and Eastern Scheldt do not affect *P. miliaris*. However, more information on the maximum salinity tolerance is needed to safely say whether salinity can at times be a limiting factor for *P. miliaris* distribution and abundance in the natural areas.

Temperature

There was no significant correlation between *P. miliaris* encounter rate and the percentage of days below 2°C in the Eastern Scheldt, but there was a strong negative correlation with the percentage days below 1°C. Although this might not necessarily be a causal relationship, this finding supports that cold winters negatively affect *P. miliaris* abundance as previously suggested by Wolff (20). This negative correlation may exist because the *P. miliaris* temperature tolerance lies somewhere around 1°C. It would however require laboratory experiments to be certain what the *P. miliaris* minimum temperature tolerance is.

There was no significant correlation between encounter rate and cold days below 1°C in the Grevelingen lake which suggests that the *P. miliaris* population in the Grevelingen is more resistant to low temperatures. However, we did observe a trend and missing data points may partly explain the lack of power. Thus, these

findings indicate that more data on temperature is required and warrants further study.

These results also agree with the findings by Ursin (1960), that *P. miliaris* is found at locations where winter temperatures are just above zero (52).

P. miliaris embryos have been shown not to develop at temperatures of 5°C and lower (22). Although the winter temperatures in the study area are often much lower than 5°C, the spawning period is not in winter and thus embryo are generally not exposed to temperatures lower than 5°C. You could therefore conclude that low temperatures do not greatly affect embryos in the study area. But it does limit the period in which spawning is possible.

So in general, depending on the severity of the winter, temperature can be quite an important limiting factor to *P. miliaris* abundance and distribution in the Eastern Scheldt, Grevelingen and North Sea. The effect of high summer temperatures on abundance and distribution is still unknown.

Substrate / Bottom type

Several sources report that *P. miliaris* prefers hard substrates (18,23,33), only a dated report by Engel (1932) states that *P. miliaris* prefers sandy grounds (40). As the bottom type of the Eastern Scheldt Grevelingen and North Sea mostly consists of soft, sandy substrates and relatively small areas consist of hard substrates, it is likely that bottom type is a very important limiting factor to *P. miliaris* abundance and distribution.

Predation

To the authors knowledge, no research was conducted towards predation on *P. miliaris* larvae specifically, but as most plankton feeders are generalists, predation on other (echinoid) larvae is likely to be very similar. Predation has been suggested to be the main source of planktonic larval mortality (61) or at least one of the most important sources of mortality (60). And thus, it may very well be a limiting factor in

determining *P. miliaris* abundance and distribution.

There is very limited knowledge on the effect of predation on adult *P. miliaris*. Only two known predators were found, *Necora puber* and *Luidia ciliaris*, of which only *N. puber* occurs in the study area. But the mortality caused by *N. puber* is unknown.

Predation is certain to affect abundance and distribution, as it causes mortality. How severe these effects are, relative to all other factors that affect abundance and distribution, is unknown and requires further research.

Debatable points of research

To the authors knowledge, the most important (a)biotic factors have been taken into account in this study. Nevertheless, there are many more (a)biotic factors that can limit a species distribution and abundance. For instance; light intensity and length of day may be important, since photoperiod is the primary stimulus for *P. miliaris* spawning (30). Also, tidal differences may affect the distribution of *P. miliaris* in the littoral zone. And the availability of nutrients both in the food and in the water around the organism may also greatly limit distribution and abundance. These factors were however hard to relate to the study area and were thus not included in the study.

The exposure times to extreme values are not reflected in minimum and maximum values. Although an organism may survive extreme values for short periods of time, when it is exposed for longer periods of time it can negatively affect survival (27,50). Also, the rate of change towards extreme values may also greatly affect an organisms response, as slower rates may allow an organism to acclimatize. When an organism is directly introduced to an experimentally altered (a)biotic factors, this may induce a stress response that may impact survival (19,27). When the organism is introduced to an altered (a)biotic factor at a slower rate, this may have a less severe effect (19). Most of the experimental studies used to determine *P. miliaris* habitat requirements, that were used for this study have not taken this into

account (22,26,49–51). It would be wise to redo these experiments with the rate of change and exposure time taken into consideration. To investigate whether the same conclusions can be drawn.

Habitat requirements can differ within the defined life stages. For instance, within the larval life stage, multiple larval stages have been defined (2-, 3-, 4-, 6- and 8-armed pluteus), and these have been shown to have different feeding rates (46,70). Hence, younger larvae require less available food. Also a juvenile of 1 week old may have relatively low survival chances when exposed to extreme (a)biotic values compared to an 8 month old juvenile.

The habitat requirements and natural values used in this study have come from many different sources using different methods, which can influence the comparability of studies. For example, differences in measuring devices, methods for determining habitat requirements and geographical areas where *P. miliaris* individuals were collected from. Hence, the values found do not fully represent reality. Therefore small trends may be hard to discover, but large differences between habitat requirements and natural values can lead to some reliable conclusions. It is also a great way of discovering knowledge gaps and can supply background for future research.

Lastly, the minimum and maximum habitat requirements may vary depending on the values of other (a)biotic factors. Such interaction effects have been reported for several (a)biotic factors including temperature and pH (71), temperature and hydrostatic pressure (22) and pH and food availability (72). This is something this study did not take into account, as interaction effects are hard to study and would require a more mechanistic approach. To later construct models that are able to make powerful predictions of abundance and distribution of marine species, one must first understand how these interaction effects work.

Knowledge gaps

Hydrodynamics and bottom type are two of the least researched habitat requirements in sea urchins (Figure 4A) and a large part of the life cycle has not been investigated (Table 2). Still, they are both very likely to be limiting factors in determining the distribution and abundance of *P. miliaris* and probably for sea urchins in general. Temperature is one of the most researched habitat requirements in sea urchins and with good reason, it is very probable to be an important limiting factor. Knowledge on temperature requirements of gametes, larvae, metamorphosis and juvenile stages is however still lacking for *P. miliaris* (Table 2). As well as the maximum temperature requirements of adults and embryos.

Table 2; Life stages per habitat requirement for which literature was available.

Habitat requirement	Gamete	Embryo	Larva	Metamorphosis	Juvenile	Adult
Diet			X		X	X
Food quantity			X			
Hydrodynamics						X
Oxygen						X
pH	X					X
Hydrostatic pressure		X	X			
Salinity	X					X
Temperature		X				X
Bottom type				X	X	X
Predation			X			X

Diet was well described for juvenile and adult *P. miliaris*, although the natural diet of larvae deserves some more attention. Information on food quantity requirements was missing for juveniles and adults (Table 2). Also, an estimation of food availability in the study area was missing for both larvae, juveniles and adults. Oxygen was also only researched for the adult life stage and the effect of longer exposure times to hypoxic conditions is unknown. Even though pH is fairly well researched for both sea urchins and *P. miliaris* (Figure 4A,B), habitat requirements were only found for the gamete and adult stages. For now, pH is unlikely to be a limiting factor to *P. miliaris* in the study area, but this may change in the future due to ocean acidification. Also, research on pH requirements is mostly focussed on the negative effects a decrease in minimum pH will have on *P. miliaris*, while there is a possibility that the maximum pH requirement would actually benefit from a decrease in pH. Predation is quite well

researched for sea urchins (Figure 4A), but little is known regarding predation on *P. miliaris*. The juvenile and metamorphosis life stages gain the least attention in sea urchin and *P. miliaris* related research (Figure 4C,D). This could indicate these stages require more attention in habitat requirement related research.

The *P. miliaris* habitat requirements of each of its life stages and the relevant natural factors in the study area were identified as far as information was available. Unfortunately a big portion of the habitat requirements per life stage was not available (Table 2). Nonetheless, this did reveal some significant knowledge gaps. And for more than half of the considered habitat requirements, it was possible to conclude with fair amount of certainty whether they are limiting factors or have minor to no

importance in controlling abundance and distribution of *P. miliaris* in the Eastern Scheldt, North Sea and Grevelingen. Also, a significant correlation was

found between winter water temperatures and *P. miliaris* observations in the Eastern Scheldt and Grevelingen.

Through this detailed case study of the echinoid *Psammechinus miliaris*, the methods of this complete life cycle approach on habitat requirements was presented. Weak points of the approach are that small trends are hard to pick up and it requires both an extensively researched organism and study area to be able to draw reliable conclusions. Also, it does not take into account the differences in habitat requirements within a species and the interaction effects between (a)biotic factors.

However, strong points include that it is a relatively easy way to get a broad understanding of the habitat requirements of an organism, to identify knowledge gaps in the research area and to prioritise further research efforts. Furthermore, it was able to identify for more than half of the discussed (a)biotic factors whether they were limiting factors or had only

minor importance. In this way, we will be able to evaluate the importance of a range of (a)biotic factors in determining a species' abundance and distribution and are a step further in understanding the drivers of population fluctuations in nature.

Conclusions and recommendations

In summary, I distinguish three groups of (a)biotic factors to answer which *P. miliaris* habitat requirements are most likely to be limiting factors in the Eastern Scheldt, Grevelingen and the North sea. **Limiting factors** that are very likely to be limiting factors to *P. miliaris*. **Debatable factors**, that require more research to be able to say for certain how important they are to *P. miliaris* abundance and distribution. And **Minor- no importance**, abiotic factors that are very likely to barely affect *P. miliaris* abundance and distribution (Table 3).

This study revealed that hydrodynamics are likely to be an important limiting factor to *P. miliaris* adult abundance and distribution. However, it has received very little research attention (Figure 4AB). I would therefore suggest an experimental study on the effects of different flow speeds on all different sea urchin life stages. Including gamete fertilization success, embryo and larval survival and development, larval settlement and recruitment success and finally the ability of adults and juveniles to remain attached to the substratum.

Bottom type was also found to be an important limiting factor to *P. miliaris* abundance and distribution. Bottom type has also received very little research attention (Figure 4AB). I would therefore suggest an observational study that relates *P. miliaris* presence/absence to the substratum or bottom type of the research area.

Table 3; importance of habitat requirements according to conclusions

Limiting factors	Debatable factors	Minor- no importance
Hydrodynamics	pH	Hydrostatic pressure
Temperature	Predation	Salinity
Bottom type	Food availability	
	Oxygen	

The approach may be improved by including a means of statistically comparing the range of habitat requirements to the range in natural values to be able to give some statistical value to the importance of the discussed habitat requirements. This would make the results more reproducible and comparable.

As a big portion of the information on *P. miliaris* habitat requirements was not available, it would be interesting to see this approach applied to a more extensively studied sea urchin, like *Strongylocentrotus purpuratus*, to see what conclusions can be drawn. This would allow a comparison between the different life stages to determine which of the life stages is most vulnerable to changes in (a)biotic factors. It also allows an interesting comparison to see both the differences and similarities between two different species of sea urchin.

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Appendix

Table 4. Natural values of abiotic factors in the Grevelingen lake.

Abiotic factor	Minimum	Average	Maximum	References
Depth (m)	0	5.3	48	(73)
Hydrodynamics (m/s)	0	<0.1	0.1	(74)
Oxygen (mg O ₂ /L)	<2	-	18	(47,75,76)
pH	7	8	9	(77)
Pressure (atm)	1	1.53	5.8	Calculated from depth
Salinity (g Cl/L)	28		34	(77)
Watertemperature (°C)	0.1	12	25.5	Own data

Table 5. Natural values of abiotic factors in the North Sea.

Abiotic factor	Minimum	Average	Maximum	References
Depth (m)	0	90	700	(78)
Hydrodynamics (m/s)	0.25	0.5	2.5	(79)
Oxygen (mg O ₂ /ml)	-	-	-	
PH	7.7	8.1	8.6	(80)
Pressure (atm)	1	10	71	Calculated from Depth
Salinity (ppt)	33.5	35	35.45	(81)
Temperature (°C)	-5	12	27	(82)

Table 6. Natural values of abiotic factors in the Eastern Scheldt

Abiotic factor	Minimum	Average	Maximum	References
Depth (m)	0	9	48.8	(83,84)
Hydrodynamics (m/s)	0	0.45	1.65	(85)
Oxygen (mg O ₂ /L)	-	-	-	
PH	7.5	7.9	8.3	(80)
Pressure (atm)	1	1.9	5.2	Calculated from depth
Salinity (ppt)	30		33	(86)
Temperature (°C)	-0.1	11.5	24	Own data

Table 7. *Psammecchinus miliaris* (measurable) habitat requirements

(A)biotic factors	Life stages	minimum	optimum	maximum	Location	References
Food quantity (cells/ml)	L	500	1500-4000	>4000	Scotland (UK)	(26)
Hydrodynamics (m/s)	A			>0.4	English channel	(23)
Oxygen (mg O ₂ /ml)	-	-	-	-	-	-
pH	A,G	6.16(A)	7.95(G)	-	Plymouth (UK)	(49,50)
Pressure (atm)	E, L	1	-	100(E), >200 (L)	Plymouth (UK)	(22)
Salinity (ppt)	A, G, E	<14.8(A), 20-26(G,E)	-	32-38 (G,E)	former Zuiderzee (NL)	(40,51)
Temperature (°C)	E, L	5(E), <5 (L)		-	Plymouth (UK)	(22)