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Colophon

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Preface

This project has received financial support from the European Maritime and Fisheries Fund and the former Danish Foreign Ministries development program for fisheries (Bæredygtigt hjertemuslingefiskeri i Limfjorden, j.nr. 33113-B-17-109).

The topic of the report on sustainable cockle fishery in the Limfjorden has been continuously discussed with the industry in an advisory board established for the project and through the participation of the Mussel Industry Association (Foreningen Muslingeerhvervet) and the Mussel Committee. During the course of the project, a follow-up project has been applied and is still ongoing to complement the findings of this project.

Each chapter reflects the views of the authors listed for a specific chapter. Authors cannot be held responsible for any views or use which may be made of the information contained in other chapters. The summary and recommendations are written by DTU Aqua.

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Content

Summary

The project is intended to improve the scientific basis for public management of mussel (*Mytilus edulis*) fisheries, and in particular the by-catch fishery for cockles (*Cerastoderma edule*) in the Limfjorden, which is undocumented and with no knowledge on its sustainability. **Two species of cockles were found in the Limfjorden during the project**: *C. edule* and *C. glaucum*, thus relevant results for both species are presented in this report.

The overall purpose of the project was to establish the scientific basis for a sustainable utilization of the cockle resource in the Limfjorden. Specifically, the aim was to estimate the size distribution of populations of cockles, estimate the proportion of cockles on the surface of the sediment and describe reasons for their presence on the surface instead of being naturally buried. Such knowledge is imperative for the development of coastal fishing and the sustainable use of an important resource.

The Limfjorden cockle fishery has become as valuable, and often more, than the blue mussel fishery since 2015 (Fiskeristyrelsen). The Limfjorden cockle fishery represented 31.5% of annual landings of bivalves in Denmark in 2013-2021, with an average value of ca. 28.2 Mio. kr. per year and was the main European cockle fishery, accounting for ca. 35% (EU 28) or 53% (EU27, excluding UK) of all European cockle landings since 2016-2020 (Eurostat).

Cockle landings in the Limfjorden have been relatively stable since 2013 at 6 700 tonnes per fishing season with 3 525 tonnes captured from the main fishing area, Kås Bredning. The contribution and importance of Kås Bredning to landings increased significantly in recent seasons, from 38% in the 2013-2017 seasons to 66% in the 2017-2021 seasons.

The 2018 survey in the Western Limfjorden estimated the cockle stock at 18 166 tonnes, and only at 6 592 tonnes in areas accessible to bivalve fishery outside Natura 2000 areas. However, the 2018 survey conducted with a **suction dredge, clearly underestimated the stock** due to low station density (283 stations) and patchiness of the cockle beds. This was substantiated by cockle landings just over 7 600 tonnes in the following fishing season. It was thus recommended another cost-efficient survey approach is developed to assess cockle populations. This was addressed in the follow-up project COCKLE II (j.nr. 33113-B-20-172).

An important question of this study concerned the surfacing behaviour of the cockle and its association with pathogens and mortality events: what are the factors facilitating surfacing and is it because of this behaviour that the blue mussel fishery is able to fish cockles as a by-catch? Although it was challenging to find surfacing beds in the Limfjorden, an *in-situ* survey of surfacing and non-surfacing cockle populations (Venø August 2019) showed surfacing areas had higher density and biomass than non-surfacing areas, but cockles in the two areas had similar size distribution, weight, and condition. However, **surfaced cockles had a lower condition than buried cockles in surfacing areas**. In addition, *C. glaucum* specimens had lower condition than *C. edule* specimens from surfacing areas, either in the surfaced or buried fractions, but not compared to those from non-surfacing areas. There was no sign that the pathogens detected in the different fractions were associated to the vertical distribution of cockles in the sediment. Laboratory surfacing and burial experiments with healthy cockles, with no pathogens and from buried beds, revealed **cockles were more prone to surfacing and less prone to re-bury at high density than at lower density**. Oxygen depletion only induced surfacing at high density. The two cockle species had different burial capacity with the lagoon cockle *C. glaucum* less able to re-bury than the common cockle *C. edule*.

The **cockle fishing efficiency** of the mussel surface dredge with an inner net inside the dredge, as commonly used in the Limfjorden cockle fisheries, was 34.0% (±9.6% SE), and not statistically different than without an inner net at 18.5% (±16.8% SE). In surfacing areas, fishing efficiency was 40.8% (±16.0% SE) almost twice as much as in non-surfacing areas with 21.8% (±9.8% SE), although not statistically different. **Buried cockles constituted a significant fraction of fished cockles**, even in surfacing areas.

It can thus be concluded that cockle **surfacing behaviour is not a prerequisite for the cockle fishery** using the surface-based blue mussel dredge. However, surfacing likely increases cockle availability and capture efficiency by the blue mussel fishery and raises concerns regarding the fate of surfaced cockles if associated with increased mortality as surfaced cockles had a lower condition than buried cockles. The surfacing behaviour was not linked to pathogens, but rather influenced by density and adverse environmental conditions such as oxygen depletion.

In relation to spawning periods, both species have their **main spawning from mid-May to mid-June**, with *C. edule* having a slightly more prolonged spawning period from April to mid-June. During the period from April to June all *C. glaucum* specimens (100%) and 95% of the *C. edule* specimens were healthy as assessed from their gamete-developmental stages.

In the seasonal and surfacing surveys conducted in Venø Bugt in the Limfjorden, no *Marteilia* sp. and **only a few macroparasites species were detected**. A few macroparasite species were recorded in the cockles: *Monorchis parvus*, *Gymnophallus choledochus* and *Himasthla* sp.. Of these, *M. parvus* and *G. choledochus* in particular are harmful as they prevent offspring production in infected cockles. However, during the present survey they both occurred in low prevalence. The commensalistic turbellaria *Paravortex cardii* was observed in the intestine of many cockles. However, this species is not expected to harm cockles. Some cockles showed the presence of *Vibrio aestuarianus* from June to October with a peak infestation in August. Surprisingly some cockles tested positive to *Bonamia ostreae* a non-cockle parasite currently affecting the European flat oyster *Ostrea edulis* population, indicating that cockle might serve as a reservoir for the parasite. Overall, there were **no sign that pathogen infesta-tion was high enough to cause mass mortality nor be responsible of surfacing events**. Future environmental changes may impact the level of infestation by *Vibrio aestuarianus*, which might also cause mortality.

Conclusions and recommendations. **Kås Bredning is fundamental for the cockle fishery**, and the high dependence on a single area poses a risk to the future stability and sustainability of the Limfjorden cockle fishery. Variations in Kås Bredning cockle stocks, either from recruitment failure or natural and fishing mortality, may compromise the sustainability of the Limfjorden cockle fishery, with the risk of significant economic and social impacts on fishermen and other industry stakeholders.

In view of the temporal and spatial offset in mussel and cockle fishing, the current blue mussel fishery with associated cockle by-catch can be viewed as a co-target fishery on two separate species. These results support that management of cockle fishing should be performed as an independent fishery in the Limfjorden. A long-term perspective in terms of development of a management practice for an independent Limfjorden cockle fishery should be developed. A new management practice for the Limfjorden cockle fishery is advised to include elements such as development of cost-efficient methods for stock assessment at least in the most important basins, a strategy for overall harvest and management, development of population indicators to support population health assessments in areas with no monitoring for stock size, detailed information on where the cockle fishery takes place based on Black Box data and control rules and limitations to the fishery.

1. Introduction

The common cockle is not only an economically important species (e.g. Carss et al., 2020 for a recent review; landings from Eurostat), it also plays an important ecological role and can significantly engineer and modify the ecosystem it inhabits (e.g. Donadi et al., 2015), namely affecting bioturbation and sediment dynamics (e.g. Ciutat et al., 2006; Dairain et al 2020). Cockles are infaunal bivalves that live buried in the top 5 cm of the bottom sediment, even though episodic emergence of cockles to the sediment surface may occur.

Cockles (*Cerastoderma edule* and *Cerastoderma glaucum,* Syn. *C. lamarcki*), are fished in significant amounts in the Limfjorden as a by-catch of the blue mussel (*Mytilus edulis*) surface dredge fishery and have been so since 2012 (Fiskeristyrelsen). This contrasts to almost all European fisheries that fish intertidal cockle populations, as in the Limfjorden cockles are exclusively fished in sublittoral areas at depths >3m. Historical information is incomplete, but landings have increased by one-order of magnitude since 2010-2012 and hover around 5 000 – 7 000 tonnes per year, representing about one third of cockle landings by weight in Europe.

In normal conditions cockles are not expected to be significantly available to the surface dredge used by the blue mussel fishery. Cockles are worth more than blue mussels and in practice cockles are almost entirely fished in the Limfjorden at locations separate from blue mussel beds. Targeted fishing of cockles is a common practice of the fishery, with fishermen adding a smaller mesh inner net to the dredge and actively searching for cockle beds. Thus, capture of infaunal cockles in the Limfjorden with a gear designed for epibenthic bivalves is not accidental.

Industry and governmental agencies are interested in more information on the Limfjorden cockle population in order to ensure a sustainable regulation and management of the cockle fishery. At present there is no or very little information about the Limfjorden cockle stock. For cockle fishing in the Limfjorden to be sustainable, information on population structure, abundance, and biology needs be assessed annually at least in main fishing grounds, to provide data-base advice for the management and regulation of the fishery (e.g. the establishment of sustainable quotas, establishment of an independent fishery).

Therefore, there is an imperative need to carry out more research on the cockle population to be able to assess the available stock, whereby a sustainable quota for fishery can be set. This information is also required in order to evaluate the potential for implementing cockle fishing in the Limfjorden as a new independent fishery. So far, the Limfjorden and cockle fishery is certified MSC, however, the development, implementation and execution of a research plan on the cockle population in the Limfjorden is a requirement to attribute certification in the future. No previous surveys of cockle distribution in the Limfjorden exist, with the exception of a few localized coastal studies from the 1970´s and 1980´s (Brock, 1979, 1980; Hylleberg, 1978; Ivell, 1981).

Census of pathogens in Limfjorden is also lacking and large survey of the western part where large cockle beds occur is necessary to obtain a baseline assessment of the pathogens and diseases present over the cockle life cycle. Very little research has been conducted on the reproductive cycle disease susceptibility of the two cockle species in Limfjorden.

It has been suggested that cockles were available to mussel fishery due to a surfacing behaviour where cockles, an infaunal species, migrate to the surface and would get caught by epibenthic fishing gear. If true, this would have consequences on the available stock for the cockle fishery and its management, as large fraction of the cockle population would be unavailable to the fishery. The cockle fishing efficiency of the surface mussel dredge needs to be evaluated to understand if only surfacing cockles are caught by the dredge or if non-surfacing cockle beds are also available and exploited by the fishery.

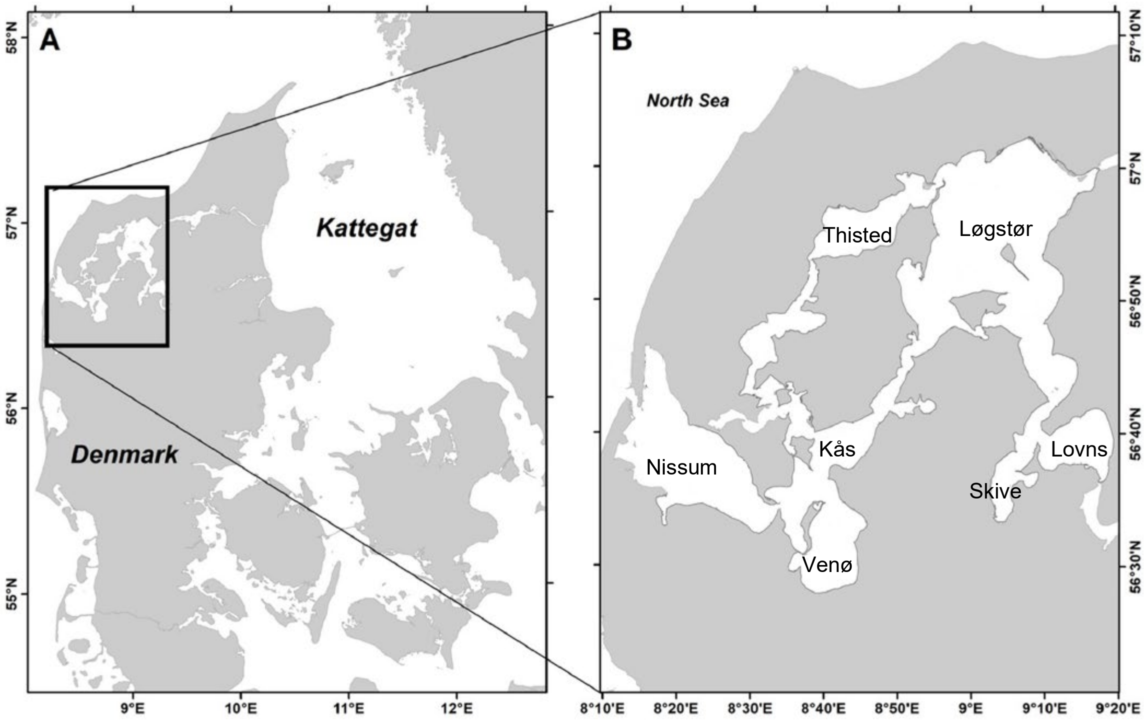

Figure 1.1. Map of Denmark (A) and the western part of Limfjorden (B) with selected broads.

Cockles are known to occasionally emerge onto the sediment surface, often in large quantities and associated with increased disease, mortality, or reduced condition (Jonsson and André, 1992; Richardson et al., 1993; Blanchet et al., 2003; Mouritsen and Poulin, 2003, Mouritsen, 2004; Tompkins et al., 2004; Thieltges, 2006; Morgan et al. 2012). Different causes have been attributed for the surfacing behaviour in cockles, from environmental factors (Richardson et al., 1993; Mouritsen, 2004; Marsden and Bressington, 2009; Lewis and DeWitt, 2017; Zhou et al., 2022), but also biotic factors (Jonsson and André, 1992; Richardson et al., 1993; Thomas and Poulin, 1998; Mouritsen, 2002; Blanchet et al., 2003; Mouritsen, 2004; Tompkins et al., 2004; Marsden and Bressington, 2009; Morgan et al., 2012).

All studies have focused on cockles in their more common intertidal sandy habitats (e.g. Dare et al., 2004; Dabouineau and Ponsero, 2011), contrasting the subtidal muddy habitat of cockle populations in the Limfjorden, Denmark, which may affect the processes of cockle surfacing and burying behaviour, and survival. A single small study indicated that surfacing cockles in the Limfjorden had similar parasite prevalence than the buried ones but had a higher proportion of mature females and a lower capacity to re-burial which worsens at low oxygen levels[1]. The causes of surfacing and the fate of the surfaced cockles in the Limfjorden are thus of relevance to the fishery: re-burial or death by predation, exhaustion, disease or fishing.

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[1] https://videnskab.dk/miljo-naturvidenskab/mystisk-er-stenalder-fisk-vendt-tilbage-med-klamme-parasitter
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A research plan on the Limfjorden cockle population and fishery was developed by DTU Aqua (Dansk Skaldyrcenter) collaboratively with FME, which resulted in a project funded by EMFF (33113-B-17-109). This report presents its results: description of the temporal evolution of cockle landings and spatial fishing patterns (Chapter 2); an assessment of cockle population structure, abundance and distribution (Chapter 3); an evaluation of potential causes and consequences of cockle surfacing (Chapters 4 and 5); a census of pathogens and the current cockle reproductive cycle (Chapter 6); an assessment of the fishing efficiency of the mussel dredge on surfaced and buried cockles (Chapter 7). Finally, results and conclusions from the project supported advice and recommendations to management (Chapter 8).

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2. Limfjorden cockle fishery status

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2.1 Rationale

The purpose of this chapter is to provide a background to the Limfjorden cockle fishery and describe: 1) its importance at national and European level; 2) its spatial and temporal fishing patterns; and 3) recent landing statistics.

The approach taken to describe the cockle fishery in the Limfjorden was to integrate information of cockle landing statistics from Fiskeristyrelsen and Eurostat, together with Black Box data (BlackBox R2, Anchor Lab, Copenhagen, described in Nielsen et al., 2021) collected from continuous registrations of cockle and mussel fishing vessels activities. Data were evaluated and crossed with input from Foreningen Muslingeerhvervet (FME) to distinguish cockle fishing from blue mussel fishing, and thus define cockle fishing beds.

2.2 Cockle fishing in the Limfjorden

In the Limfjorden, cockles are fished as a by-catch of the blue mussel (*Mytilus edulis*) fishery with a daily restriction of no more than 49% of total landings in weight per vessel. As a by-catch, cockle fishing in the Limfjorden is tied to fishing practices, management and harvest control rules of the blue mussel fishery (e.g. TAC, daily cockle landing limits). Such a management structure is not necessarily best adapted to cockle biology, cockle abundance and cockle fishing practices in the Limfjorden, and often can result in inefficient fishing of both cockles and blue mussels e.g., from excessive travelling or fishing sub-optimal mussels to maximize more valuable cockle landings.

Two cockle species, mainly the common cockle *Cerastoderma edule*, but also the lagoon cockle *Cerastoderma glaucum* in lower amounts, have been significantly fished in the Limfjorden since 2012 (Fiskeristyrelsen). In contrast to almost all European fisheries that fish intertidal cockle populations, cockles are exclusively fished in the Limfjorden in sublittoral areas at depths >3m.

The blue mussel fishery uses a lightweight surface dredge introduced in 2010 (Chapter 7), assumed not to dig into the sediment. Cockles as infaunal species living just below the sediment surface to 5 cm deep were not expected to be significantly available to capture by the surface dredge, except during episodic emergence to the sediment surface. However, fishing trials (Chapter 7), fishing patterns and information provided by the fishery all support that fishing of non-surfacing cockles is a normal practice of the fishery.

Most if not all of cockle fishing in the Limfjorden occurs as targeted fishing at different locations other than blue mussel fishing. Therefore, cockle fishing in the Limfjorden can be seen as part of a two species fishery, with cockles being a co-target species rather than a by-catch species.

2.3 The Limfjorden cockle fishery in DK and Europe

Cockle fishing in the Limfjorden has become the most valuable individual bivalve fishery in Denmark since 2015, often at the level or above the Limfjorden blue mussel fishery. Cockle fishing in other Danish coastal areas is negligible or non-existent in most years (<0.4% since 2013).

The value of cockle landings in the Limfjorden averaged 30.4 mio. kr. per year or 34.4% of all bivalve landings in Denmark between 2015-2021 (Figure 2.1; Fiskeristyrelsen, 2022). In 2019, Limfjorden cockle landings reached a maximum value of 51.4 mio. kr., constituting 61.3% and 48.4% of all bivalve landings in the Limfjorden and Denmark in that year, respectively (Figure 2.1.).

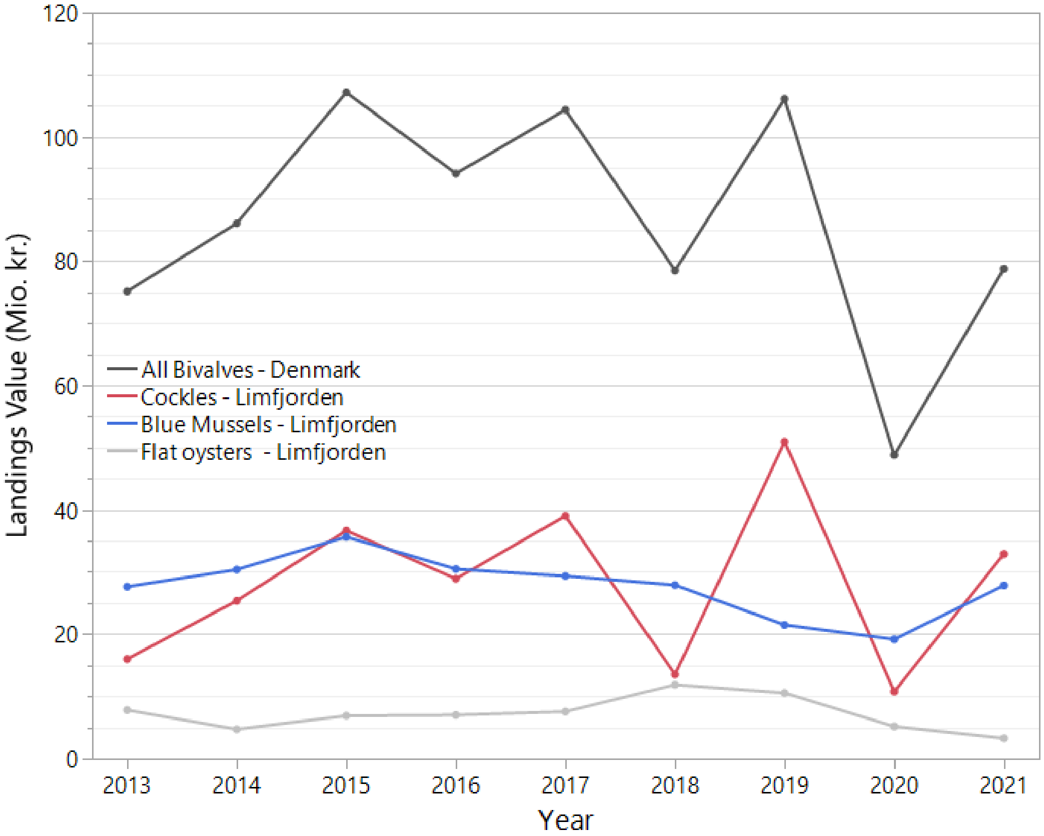

Figure 2.1. Annual bivalve landings in Denmark since 2013 (Mio. kr.). All bivalve species in Denmark (black) and only in the Limfjorden: cockles (red), blue mussels (blue) and European flat oysters (grey). (Fiskeristyrelsen, 2022).

Since 2013, the importance of the Limfjorden cockle fishery to cockle landings in Europe has increased significantly, in parallel with a decline in cockle landings in other EU countries (mainly the UK; Figure 2.2).

The Limfjorden, as it supplies almost all Danish cockle landings, is currently the single largest cockle fishery in the EU. The Limfjorden accounted for 31.6% of all cockle landings between 2013-2019 in the EU with 28 countries or 53.4% of all cockle landings between 2013-2020 excluding the UK (Figure 2.2; Eurostat, 2021).

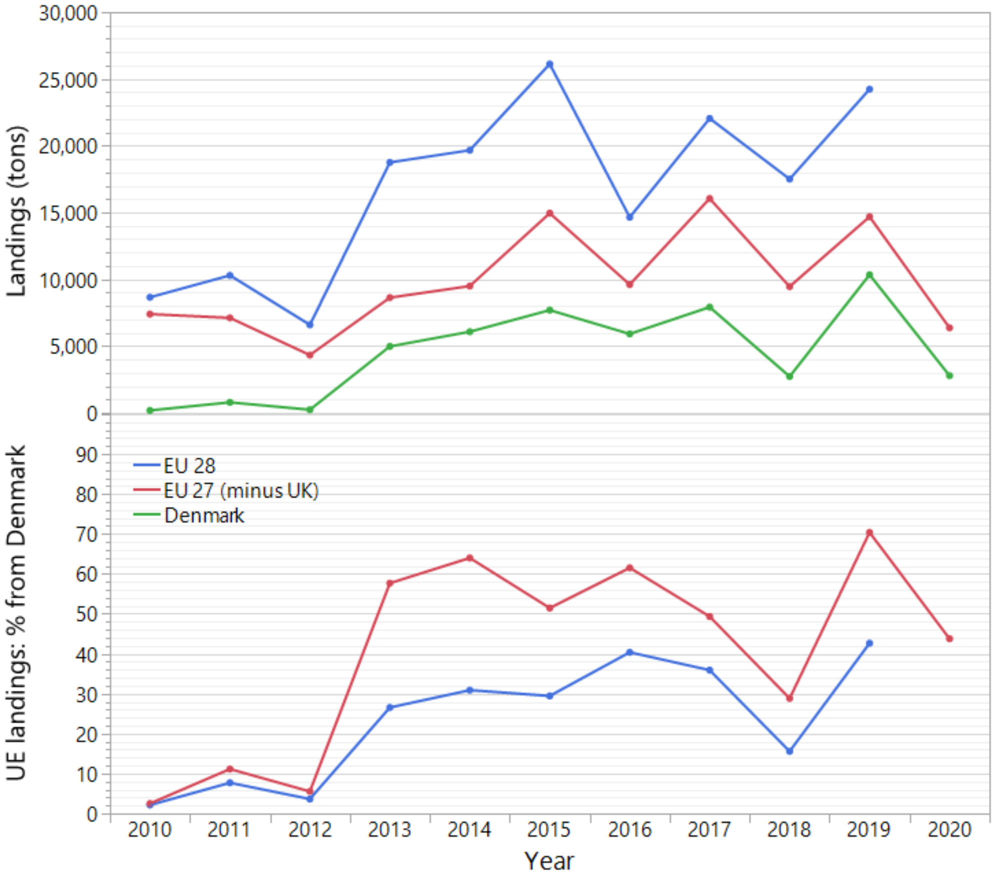

Figure 2.2. Annual cockle (*Cerastoderma* sp.) landings (tonnes) in Europe and Denmark (top) and the % of European landings from Denmark (bottom). EU 28 includes, and EU 27 excludes landings form the United Kingdom (Eurostat, 2021).

2.4 Fishery in Limfjorden: Temporal and spatial fishing patterns

2.4.1 Landings per fishing season

The cockle fishing season, as with mussel fishing, is from September to the following June, and thus cockle fishing patterns and population dynamics cockle are better described by fishing season than by calendar year.

Since 2015, most landings occurred in the months of January, April, May, October and November ranging between 980 and 1 490 tonnes (Figure 2.3). However, a large variability of landings occurred in in May, September, and October, likely reflecting the timing of gonad development and spawning that affects meat quality in spring, and in autumn occasional low meat content in blue mussels that limit cockle fishing (Figure 2.3; information from FME).

Cockle landings average 6 723 tonnes per season (±389 tonnes, SE) from 2013–2014 to 2020–2021, ranging from 4,993 to 8 608 tonnes per season (Table 2.1 and Figure 2.4). Landings have remained above 6 000 tonnes in the last five seasons, with a maximum of 7 647 tonnes (2018–2019) and a minimum of 6 182 tonnes (2019–2020).

2.4.2 Landings per production area

The cockle fishery relied in only a few areas in each season with 94.2% of landings in the four seasons between 2017–2021 originating from just 4 fishing areas (areas 9, 15, 7 and 8).

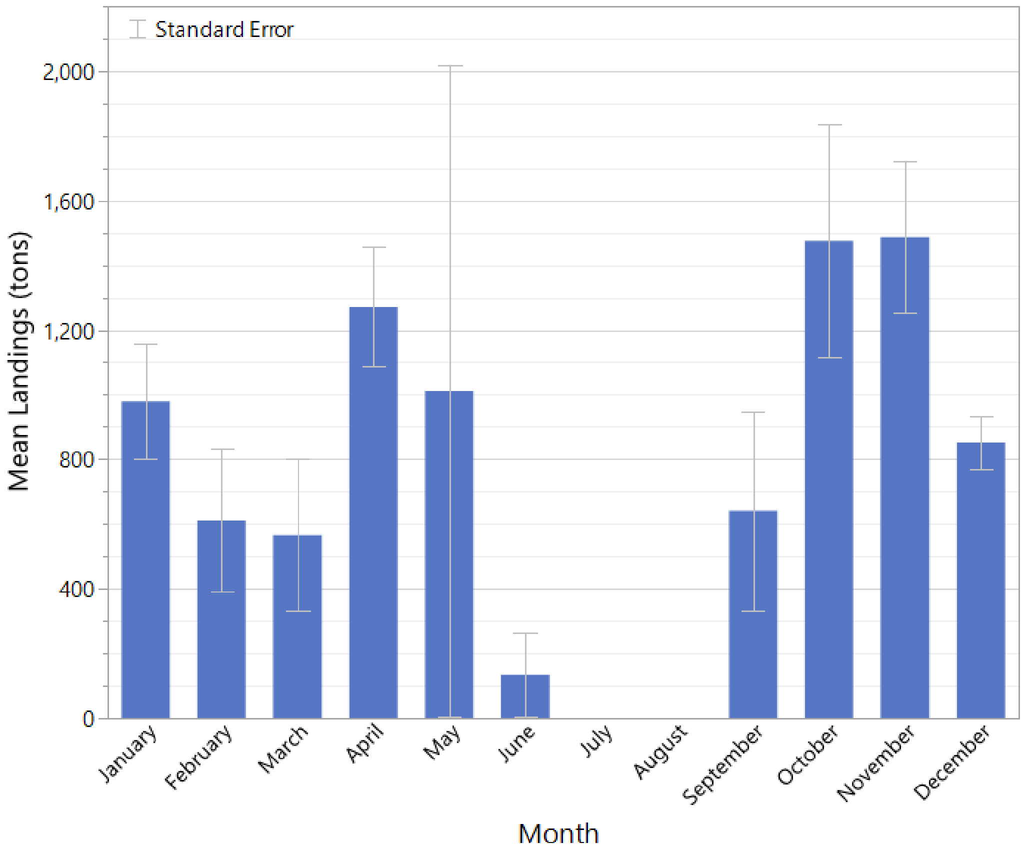

Figure 2.3. Cockle landings per month between the 2015–2016 to 2020–2021 fishing seasons (Fiskeristyrelsen, 2022).

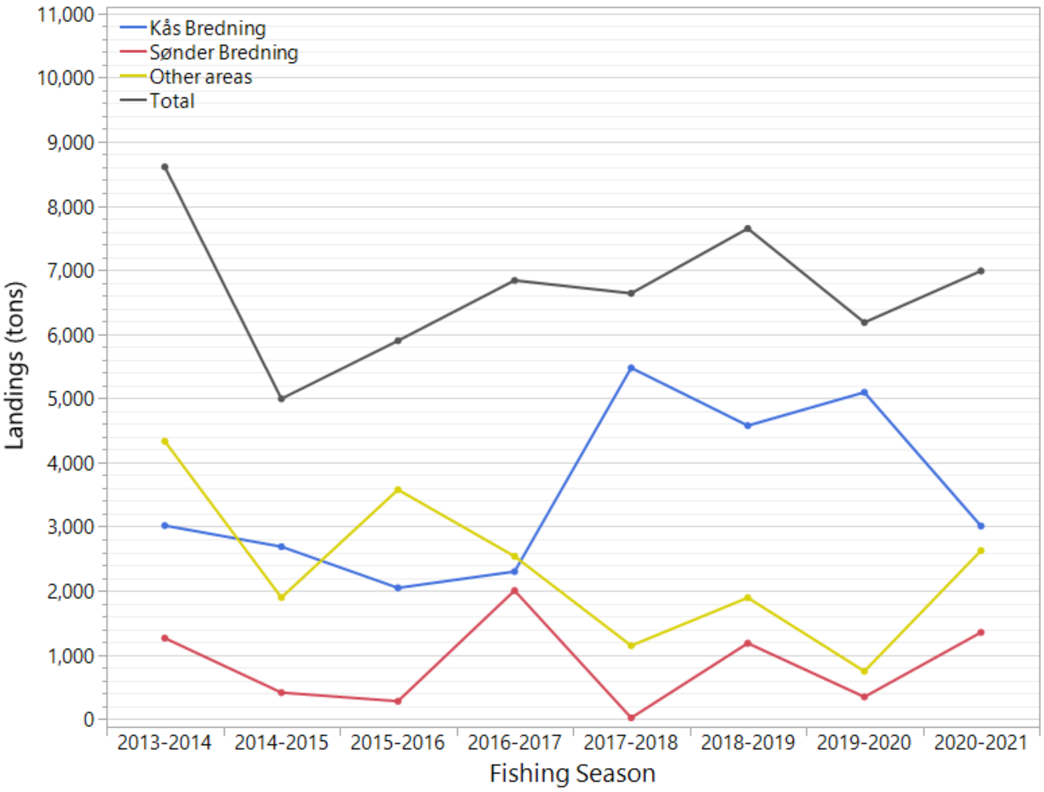

Figure 2.4. Cockle landings per fishing season between the 2013–2014 to 2020–2021 fishing seasons (Fiskeristyrelsen, 2022).

Table 2.1. Cockle landings (tonnes) from the Limfjorden per fishing season (September-June) since 2013. Landings in tonnes of live weight.

	Landings per Fishing Season (tonnes)									
Production Area	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019	2019-2020	2020-2021	**All**	**%**
Kås (9)	3 016	2 687	2 044	2 298	5474	4 574	5 093	3 009	28 196	52.4
Sønder (15)	1 261	412	278	2 003	19	1 182	345	1 350	6 848	12.7
Salling Syd (11)	3 181	0	0	0	0	739	0	0	3 919	7.3
Visby (25)	0	0	1 342	2 466	72	9	621	0	4 510	8.4
Livø Vest (35)	947	1 465	1 334	34	27	19	13	6	3 845	7.1
Venø Bugt (7)	0	0	0	0	4	1 096	0	1 636	2 736	5.1
Venø Bugt (8)	0	0	8	0	1 016	28	97	946	2 094	3.9
Dragstrup (26)	0	0	869	11	3	0	0	0	883	1.6
Venø Sund (5)	0	256	7	0	0	0	0	0	263	0.5
Venø Sund (6)	97	150	0	0	0	0	0	0	247	0.5
Andre Områder	106	23	15	26	21	0	14	37	243	0.5
Total	**8 608**	**4 993**	**5 897**	**6 838**	**6 636**	**7 647**	**6 182**	**6 985**	**53 785**	

Table 2.2. Relative landings in percentage of total landings from the Limfjorden per fishing area (muslingeområder) for the four seasons between 2017–2018 to 2020–2021, the first four seasons between 2013–2014 to 2016–2017, and all seasons (2013 to 2022).

Fishing Area	2017–2021 %	2013–2017 %	All %
Kås Bredning (9)	66.1	38.1	52.4
Sønder Bredning (15)	10.5	15.0	12.7
Salling Sund syd (11)	2.7	12.1	7.3
Visby Bredning (25)	2.6	14.5	8.4
Livø Bredning vest (35)	0.2	14.4	7.1
Venø Bugt nord (7)	10.0	0.0	5.1
Venø Bugt syd (8)	7.6	0.0	3.6
Salling Sund nord (13)	0.0	0.0	0.0

A change in landing patterns was observed between the first four seasons 2013–2017 and the seasons 2017–2021 (Tables 2.1 and 2.2). While between 2013–2017, Kås Bredning accounted for only 38% of landings, between 2017–2021 Kås Bredning accounted for the majority of landings,66% but reaching up to 82% in 2019–2020.

The contribution of secondary areas was significant between 2013–2017, with the 56% of landings coming from four areas (11, 15, 25 and 35; Tables 2.1 and 2.2). Between 2017–2021, secondary areas supplied a minority of landings, 34% (Tables 2.1 and 2.2). A change in fishing patterns of secondary areas between 2017–2021 was also clear, with areas 25 and 35 producing only residual landings and being replaced by areas 7 and 8 (Tables 2.1 and 2.2)

2.4.3 Kås Bredning relevance to the fishery

The Limfjorden cockle fishery has heavily relied on a single fishing area, Kås Bredning (production area 9) to supply the majority of landings: 52.4% of all cockle landings between 2013–2021 at 3 524 tonnes/season (±468 tonnes, SE; Tables 2.1 and 2.2, Figure 2.4).

Kås Bredning, was the only area with significant regular landings in every season (several 1 000 tonnes). Of the secondary areas, only Sønder Bredning (area 15) supplied relevant landings every season, albeit lower than 500 tonnes in several seasons (Table 2.1). It is thus critical for the Limfjorden fishery to find beds with abundant cockles of the right size and meat content in Kås Bredning and to a lesser extent in Sønder Bredning.

Other fishing areas produced variable landings >100 tonnes/season, but only for one or two seasons then often followed by several seasons with no or very small landings (Table 2.1). Such fishing pattern may result from a lack of fishing effort in those areas following successful fishing seasons but can also be interpreted as an indication of fishing mortality pushing local cockle populations past their renewal capacity, which then take several seasons to recover.

Since 2017, the importance of Kås Bredning has increased, even if its contribution in 2020–2021 decreased to pre-2017–2018 levels and the fishery has become less reliant on secondary fishing areas (Table 2.1 and Figure 2.4).

Kås Bredning has ca. 44 km^2 of fishable area, however cockle catches originate from a smaller area. Fishing grounds between 2013–2020 cover ca. 18.42 km^2 or ca. 42% of the total fishable area (Figure 2.5). In each fishing season, an even smaller area was fished of 3.9 ±0.4 km^2 (SE, n=7) or 8.8% of the total fishable area.

If Kås Bredning cannot provide sufficient landings, the fishery becomes dependent on other areas to compensate the reduction in landings. A situation that occurred in the recent seasons of 2020–2021 and the ongoing 2022–2023, when areas that historically had only been fished twice (area 7) or only once (area 11), respectively, compensated reductions in landings from Kås Bredning (Table 2.1).

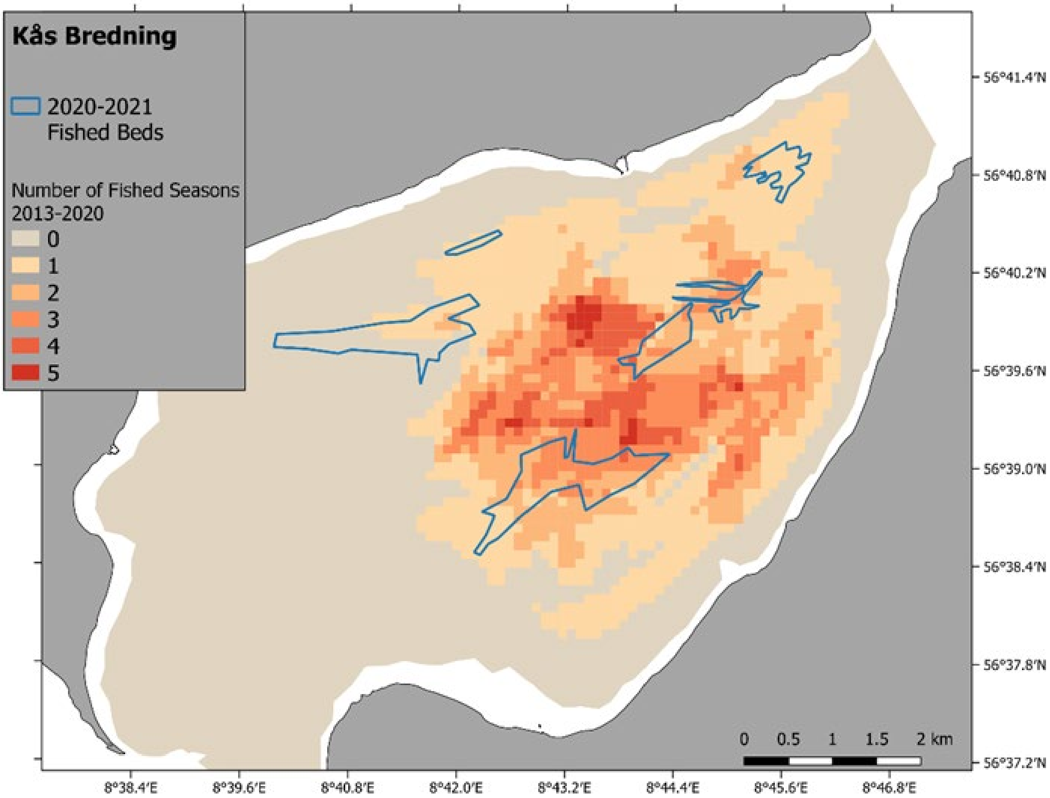

Figure 2.5. Location of fished cockle beds in Kås Bredning between the 2013 to 2020 seasons (colour map). Colour code indicates the number of seasons each cell in a 100x100 grid was fished (i.e. if > 5 tracks per season). Overlaid are the fished cockle beds in the 2020-2021 season (blue line). A cockle bed is defined as the area encompassing dense fishing tracks and thus includes non-dredged areas between tracks.

2.5 Conclusions

The Limfjorden cockle fishery has become the most valuable bivalve fishery in Denmark and plays a major role in the EU landings and represented up to 70% of EU landings (excl. UK).

As cockle fishing is a by-catch from blue mussel fishery, it is difficult to distinguish patterns of cockle fishing from blue mussel fishing, even using Black Box data and input from stakeholders.

It is evident from previous landing statistics that Kås Bredning is the main cockle producing area. Variations in Kås Bredning cockle stocks, either from recruitment failure or natural and fishing mortality, may thus compromise the sustainability of the Limfjorden cockle fishery. Although such situation has not yet occurred, it could pose a risk of significant economic and social impacts on fishes and industry stakeholders.

2.6 References

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3. Cockle populations in the Western Limfjorden in 2018

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3.1 Rationale

Cockles (*Cerastoderma* sp.) are infaunal suspension feeding bivalves, living just below the sediment surface to 5 cm depth in sandy to muddy sediments of inter-tidal and subtidal areas of NW Europe. Cockles can episodically emerge from the sediment (Chapters 4 and 5, this report), often associated with density, increased disease and mortality events (Burdon et al 2014). Cockle growth is highly variable spatially and temporally, depending on food supply and density driven food and space competition (André and Rosenberg, 1991; Hylleberg et al., 1978; Ivell, 1981; Jensen 1991, 1993). Cockles usually mature during the second year (13-16 cm, 15-18 months) depending more on size than age, spawning from May to August in the Limfjorden (Ivell, 1981), have high fecundity, a longevity of 5-8 years, and highly variable recruitment as with most bivalves (see Dare et al., 2004; Dabouineau and Ponsero, 2009 and Malham et al. 2012, for reviews of cockle biology).

Two cockle species, the common cockle *Cerastoderma edule*, and the lagoon cockle *Cerastoderma glaucum* (syn. *C. lamarcki*) are fished in the Limfjorden as a by-catch of the blue mussel (*Mytilus edulis*) surface dredge fishery, but are more or as valuable to the fishery as blue mussels. However, most if not all of cockle fishing in the Limfjorden is targeted fishing at different locations and times than blue mussel fishing. As infaunal species cockles were not expected to be significantly captured by the blue mussel fishery surface dredge, except during episodic emergence of cockles to the sediment surface. However, cockle fishing efficiency trials showed that a significant fraction of buried cockles is captured by the surface dredge (Chapter 7, this report), while fishing patterns and information provided by the fishery indicate targeted fishing of non-surfacing cockles is a normal practice of the fishery.

By 2018 there was no or very little information on cockle populations, including that the two species of cockles are fished, on fishing practices and behaviour of the fishery and on the impact of being managed according to the requirements specific to blue mussel populations and fishery. No previous surveys of cockle distribution in the Limfjorden exist, except for a few localized coastal studies from the 1970´s and 1980´s (Brock, 1979, 1980; Hylleberg, 1978; Ivell, 1981).

The aim of this task was to evaluate the large-scale cockle distribution over most of the western and central Limfjorden in 2018.

3.2 Methods

Survey approach

To fulfil the requests presented at the time by Fiskeripolitisk Kontor, formerly part of the Udenrigsministeriet (UM), DTU Aqua planned and executed a survey on the basis of: 1) to cover most of the western and central Limfjorden (ca. 76% of the area deeper than 3 m); 2) to include most of the shellfish production areas where cockle fishing has occurred (excluding production areas 12, 14, 16 to 22 and 28 to 31 based on cockle landings statistics and cockle fishing patterns, as well as information from Foreningen Muslingeerhvervet, FME); 3) to use a boat-based survey to cover the large sub-tidal area; and 4) to use a sampling method that captured 100 % of the cockles in the sediment.

The fishing vessel 'Wilhelmina' from Fiskeriselskabet Cardium ApS was chartered to perform a survey using a hydraulic suction dredge as used in the Wadden Sea. The suction dredge is assumed to have 100% efficiency for cockles larger than 15 mm in shell width, the smallest shell dimension captured by the spacing of the bars in the dredge. Below 15 mm in shell width, the efficiency of the suction dredge decreases and is variable. An eleven-day survey was done from 9 to 20 of April 2018, with a total of 283 stations sampled (Table 3.1 and Figure 3.1).

Table 3.1. Number of stations, station area density per production area (muslingeområde).

Production Area			Stations		
Name	ID	km^2	Number	stations /km^2	m^2/km^2
Nissum Bredning	1	49.8	19	0.38	37.5
Nissum Bredning	2	46	13	0.28	31.0
Nissum Bredning	3	20.1	11	0.55	59.0
Nissum Bredning	4	19.3	10	0.52	53.3
Nissum Bredning, Ud			6		
Venø Sund, Syd	5	16.2	4	0.25	25.0
Venø Sund, Nord	6	30	10	0.33	39.5
Venø Bugt, Nord	7	39.6	11	0.28	40.4
Venø Bugt, Syd	8	31.9	11	0.35	44.2
Kås Bredning	9	43.9	52	1.18	117.8
Salling Sund, Syd	11	12.4	7	0.56	61.1
Salling Sund, Nord	13	10.2	5	0.49	55.1
Sønder Bredning	15	30.4	9	0.3	33.2
Agerø Sund,Vest	23	12.6	2	0.16	18.4
Agerø Sund,	24	6.5	1	0.15	25.1
Visby Bredning	25	19.1	18	0.94	134.1
Dragstrup Vig	26	18.3	12	0.66	91.3
Vilsund	27	12	7	0.58	67.3
Thisted Bredning, Øst	30	27.5	2	0.07	16.1
Feggesund	32	14	1	0.07	9.1
Løgstør Bredning, Vest	33	40.5	8	0.2	26.2
Løgstør Bredning	34	50.4	16	0.32	42.5
Livø Bredning, Vest	35	35.8	16	0.45	42.7
Livø Bredning, Øst	36	34.2	8	0.23	28.4
Bjørnsholm Bugt	37	34.7	6	0.17	19.2
Løgstør Bredning, Øst	38	46.3	10	0.22	48.4
Løgstør Grunde	39	36.2	8	0.22	30.6
Total/Average		737.9	283	0.38	45.0

Cockle biomass estimates reported here have to be considered under the requirement and constraints presented above, and a significant variability must be expected as commonly occurs in cockle surveys even in inter-tidal habitats (e.g. Dare et al., 2004), due to the high patchiness in the distribution of cockles.

Once on-board, cockles were sorted, weighed and frozen. Shell size, shell length, height, and width (mm) were measured in the laboratory after thawing. Age was determined by counting the number of annual winter growth lines (Richardson et al., 1980).

The von Bertalanffy growth function was fitted to shell width and age data: $\text{Length}_{(\text{age})} = L_\infty\ (1\text{-}e^{-K(\text{age-}t_0)}$, where age is age in years, L_∞ is length at infinity when growth approaches 0, K is the growth coefficient and t_0 is theoretical age when size is 0. ϕ is the growth performance index (Pauly and Munro, 1984) = 2 * $\log(L_\infty) + \log(K)$.

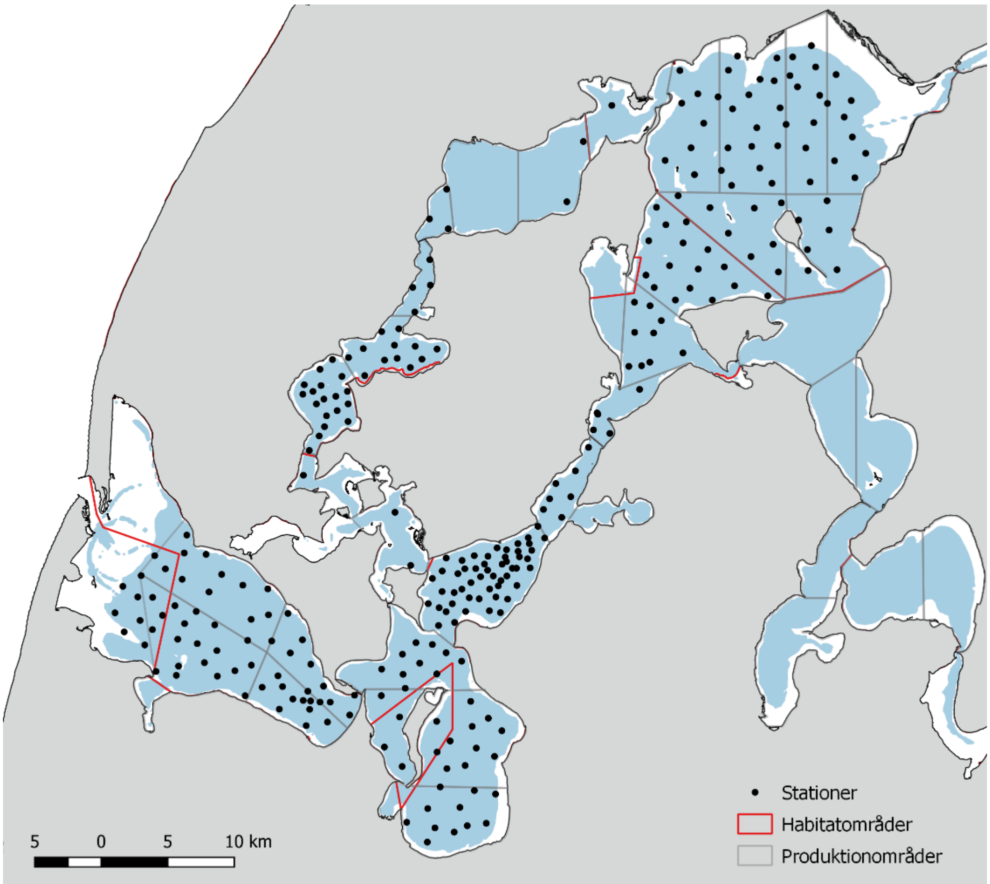

Figure 3.1. Location of the 2018 survey stations in black in the western Limfjorden. In blue, the area deeper than 3 m. Grey lines delimit production areas and red lines delimit Natura2000 areas. Fishing production area number in Figure 3.3.

3.3 Results and Discussion

3.3.1 Spatial distribution

The spatial distribution of cockles in the Limfjorden in April 2018 was highly clumped with a few high abundance stations accounting for most of the cockle abundance, while the remainder areas form a background of low or zero abundance matrix (Figures 3.2 and 3.3).

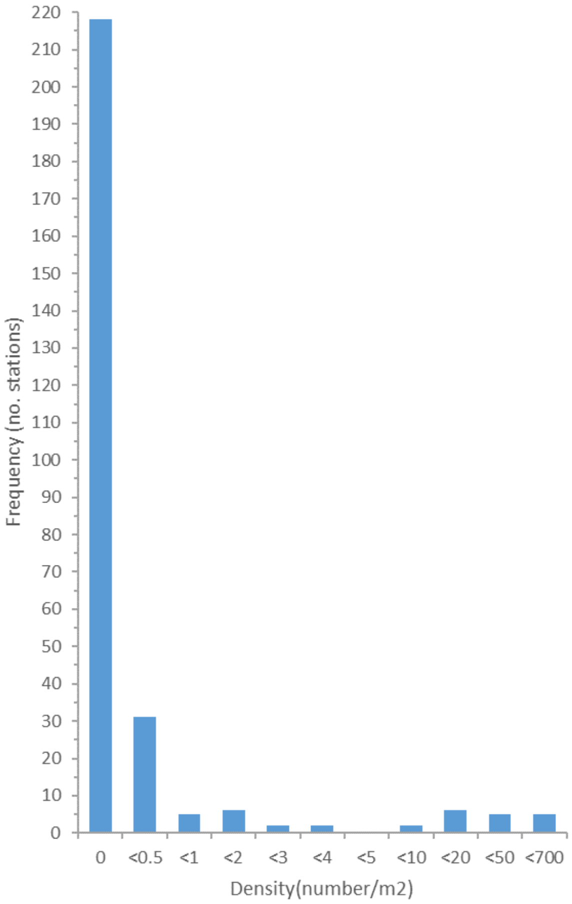

Figure 3.2. Frequency of station per cockle density class (cockles/m^2). X-axis is not linear.

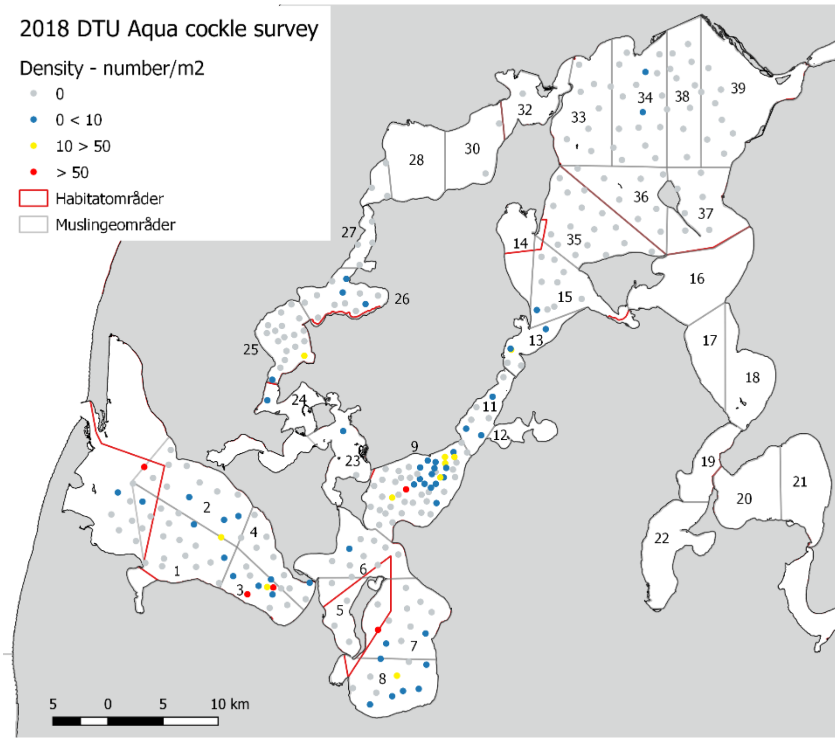

Figure 3.3. Density (number/m^2) of cockles per station in the 2018 DTU Aqua cockle survey of the Limfjorden.

Table 3.2. Number and proportion of stations with cockles, mean cockle biomass (g/m^2) and density (cockles/m^2) per production area in the western Limfjorden in April 2018. Error is standard error (SE).

Production Area		Stations			Biomass		Density	
Name	ID	Total	With cockles	%	g/m^2	SE	cockles/m^2	SE
Nissum Bredning	1	19	3	15.8	0.89	±0.87	0.08	±0.07
Nissum Bred.	2	13	6	46.2	45.81	±35.66	7.64	±6.78
Nissum Bred.	3	11	7	63.6	435.7	±255.1	100.05	±62.23
Nissum Bred.	4	10	1	10	0.01	±0.10	0.002	±0.002
Nissum Bred. Ud		0	1	16.7	0.2	±0.20	0.06	±0.06
Venø Sund	5	4	0	0				
Venø Sund	6	10	1	10	0.003	±0.003	0.001	±0.001
Venø Bugt	7	11	3	27.3	85.78	±85.70	19.42	±19.39
Venø Bugt	8	11	7	63.6	12.86	±9.46	1.82	±1.59
Kås Bredning	9	52	20	38.5	57.16	±31.80	5.09	±2.50
Salling Sund	11	7	3	42.9	0.73	±0.61	0.09	±0.07
Salling Sund	13	5	3	60	68.54	±60.00	7	±5.24
Sønder Bredning	15	9	1	11.1	0.01	±0.01	0.003	±0.003
Agerø Sund	23	2	1	50	0.49	±0.49	0.17	±0.17
Agerø Sund	24	1	1	100	16.4	0	3.64	0
Visby Bredning	25	18	2	11.1	5.31	±4.50	1.1	±0.93
Dragstrup Vig	26	12	3	25	0.65	±0.63	0.11	±0.11
Vilsund	27	7	0	0				
Thisted Bredning	30	2	0	0				
Feggesund	32	1	0	0				
Løgstør Bredning	33	8	0	0				
Løgstør Bredning	34	16	2	12.5	0.06	±0.06	0.003	±0.002
Livø Bredning	35	16	0	0				
Livø Bredning	36	8	0	0				
Bjørnsholm Bugt	37	6	0	0				
Løgstør Bredning	38	10	0	0				
Løgstør Grunde	39	8	0	0				
Total		**283**	**65**	**23.0**				

Of the 283 stations surveyed (Figures 3.2 and 3.3, Table 3.2), 77.4% or 220 stations had no cockles, 9.9% or 28 stations had ≥1 cockles/m^2, 5.7% or 16 stations had ≥10 cockles/m^2, while only 1.8% or 5 stations had ≥50 cockles/m^2.

Cockle biomass and density in the Limfjorden were strongly skewed (skewness of 3.77 and 6.22, respectively). Two stations in Nissum Bredning accounted for 42.6% and 55.2% of all the cockle biomass and density sampled during the DTU Aqua cockle survey. Four stations accounted for 68.1% and 76.6% of all the cockle biomass and density. The percentage of stations with live cockles in each production area varied between 0 and 63.6% (Table 3.2) and reflected the variation in cockle abundance (Figures 3.3 and 3.4).

In the 2018 DTU Aqua cockle survey, cockles were most abundant in production areas 2, 3, 7, 8, 9, 13, 24 and 25, particularly in Nissum Bredning, Venø Bugt, Kås Bredning and Salling Sund (Figures 3.3 and 3.4, Table 3.2).

In stations with live cockles, cockle biomass ranged from 0.02 to 2 192 g/m^2, with a mean of 152.7 g/m^2 and a median of 3.91 g/m^2 (Table 3.2). Cockle density ranged from 0.01 to 683 cockles/m^2, with a mean of 24.4 cockles/m^2 and a median of 0.53 cockles/m^2 (Table 3.2).

3.3.2 Cockle abundance

Cockle biomass was estimated for 26 production areas and of these, only six areas accounted for 98.4% of the total biomass (Table 3.3): areas 2 and 3 (Nissum Bredning), 7 and 8 (Venø Bugt), 9 (Kås Bredning) and 13 (Salling Sund). The total cockle biomass below 3 m depth was estimated at 18 166 tonnes ±13,104 (95% CI; Table 3.3).

However, of the cockle biomass estimate, ca. 11 025 tonnes or 60.7% were in Natura 2000 areas (Nissum Bredning, Mors vest and Nees Sund) and thus not currently fished. In addition, ca. 69.7% of area 5, ca. 5.3% of area 6 and ca. 16.2% of area 7 are also Natura 2000 areas and thus not fished. In particular, Nissum Bredning (areas 1-4) where no cockle fishing has occurred, had the largest cockle stock biomass, 10 914 tonnes. Cockle biomass in fishing areas and excluding Natura 2000 areas is estimated at ca. 6 592 ±4 761 tonnes (Table 3.4).

A crude evaluation of the impact of low station density, i.e. likelihood of missing significant cockle beds, leading to underestimation on the cockle biomass estimates can be obtained by comparing it with cockle landings (Table 3.4).

Cockle landings for the six fishing seasons since 2013-2014 to 2018-2019 were a total of 40 618 tonnes, averaging 6 770 tonnes per fishing season that is of similar magnitude as the cockle biomass estimate outside Natura 2000 areas of ca. 6 592 ±4 761 tonnes.

Cockle landings in 2017-2018, the fishing season preceding the 2018 cockle survey, were 6,636 tonnes, and again similar in magnitude to the biomass estimate ca. 6 592 ±4 761 tonnes outside Natura 2000 areas. While landings in 2018-2019, the fishing season following the 2018 survey, were 7 647 tonnes, supporting a significant underestimation of cockle biomass in the 2018 survey.

For the main cockle fishing area of Kås Bredning, 5 474 tonnes were landed in 2017-2018 and 4 574 tonnes in 2018-2019, respectively 218% and 182% of the 2018 survey biomass estimate of 2 512 ±2 795 tonnes (Table 3.4).

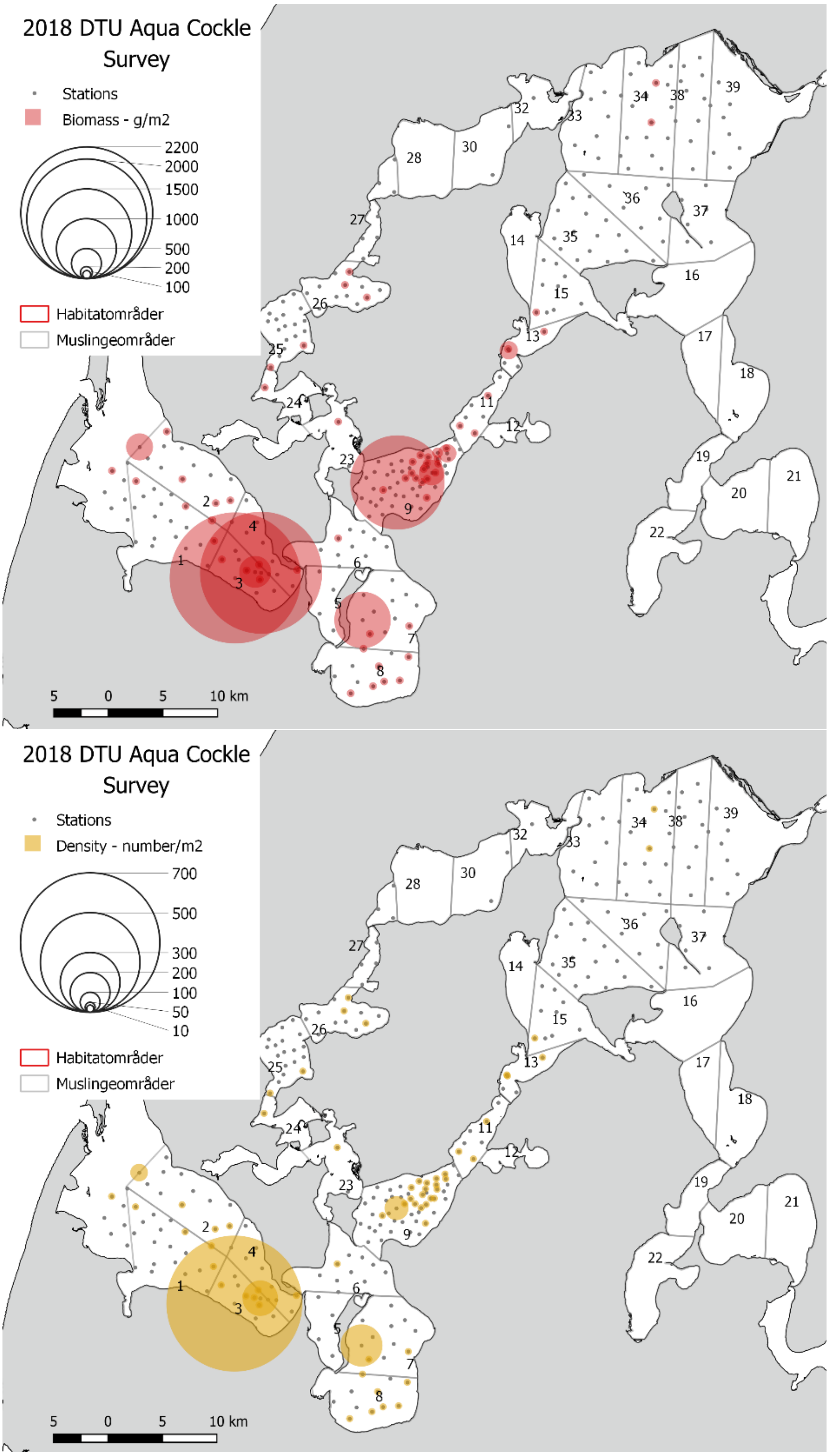

Figure 3.4. Abundance and distribution of cockles in 2018 in the Limfjorden: biomass (top, g/m^2) and density (bottom, number/m^2).

Similarly, landings from areas 11 and 15 in the 2018-2019 (739 and 1 182 tonnes, respectively) were also significantly higher than the 2018 cockle biomass estimates (9 and 0.2 tonnes, Table 3.4). Again, supporting the 2018 survey significantly underestimated cockle biomass.

Such comparisons are biased by the different timing of the survey (April) compared to the normal fishing season (October to March-April), and by recruitment, growth and mortality of cockles, and the uncertainty in biomass estimates. Some of these factors contribute to increase in cockle biomass, i.e. recruitment and growth, while others contribute to decrease in cockle biomass, i.e. natural and fishing mortality. However, with no quantification of these factors available, their impact on cockle biomass before and after the 2018 survey cannot be evaluated.

Table 3.3. Cockle biomass estimates (tonnes ±95% CI) per production area in the Limfjorden in April 2018.

Production Area		**Biomass**		
Name	ID	tonnes	95% CI	%
Nissum Bredning	1	44.3	±86.7	0.2
Nissum Bredning	2	2 107	±3 285	11.6
Nissum Bredning	3	8 762	±10 256	48.2
Nissum Bredning	4	0.2	±0.5	<0.01
Venø Sund, Syd	5	0		0
Venø Sund, Nord	6	0.1		<0.01
Venø Bugt, Nord	7	3 396	±6 785	18.7
Venø Bugt, Syd	8	410	±603	2.3
Kås Bredning	9	2 512	±2 795	13.8
Salling Sund, Syd	11	9	±15.2	0.05
Salling Sund, Nord	13	697	±1 220	3.8
Sønder Bredning	15	0.2		<0.01
Agerø Sund, Vest	23	6	±12.4	0.03
Agerø Sund,	24	106		0.6
Visby Bredning	25	102	±172.3	0.6
Dragstrup Vig	26	12	±23.1	0.07
Vilsund	27			
Thisted Bredning, Øst	30			
Feggesund	32			
Løgstør Bredning, Vest	33			
Løgstør Bredning	34	3	±5.7	0.02
Livø Bredning, Vest	35			
Livø Bredning, Øst	36			
Bjørnsholm Bugt	37			
Løgstør Bredning, Øst	38			
Løgstør Grunde	39			
Total		18 166	±13 104	100

Table 3.4. Cockle stock estimates per production area in 2018 and landings for the two fishing seasons before and after the 2018 survey, as well as from 2013 to 2019 in the Limfjorden. In bold, underestimated stock relative to landings.

Area		**Stock estimate**	**Landings**		
			2018-2019	**2017-2018**	**since 2013**
Name	number	tonnes	tonnes	tonnes	tonnes
Nissum Bredning	1	44 ±86.7			16
Nissum Bredning	2	2 107 ±3 285		10	10
Nissum Bredning	3	8 762 ±10 256			
Nissum Bredning	4	0.2 ±0.5		8	8
Venø Sund, Syd	5				263
Venø Sund, Nord	6	0.1			247
Venø Bugt, Nord	7	3 396 ±6 785	1,096	4	1 100
Venø Bugt, Syd	8	410 ±603	28	1 016	1 052
Kås Bredning	9	**2 512 ±2 795**	**4 574**	**5 474**	20 094
Salling Sund, Syd	11	**9 ±15.2**	**739**		3 919
Salling Sund, Nord	13	697 ±1 220	0		
Sønder Bredning	15	**0.2**	**1 181**	19	5 153
Agerø Sund, Vest	23	6 ±12.4			
Agerø Sund	24	106			
Visby Bredning	25	102 ±172.3	9	72	3 889
Dragstrup Vig	26	12 ±23.1		3	883
Løgstør Bredning	34	3 ±5.7			
Livø Bredning, Vest	35		19	27	3 828
Other areas					157
All areas		18 166 ±13 104			
Minus N2000		**6 592 ±4 761**	**7 647**	**6 636**	**40 618**

3.3.3 Clumped distribution of cockles and limitations of survey design

To demonstrate the impact of the clumped cockle distribution and station density on cockle biomass estimates, cockle biomass was estimated with additional non-quantitative cockle abundance data obtained by DTU Aqua monitoring of blue mussels and oysters in 2018. This survey identified up to 20 dense cockle beds not captured by the 2018 cockle survey. However, data was collected with an oyster surface dredge, with low and variable fishing efficiency, and thus it provides only proof of presence and not proof of absence of cockles. In this exercise, 100% cockle fishing efficiency of the oyster surface dredge and thus minimum cockle abundance were assumed.

Under such scenario, depending on the number of additional beds added, the proportion of stations with cockles increases by 8 to 43% and biomass estimates significantly increase in seven production areas (1, 2, 5, 6, 8, 11 and 15). Production areas 6 and 15 are good examples of such impact, where one or two additional cockle beds found in the monitoring of blue mussels and oysters increased cockle biomass estimates from 0.1 tonnes to 1 276-3 365 tonnes for area 6 and from 0.2 tonnes to between 1 679-2 285 tonnes for area 15.

Total cockle biomass would increase by 24.7-60.3%, from 18 166 tonnes to 22 658-29 128 tonnes. The cockle stock in areas outside Natura 2000 areas would increase from ca. 6 592 tonnes to 10 324-14 801 tonnes.

Relative to blue mussels, which are epibenthic and have a highly gregarious and more stable distribution, cockles are infaunal with spatially and temporally unstable populations in response to favourable and unfavourable conditions (e.g. Dare et al., 2004). The highly clumped distribution of cockles in the Limfjorden (Ivell, 1981, this report) and elsewhere (Dare et al., 2004), requires high-spatial resolution sampling to reduce the likelihood of missing and thus sub-sample dispersed and patchy high-density cockle beds.

The sub-tidal nature of cockle populations in the Limfjorden adds complexity and difficulty to surveys of cockle populations, requiring costly and slower boat-based sampling methods that strongly limit sampling intensity and resolution. In contrast, the vast majority of monitoring or stock assessment programs in European cockle fisheries benefit from intertidal conditions to directly and cost-effectively access and sample cockle beds (e.g. UK, Ireland, Netherlands, France, Spain). Even then, large and intensive surveys are required, which often cannot be implemented.

Relative to intertidal cockle monitoring or stock assessment programs normally conducted in Europe, the 2018 Limfjorden cockle survey had one to two order of magnitude lower station density (0.38 stations/km^2) due to the constraints of boat-based subtidal surveying, but higher sampling area intensity (45 m^2/km^2) from sampling with a dredge (Table 3.1). For comparison (Dare et al., 2004 for a review), the Dutch Wadden Sea (RIVO) with twice the area (ca. 1 400 km^2) surveyed 4,200 stations at 3 stations/km^2, but only at 0.3 m^2/km^2. Similarly, monitoring programs in the UK (e.g. CEFAS) sampled 1.7 to 47 stations/km^2 at 0.2 to 4.8 m^2/km^2. The sampling intensity of the Dutch and UK cockle monitoring programs, together with large area surveyed and the clumped/aggregated distribution of cockle populations, also often resulted in low precision of stock estimates (> 100%; Dare et al., 2004).

The 2018 Limfjorden cockle survey thus had a higher probability of sub-sampling (missing) the dispersed high-density cockle beds than other European surveys, but the higher area sampled in each station integrated the local small-scale patchiness. Unrealistic and impracticable dredge-based large-scale boat surveys would be needed to resolve the scale of spatial variability of cockle populations in the Limfjorden.

3.3.4 Cockle species

Two species of cockles are present in the Limfjorden (Brock 1979, 1980; Ivell 1981), the common cockle *C. edule* and the lagoon cockle *C. glaucum* (see Boyden, 1971 for a description of morphological differences and Figure 3.5). However, both cockle species are fished in the Limfjorden, but are not distinguished by the fishery and landed as the common cockle *C. edule*. Since *C. glaucum* has slower growth and thus lower size-at-age than *C. edule* (Brock, 1979), *C. glaucum* populations likely have longer renewal times than *C. edule* populations. Data obtained in the 2018 cockle survey found that the mean frequency of *C. glaucum* in the Limfjorden was 4.6 ±1.9% (SE) of all cockles but reached 34.9% (±4.0%, SE) in Venø Bugt (7, 8) that supplied 15% of cockle landings in the two previous fishing seasons.

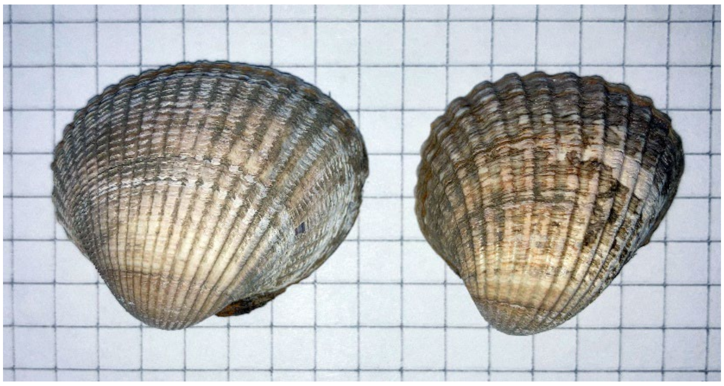

Figure 3.5. Left *Cerastoderma edule*, right *Cerastoderma glaucum* collected in the Limfjorden 2019. Photo P. Freitas.

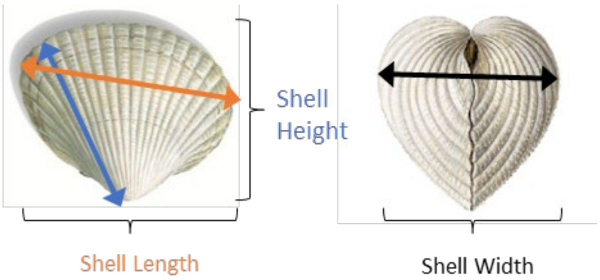

Figure 3.6. The three shell size metrics: Shell height (blue), shell length (orange) and shell width (black).

3.3.5 Size structure

Most cockle fisheries use minimum legal or reference sizes, ranging between 14-22 mm shell width (Figure 3.6), to protect spawning potential and ensure a significant proportion of cockles reach maturity and reproduce (Dare et al. 2004; Southall and Tully, 2014; Hervas et al, 2008). A reference minimum size of 16 mm shell width was assumed for *C. edule* cockles in the Limfjorden, even though it may change in the future if supported by maturity at size/age.

Shell length, height and width ranged between 14.3–48.5, 13.2–41.0 and 9.4–31.1 mm, respectivelly (Figures 3.6 and 3.7). Histograms of shell size, identified three clear modes in shell length and shell height frequencies, but not in shell width (Figure 3.7), supporting the measurement of the three shell linear dimensions to evaluate *C. edule* size structure.

Allometric linear regressions between *C. edule* shell length and height with shell width were significant and strong, allowing the interconversion of the three shell size dimensions (Figure 3.8):

$SL = 1.147\ (\pm 0.221\ SE) + 1.394\ (\pm 0.011\ SE) * SW$

Shell Length ($F_{(1,918)} = 15{,}992$, $p < 0.0001$, RMSE = 1.52, $r^2 = 0.946$)

$SL = 2.495\ (\pm 0.165\ SE) + 1.153\ (\pm 0.008\ SE) * SW$

Shell Height ($F_{(1,915)} = 19{,}674$, $p < 0.0001$, RMSE = 1.13, $r^2 = 0.956$)

The *C. edule* total fresh weight and shell width relationship in the Limfjorden was described by an allometric power equation (Figure 3.9):

$TFW = 0.0022112\ (0.001641\text{–}0.02677\ CI) * SW^{2.7175\ (2.641,\ 2.793\ CI)}$

$r^2 = 0.922$, n = 958

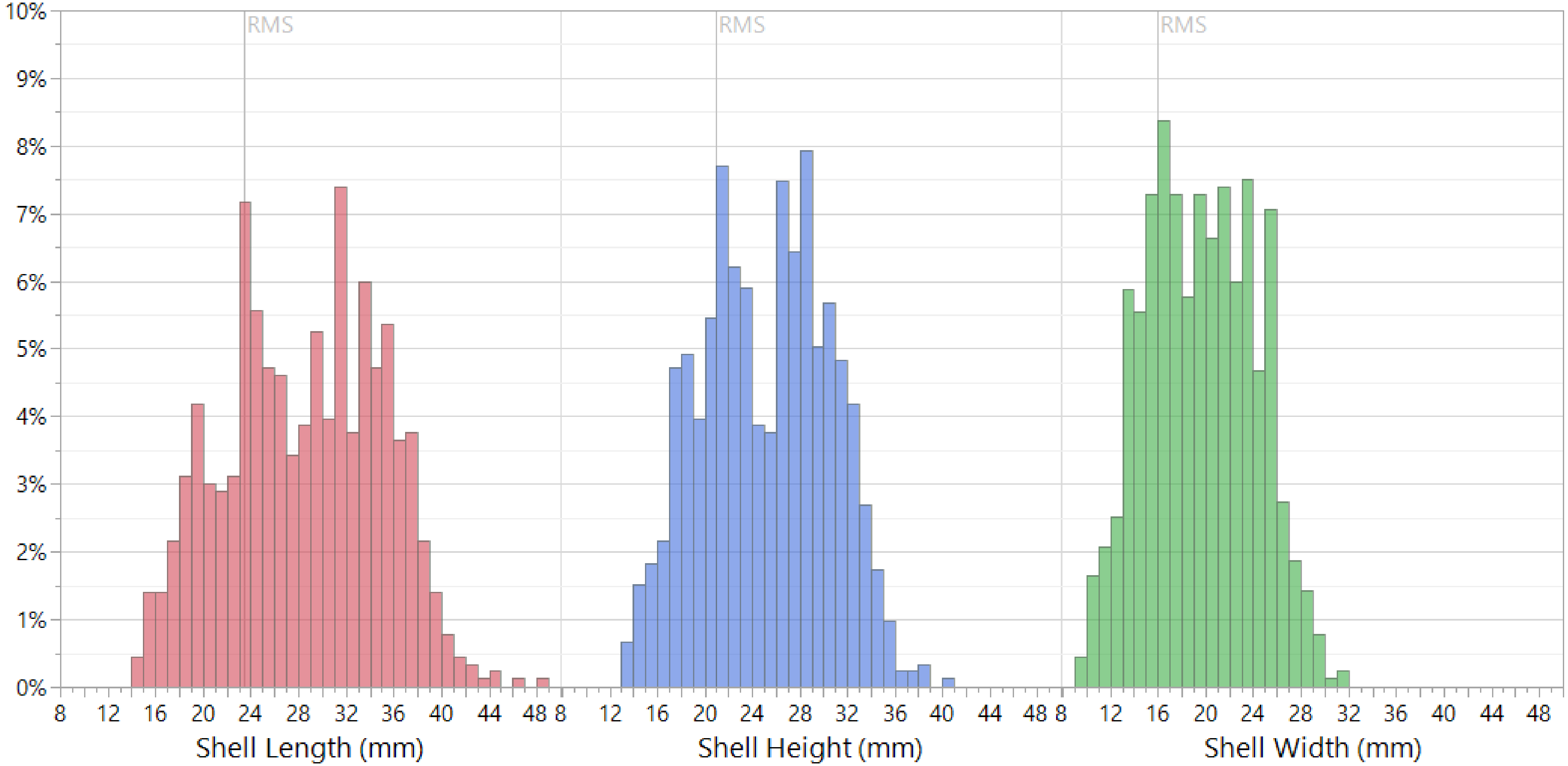

Figure 3.7. Histograms of shell length, shell height and shell width (mm) of the common cockle *C. edule* in the Limfjorden in April 2018. Grey lines mark reference minimum size based on 16 mm shell width, of 23.4mm shell length and 20.9 mm shell height.

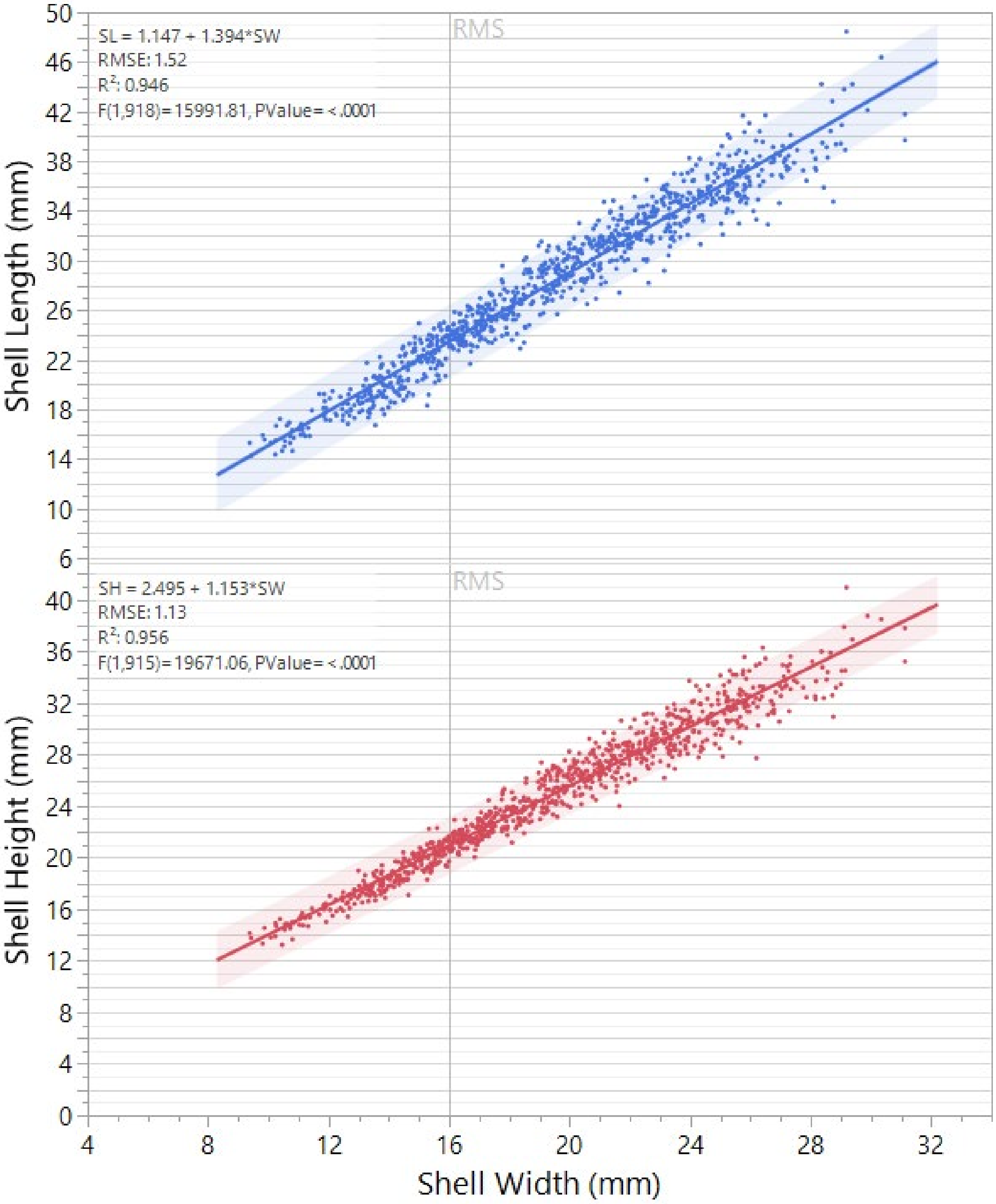

Figure 3.8. Allometric linear regression of shell width (mm) with shell length and height (mm) of the common cockle *C. edule* in the Limfjorden. Shaded areas are 95% CI of fit (dark) and 95%CI if prediction (light). Grey line is the reference minimum size of 16 mm shell width.

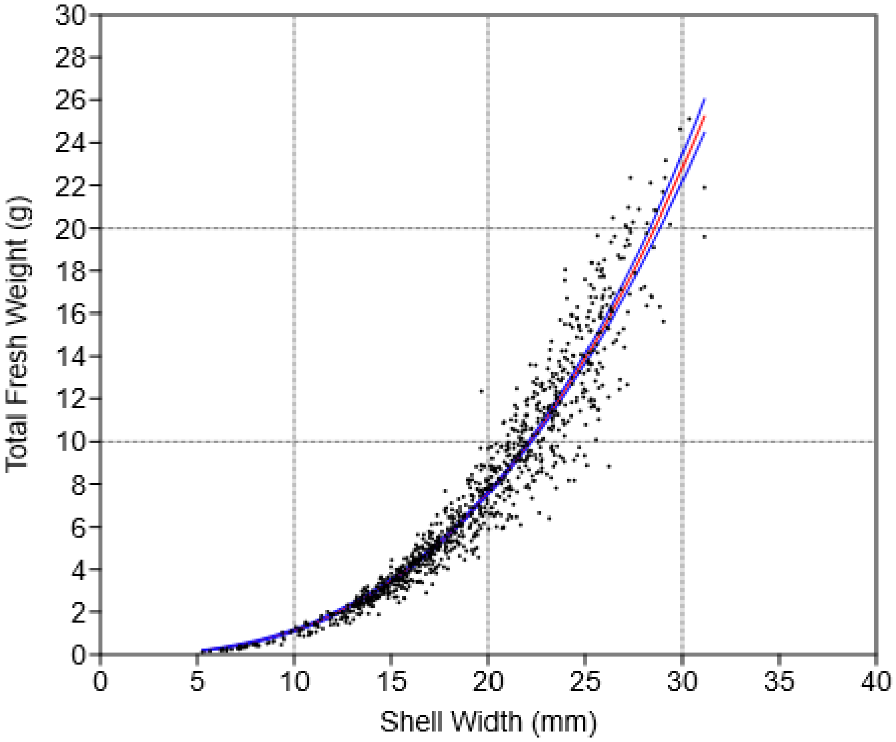

Figure 3.9. Total fresh weight and shell width allometric relationship for the common cockle *C. edule* in the Limfjorden. Blue lines are 95% CI of fit.

3.3.6 Age structure

Multiple age cohorts of the common cockle *C. edule* occurred in most basins, indicating successful recruitment in several previous years (Table 3.5). Two, three and four year old cohorts dominated age structure across the Limfjorden, with a small proportion being 5 years or older.

In individual basins, one to two age cohorts usually dominated the population age structure. The most recent age cohort (one year-old) is underrepresented since it was not quantitatively sampled (i.e. smaller than the dredge grid size).

Size-at-age showed that 54% and 94% of cockles at age of 2 and 3 years were above the reference minimum size of 16 mm shell width (Table 3.6).

The relationship between shell size and age (i.e., growth plots) for the common cockle *C. edule* is shown in Figure 3.10. Shell length was used instead of shell width to allow comparison with literature. As expected, shell length increased with age, but with significant variability and strength of the fit was low ($r^2 = 0.46$; Table 3.7). Growth rate (*K*) was 1.592 year^{-1} while overall growth performance (ϕ, Pauly and Munro, 1984), which reflects growth rate and maximum length, was 3.20 (Table 3.7). Growth rate was the highest and overall growth performance was one of the highest reported in the literature (see Mahony et al., 2022 for a review), even compared to other subtidal *C. edule* populations (Cardoso, 2007).

Table 3.5. Proportion of the common cockle *C. edule* in each age cohort in the Limfjorden. Age corresponds to the number of annual winter growth lines in the shell, e.g. a cockle with age 2 year settled in the spring-summer of 2016, deposited two winter growth lines during 2017 and 2018 and was sampled in spring 2018. Data normalized by cockle density.

	Settlement	2017	2016	2015	2014	2013	≤ 2012	**Biomass**		
Fishing area	**Age**	1	2	3	4	5	≥ 6	**%**	**Stations**	**N**
Nissum Bredning (1-4)			1.5	**93.5**	5.1			60.1	5	123
Venø Bugt (7-8)			1.8	**62.8**	**31.7**	3.7	0.02	21.0	5	153
Kås Bredning (9)		6.8	**29.0**	1.8	**40.3**	16.5	5.5	13.8	14	563
Sallingsund (13)			**55.1**	**37.3**	7.5			3.9	2	34
Agerø Sund (23, 24)		**96.4**	3.6					0.6	2	50
Visby Bredning (25, 26)		**100**						0.6	2	50
All Limfjorden		2.7	7.4	**70.2**	15.2	3.4	1.0	100	30	973
Fishing areas (7-9, 13, 25-26)		6.9	**17.9**	**29.9**	**33.2**	9.5	2.7	39.2	23	800
Non-fishing areas (1-4, 23-24)		0.4	1.5	**93.1**	5.1			60.7	7	173

Table 3.6. Proportion of cockles in each age cohort larger than reference minimum size (shell width > 16 mm). Derived from all annual increments in each cockle shell, e.g. a cockle with an age of 3 years has 3 size measurements at the annual winter lines corresponding to ages 1, 2 and 3 years. Two stations were excluded as their extremely small size-at-age with the highest densities in the survey strongly skewed the analysis. Data normalized by cockle density.

	% > reference minimum size					
Fishing Area Age Cohort	1	2	3	≥4	Stations	N
Nissum Bredning (1-4)	0.2	54	89	100	4	98
Venø Bugt (7-8)		52	99	100	4	103
Kås Bredning (9)	3	54	99	100	14	563
Sallingsund (13)	4	79	100	100	2	34
Agerø Sund (23, 24)	48	100			2	50
Visby Bredning (25, 26)	75				2	50
All Limfjorden	4	56	94	100	28	8
Fishing areas (7-9, 13, 25-26)	7	57	99	100	22	750
Non-fishing areas (1-4, 23-24)	1	55	89	100	6	148

Table 3.7. Von Bertalanffy growth constants for the common cockle C. edule in the Limfjorden using shell length (longest growth axis).

	C. edule
N	700
L_∞ (mm)	31.65
K (year^{-1})	1.592
t_0 (year)	0.3156
ϕ	3.203
r^2	0.461
p	0.001

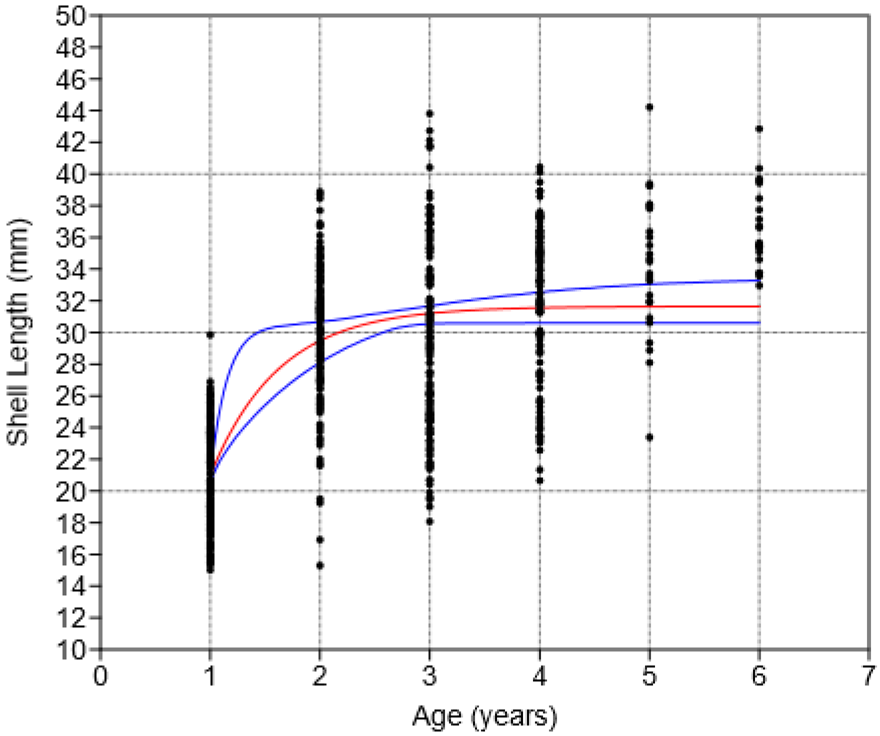

Figure 3.10. Relationship between shell length and age of the common cockle *C. edule* (n = 689).

3.4 Conclusions

Cockle biomass estimates as described by the DTU Aqua 2018 cockle survey must be considered as a significant underestimation, which resulted both from the compromise and constraints presented above that determined survey sampling approach and from the patchy distribution of cockles. A significant variability is always expected and commonly occurs in cockle stock assessments even in intertidal habitats (e.g. Dare et al., 2004). Future assessment of cockle populations in the Limfjorden must use an alternative cost-effective approach that ensures high station density to avoid missing any significant cockle beds, increasing the accuracy and precision of estimates.

Multiple cohorts were observed, indicating successful recruitment over several years, usually with one or two cohorts dominating in individual basins of the estuary. Growth parameters indicated high overall growth performance compared to other locations in Europe. At least 54% and 94% of 2 and 3 year old cockles were larger than the reference minimum size of 16 mm shell width.

Two cockle species are fished in the Limfjorden but are not distinguished by the fishery and landed as the common cockle *C. edule*. While the prevalence of the lagoon cockle *C. glaucum* was low at ca. 5%, it reached ca. 35% in one basin that has at times provided significant landings.

3.5 References

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4. Surfaced and buried cockles, *Cerastoderma edule* and *Cerastoderma glaucum*, in the Limfjorden

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4.1 Rationale

The common cockle (*Cerastoderma edule*) is a widespread and abundant bivalve present in estuaries and coastal European waters, ranging from the Barents Sea to West Africa and the Mediterranean Sea (Dabouineau and Ponsero, 2009; Malham et al., 2012 for reviews of cockle biology). The common cockle is an infaunal bivalve mostly buried in the top 5 cm of the sediment, with preferred habitats including sandy sediments, but also gravel and mud (http://www.fao.org/fishery/species/3535/en). The common cockle is an ecologically and economically important species, (e.g. Carss et al., 2020 for a recent review; landings from Eurostat), which can significantly engineer and modify the ecosystem it inhabits (e.g. Donadi et al., 2015), namely affecting bioturbation and sediment dynamics (e.g. Ciutat et al., 2006; Dairain et al 2020).

The lagoon cockle (*Cerastoderma glaucum, syn. C. lamarcki*) is morphologically similar and close relative of the common cockle, often with overlapping distributions, but preferring brackish water habitats further inshore (Boyden, 1971; Boyden and Russel, 1972; Russel and Petersen, 1973; Brock 1979; Brock, 1980; Malham et al., 2012). Both cockle species occur in the Limfjorden, often together in sympatric populations (Brock 1980; Ivell, 1981), and can be distinguished morphologically (Parada, 2018). *C. edule* has been described as growing faster than *C. glaucum*, with the latter species having a lower burrowing ability (Brock, 1979).

Several cockle species, including *C. edule*, are known to occasionally emerge onto the sediment surface, often in large quantities and associated with increased mortality or reduced condition (Trueman et al., 1966; 1983; Jonsson and André, 1992; Richardson et al., 1993; Blanchet et al., 2003; Mouritsen and Poulin, 2003, Mouritsen, 2004; Tompkins et al., 2004; Thieltges, 2006; Morgan et al. 2012).

Different causes have been attributed for the surfacing behaviour in bivalves and in cockles in particular, from environmental factors such as thermal stress (Zhou et al., 2022); a response to sediment cover and physical disturbance (Richardson et al., 1993; Richardson et al., 1999); hypoxia (Mouritsen, 2004; Marsden and Bressington, 2009 Lewis and DeWitt, 2017), but also biotic factors, such as density (Richardson et al., 1999), bioturbation or macroalgae cover (Mouritsen, 2004; Marsden and Bressington, 2009), neoplasia disease along with spawning related stress (Morgan et al., 2012), and even as part of normal behaviour, which can be modulated by diurnal and tidal cycles (Richardson et al., 1993).

Cockle surfacing behaviour has often been attributed to parasite or bacteria infestation (Jonsson and André, 1992; Thomas and Poulin, 1998; Mouritsen, 2002; Blanchet et al., 2003; Tompkins et al., 2004) acting through a reduced ability to re-burrow by damage to the foot tissue and functionality (Jonsson and André, 1992; Thomas and Poulin, 1998; Desclaux et al., 2002; Mouritsen, 2002; Blanchet et al., 2003; Tompkins et al., 2004) or a parasite-induced “favourisation/facilitation” mechanisms or impaired burial ability (Jonsson and André, 1992; Thomas and Poulin, 1998; Desclaux et al., 2002; Mouritsen, 2002; Mouritsen, 2004; Thieltges, 2006; Fermer et al., 2011). Neoplasia disease

(Morgan et al., 2012); and bacteria (Blanchet et al., 2003), increase the likelihood of parasite transmission (Bowers et al., 1996; Thomas and Poulin, 1998; Thomas et al., 1998). Parasite “favourisation”, however, has been discarded as a cause of cockle surfacing in more recent studies (Desclaux et al. 2002; Tompkins et al 2004; Fermer et al., 2011). Additionally, opposing results on the role of parasites and bacteria as a cause or consequence of cockle surfacing have been found (e.g. Blanchet et al., 2003; Malham et al., 2012; Morgan et al., 2012).

However, all previous studies have focused on cockles in their more common intertidal sandy habitats with significant tidal regimes (e.g. Dare et al., 2004; Dabouineau and Ponsero, 2011; Malham et al., 2012), contrasting with the subtidal muddy habitat of cockle populations in the Limfjorden, Denmark, which may affect processes of cockle surfacing and burying behaviour and survival.

Anecdotal observation of surfaced cockles in the Limfjorden was first reported by fishermen and divers since the late 2000’s and early 2010’s in parallel with increasing catches of cockles in the blue mussel fishery. In the Limfjorden, the surfacing behaviour of cockles presents two distinct considerations that are relevant from the point of view of the cockle fishery. Once on the surface of the sediment, cockles were expected to have an increased availability to the surface dredge used in the blue mussel fishery, even though the dredge also captures buried cockles (Chapter 7, this report). The increased mortality of surfaced cockles, often in mass mortality events (e.g. Thieltges 2006), has often been seen as a justification of fishing surfaced cockles which would otherwise likely die.

In this task we approached and investigated the phenomenon of cockle surfacing in the Limfjorden from three complementary perspectives with the purpose of evaluating differences between surfaced and buried cockles and possible causes of surfacing:

1. An *in-situ* assessment of differences in abundance, size, condition, and pathogen and parasite load of surfaced and buried fractions of a cockle population (presented in this chapter).
2. An experiment on the effect of density and oxygen depletion in the surfacing and burial of *C. edule* and *C. glaucum* (presented in chapter 5).

4.2 Aims

The aim of this task was to observe potential differences in cockle abundance between surfacing and non-surfacing areas, but also differences in size, age and condition between surfaced and buried cockles. Even though causation cannot be attributed to these variables, as they may reflect either a cause or a consequence of surfacing, results contribute to the understanding of potential causes and impacts of cockle surfacing in the Limfjorden. This task surveyed a cockle bed with surfacing and non-surfacing cockle areas in relative proximity (up to 100 m apart), sampling separately cockles that were surfaced out of the sediment from buried cockles. Collected cockles were also screened for pathogens and maturity (Chapter 5, this report)

4.3 Methods

A non-fished cockle bed in a Natura 2000 area was sampled at ca. 5.5 m depth in parallel with the fishing efficiency trials (Chapter 7, this report) in Venø Bugt fishing area 7 (“Muslingeområde” 7, Figure 4.1) on the 26-28 August 2019.

Figure 4.1. The location of fishing efficiency trial site (white area) in Venø Bugt, production area 7. Yellow lines delimit Natura 2000 area. Image from Google Earth.

Samples were collected by divers using quadrat frames (0.25 m^2) and two vertical fractions of the cockle population were sampled separately with a sliding metal plate to guide the depth of sampling into the sediment: A surface fraction (any cockles emerged from the bottom surface) and a buried fraction (cockles buried and covered by sediment). The latter was separated in two separate fractions: cockles from the top 2 cm and from 2-6 cm from the surface.

Surfacing definition

The cockle population were considered as surfacing or non-surfacing based on analysis of video images and on cockle density in the surface fraction. Samples were subjectively classified as surfacing, thus assumed to represent massive surfacing, if the number of cockles exposed on the sediment surface was >28 cockles/m^2 (e.g. 7 cockles in the 0.25 m^2 sampling quadrat; Table 4.1 and Figures 4.2 & 4.3). Samples were subjectively classified as non-surfacing if the number of fully exposed cockles on the sediment surface was <28 cockles/m^2 (Table 4.1 and Figures 4.2 & 4.3).

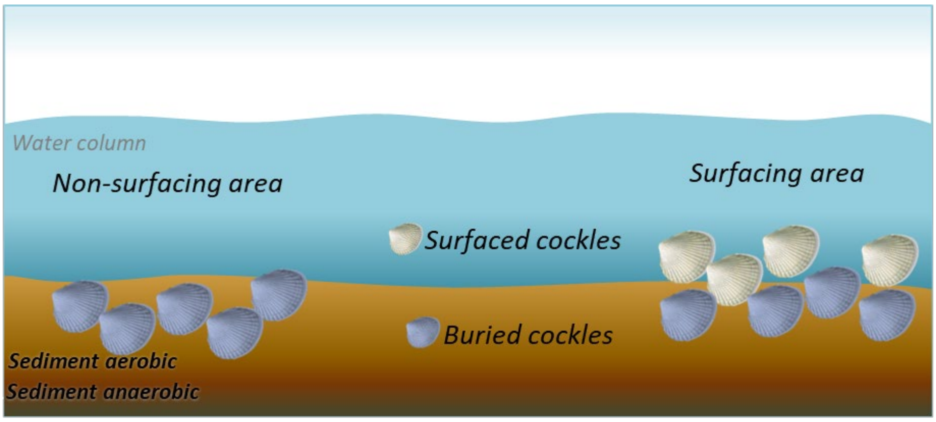

Figure 4.2. Schematic representation of surfacing cockles vs buried cockles in a non-surfacing area vs a surfacing area.

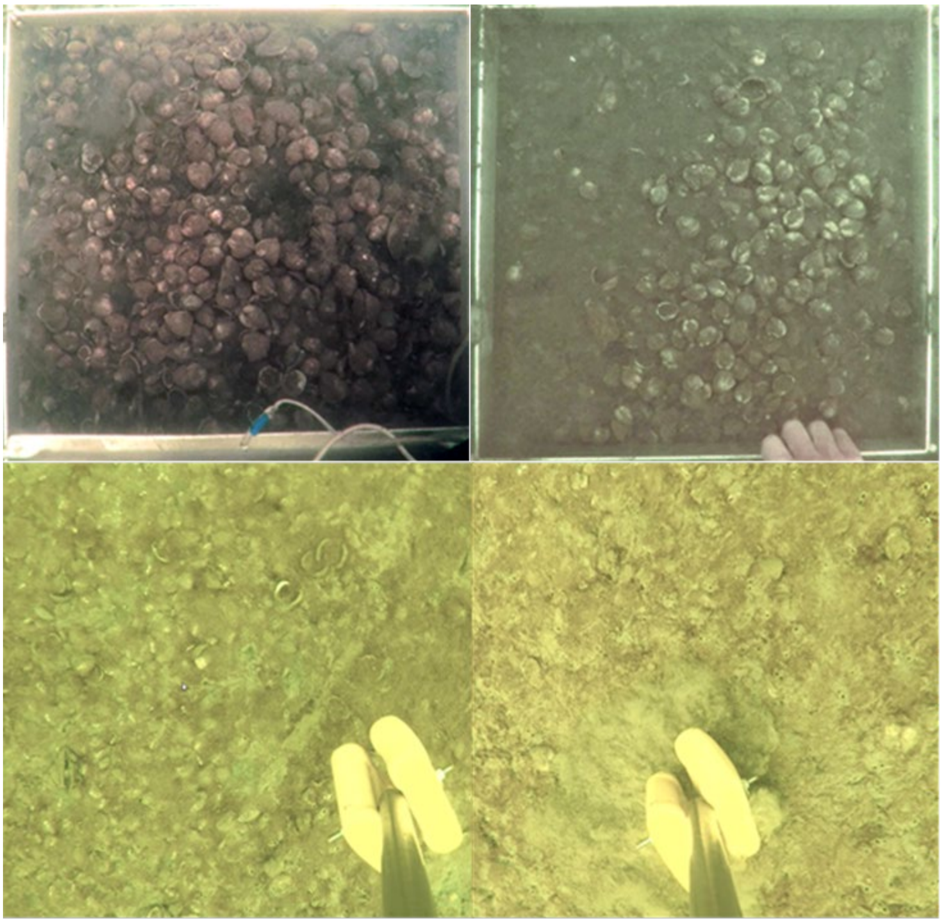

Figure 4.3. Examples of surfacing and non-surfacing areas. Top: massive surfacing of cockles, with a significant area of the bottom is covered with live cockles. Bottom: two common distinct visual aspects of non-surfacing areas. Left: most cockles are at least partially buried with only a few out of the sediment (e.g. left of the yellow weights). Right: no cockles on the surface, with only siphons of buried cockles visible (e.g. above the yellow blocks). Photos Top C. Saurel, Bottom P. Freitas.

Cockle abundance, size, weight and condition

The fresh weight and number of live cockles, as well as wet weight of dead cockle shells of the two cockle population fractions were measured on board. Cockles were frozen and morphometric measurements done in the laboratory after thawing: shell width (SW), height and shell length (SL), and shell dry weight (SDW) and tissue ash-free dry weight (AFDW).

A condition index as calculated as the AFDW/SDW * 1000 ratio. Volume of cockles was determined assuming an ellipsoid shape from shell width, height and length, and cockles were assumed to be in the top 4 cm of sediment.

Pathogens and life cycle

Sampled cockles were dissected and pathogens and sex determination and maturity analysed following the methodology described in Chapter 6 in this report.

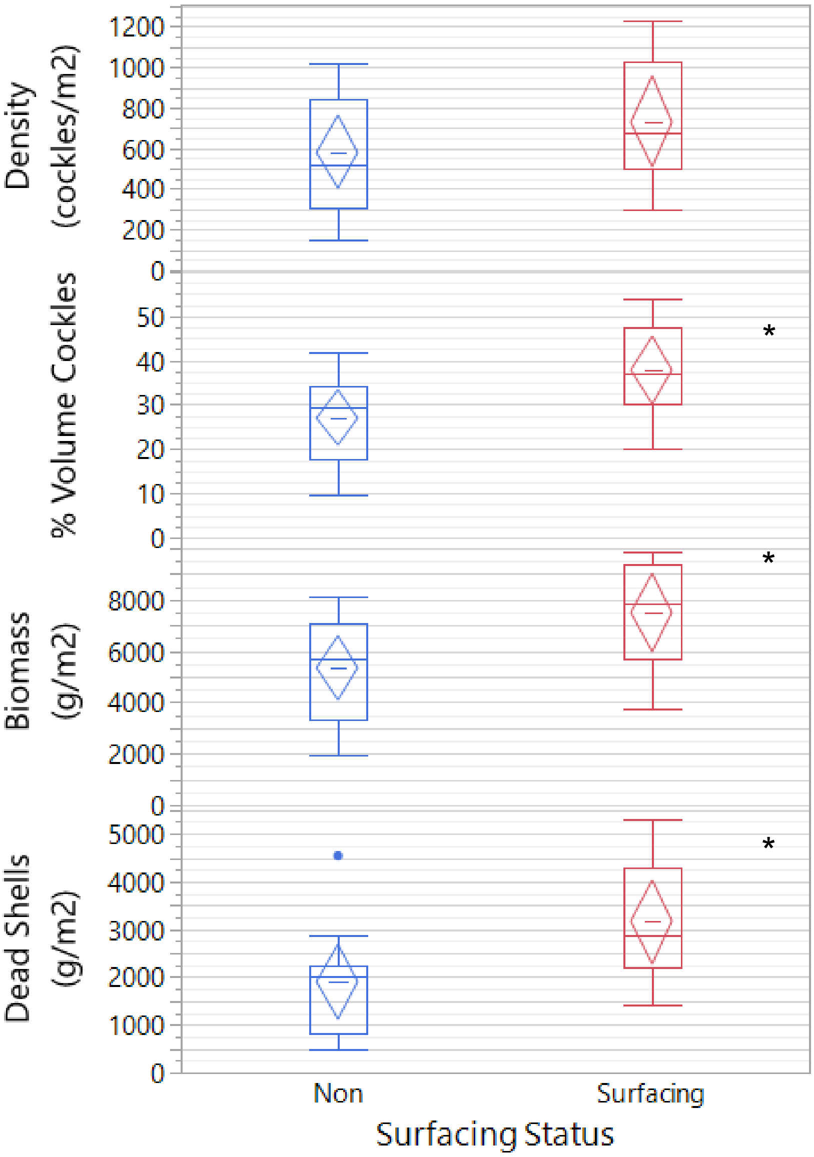

Figure 4.4. Box-plots of cockle density (cockles/m^2), % of top two cm sediment volume occupied by cockles, biomass (g/m^2) and dead shell abundance (g/m^2) in surfacing and non-surfacing areas. Open diamonds are 95% confidence intervals for the mean. *Significant at $p < 0.05$.

4.4 Results

Surfacing and non-surfacing areas: Density and biomass

Comparison of cockle abundance in surfacing and non-surfacing areas, considering total abundance of both cockle species, showed that abundance varied according to surfacing status, albeit not always statistically significant (Table 4.1 and Figure 4.4).-Cockle density did not differ between non-surfacing and surfacing areas (ANOVA, $F_{(1, 21)}$ = 1.38, p = 0.253).

However, cockle biomass was significantly higher in surfacing areas than in non-surfacing areas (ANOVA, $F_{(1, 21)}$ = 5.82, p = 0.026), as was also the mass of dead shells (ANOVA, $F_{(1, 21)}$ = 5.73, p = 0.027; Table 4.1 and Figure 4.4). The abundance of surfaced cockles in both areas was significantly correlated to the combined cockle biomass and dead shells mass (g/m^2; $\ln_{x+1}$ transformed cockle abundance: r^2 = 0.53, RMSE = 1.34, p = 0.0002, n = 21).

The volume occupied by cockles in the top two cm of the sediment was significantly higher in surfacing than in non-surfacing areas (ANOVA, $F_{(1, 21)}$ = 6.05, p = 0.023; Table 4.1).

Surfacing and non-surfacing areas: size, weight and condition
Comparison between surfacing (including both surface and buried fractions) and non-surfacing (including only buried fraction) areas of the same bed showed that cockle size, weight and condition varied with surfacing status, although not always in the same way in the two cockle species (Table 4.2 and Figure 4.5):

Regarding body size, both the surfacing status (log-transformed ANOVA, $F_{(1, 469)}$ = 12.92 and 6.07, p < 0.001 and p = 0.014, respectively) and species had significant effects on shell width and length ($F_{(1, 469)}$ = 8.99 and 35.32, p =0.003 and p < 0.0001, respectively), i.e. **cockle shells were larger and wider in surfacing areas and in *C. edule*** relative to buried and *C. glaucum* respectively (Table 4.2 and Figure 4.5). Body size as indicated by shell width and shell length were not significantly influenced by the interaction of species with the surfacing status of the population (log-transformed ANOVA, $F_{(1, 469)}$ = 0.40 and 0.22, p = 0.526 and p = 0.643, respectively).

Shell dry weight was significantly affected by both the surfacing status and species (log-transformed ANOVA, $F_{(1, 471)}$ = 9.46 and 9.50, p = 0.002, respectively), i.e. **cockle shells were heavier in surfacing areas and in *C. edule*** relative to buried and *C. glaucum* respectively (Table 4.2 and Figure 4.5). This result is expected since shell size was larger in surfaced cockles and in *C. edule* as described in the previous paragraph, and thus shell dry weight should be heavier. However, relative to shell size, shell dry weight was not affected by surfacing status, but was affected by species being heavier in *C. glaucum* (log-transformed ANCOVA, shell length as covariate, $F_{(1, 470)}$ = 3.55, p = 0.060 and 34.54, p < 0.0001, respectively). Shell dry weight was not significantly influenced by the interaction of species with the surfacing status of the population (log-transformed ANCOVA, $F_{(1, 469)}$ = 0.33, p = 0.563).

The amount of soft tissue as indicated by ash-free dry weight was not signficantly different according to surfacing status (ANOVA, $F_{(1, 190)}$ = 1.80, p = 0.181), but was significantly different between species (ANOVA, $F_{(1, 190)}$ = 14.46, p = 0.0002), i.e. **soft tissue was heavier in *C. edule* than in *C. glaucum*** (Table 4.2 and Figure 4.5). However, relative to shell size, ash-free dry weight was affected by both surfacing status and species (ANCOVA, shell length as covariate, $F_{(1, 188)}$ = 9.45, p = 0.0024 and 7.81, p = 0.0057, respectively). Ash-free dry weight was not significantly influenced by the interaction of species with the surfacing status of the population (ANCOVA, $F_{(1, 188)}$ = 0.02, p = 0.897).

Condition of cockles was significantly different according to surfacing status and species (ANOVA, $F_{(1, 188)}$ = 12.94 and 41.36, p = 0.0004 and p < 0.0001, respectively), i.e. , **Condition was lower in surfacing areas than in non-surfacing areas and in *C. glaucum* than in *C. edule*** (Table 4.2 and Figure 4.5). The condition of cockles, was not significantly influenced by the interaction of species with the surfacing status of the population (ANOVA, $F_{(1, 187)}$ = 0.64, p = 0.423).

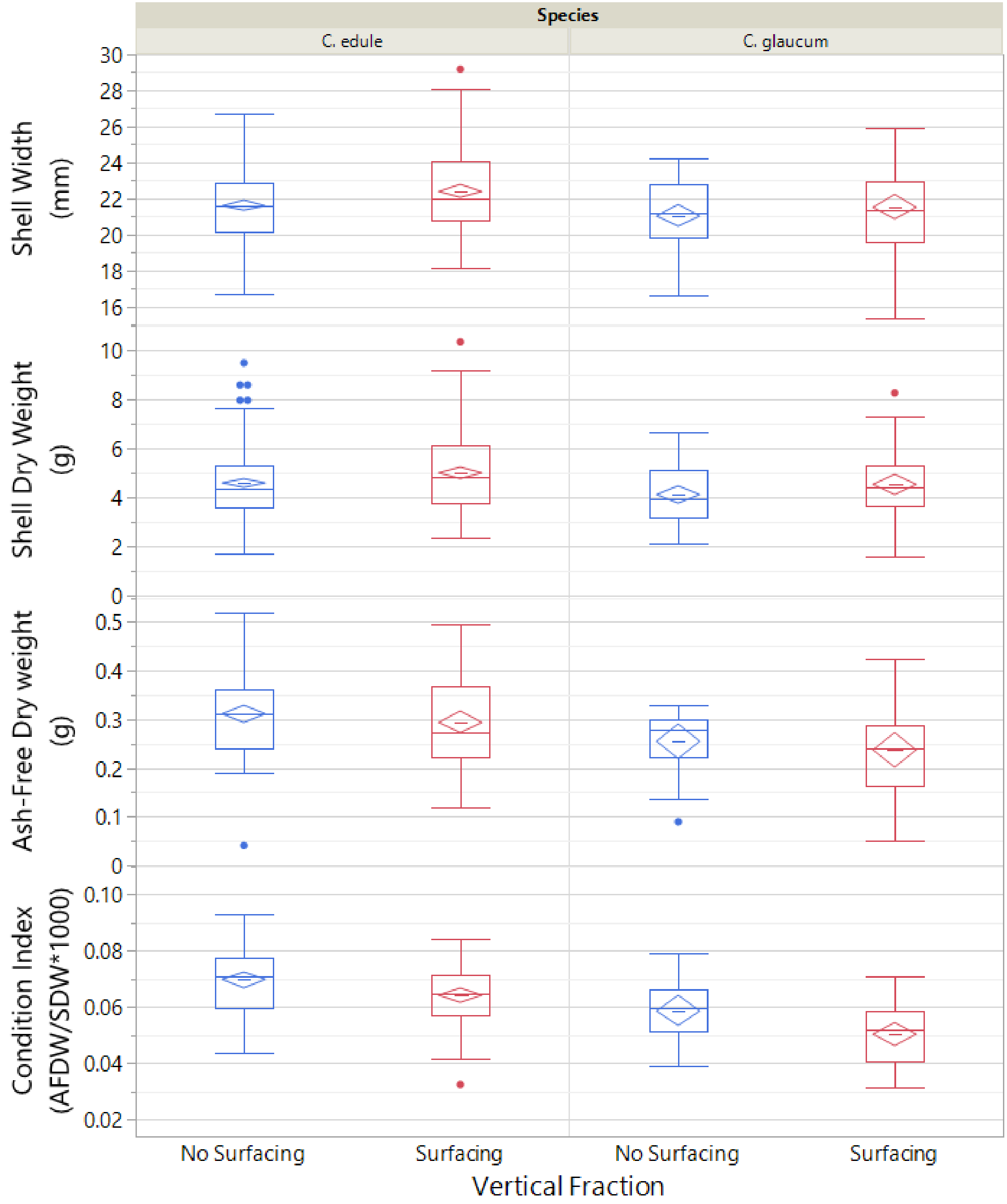

Figure 4.5. Box-plots of cockles *C. edule* and *C. glaucum* shell width, shell dry weight, ash-free dry weight and condition index in surfacing and non-surfacing areas. Open diamonds are 95% confidence intervals for the mean. Refer to text for statistical significance.

Regarding buried cockles only, the size of buried cockles was different between surfacing and non-surfacing areas, with **shell width, shell length and shell dry weight were all larger in surfacing areas** (log-transformed, ANOVA, $F_{(1,\ 358)} > 7.42$, $p < 0.007$ for all). Only shell length was different between species, being larger in *C. edule* (log-transformed, ANOVA, $F_{(1,\ 358)}$ 16,99, $p < 0.0001$).

Table 4.1. Cockle density (cockles/m^2), % of top two cm sediment volume occupied by cockles, biomass (g/m^2) and dead shells (g/m^2) per surfacing status. Error is standard error, Min. is minimum and Max. is maximum value. * Significantly different at $p < 0.05$.

		Density			**% Volume**			**Biomass**			**Dead Shells**		
	N	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Non-surfacing	12	583	152	1016	27.2	9.8	41.8	5372*	1920	8108	1903*	488	4544
		±81			±2.8			±572			±347		
Surfacing	10	732	296	1232	37.8	20.0	53.9	7491*	3720	9892	3158*	1412	5288
		±99			±3.4			±674			±396		

Table 4.2. Cockle size as shell width (SW, mm) and shell dry weight (SDW, g), ash-free dry weight (AFDW, g) and condition index (CI: AFDW/SDW) of surfaced and buried cockles in surfacing areas. Error is standard error, Min. is minimum and Max. is maximum value. Refer to text for statistical significance.

Species		**SW**			**SL**			**SDW**				**AFDW**			**CI**		
Status	N	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	N	Mean	Min.	Max.	Mean	Min.	Max.
C. edule																	
Non-surfacing	209	21.61	16.68	26.74	30.79	23.20	38.24	4.57	1.68	9.48	80	0.311	0.041	0.520	69.67	43.97	93.07
		±0.14			±0.20			±0.09				±0.009			±1.36		
Surfacing	171	22.41	18.14	29.18	31.54	23.50	38.53	5.02	2.36	10.34	68	0.295	0.119	0.497	64.39	32.45	84.46
		±0.18			±0.24			±0.12				±0.011			±1.35		
C. glaucum																	
Non-surfacing	43	21.04	16.58	24.22	28.94	23.66	34.10	4.13	2.11	6.63	16	0.256	0.090	0.331	58.80	39.34	79.28
		±0.29			±0.38			±0.17				±0.017			±2.56		
Surfacing	51	21.53	15.31	25.91	29.38	20.45	34.99	4.52	1.58	8.26	27	0.237	0.050	0.425	50.27	31.47	71.17
		±0.33			±0.41			±0.21				±0.018			±1.99		

Relative to shell size, **shell dry weight of buried cockles was not affected by surfacing status, but was affect by species being heavier in *C. glaucum*** (ANCOVA, shell length as covariate, $F_{(1,\,357)} = 2.20$, $p = 0.139$ and 33.19, $p < 0.0001$, respectively). The interaction of species with the surfacing status of the population did not affect shell size and dry weight (log-transformed ANOVA, $F_{(1,\,358)} = 0.32$, $p = 0.572$; 0.11, $p = 0.735$ and ANCOVA, shell length as covariate $F_{(1,\,357)} = 1.68$, $p = 0.196$, respectively).

The amount of soft tissue in buried cockles, ash-free dry weight, was not different according to surfacing status (ANCOVA, shell length as covariate $F_{(1,\,143)} = 0.43$, $p = 0.514$), but **was different by species being larger in *C. edule*** (ANCOVA, shell length as covariate $F_{(1,\,143)} = 6.00$, $p = 0.0156$).The interaction of species with the surfacing status of the population did not affect ash free dry weight (ANCOVA, shell length as covariate $F_{(1,\,142)} = 0.034$, $p = 0.854$, respectively).

Similarly, the **condition of buried cockles was not different according to surfacing** status (ANOVA, $F_{(1,\,142)} = 1.58$, $p = 0.210$), but **differed significantly by species being greater in *C. edule*** (ANOVA, $F_{(1,\,142)} = 26.73$ $p < 0.0001$). The interaction of species with the surfacing status of the population did not affect the condition of buried cockles (ANOVA, $F_{(1,\,141)} = 0.30$, $p = 0.586$).

C. edule was mainly two years old, followed by three years old (Figure 4.5). In surfacing areas, the prevalence of two-year-old cockles increased, while that of 3 years old decreased (Figure 4.5). ***C. glaucum* were older than *C. edule*** with mainly at three and four years of age, but more variable between non-surfacing and surfacing areas (Figure 4.5).

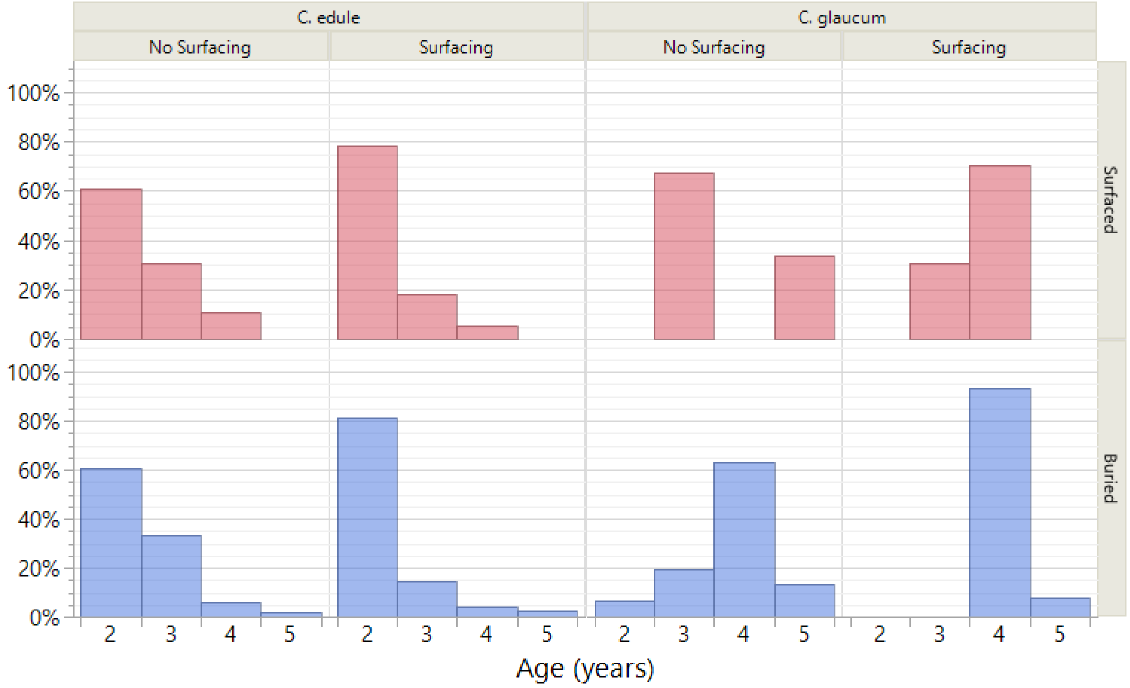

Figure 4.6. Frequency of age of *C. edule* and *C. glaucum* in non-surfacing and surfacing areas and in the buried and surfaces vertical fractions of the population.

C. edule had a higher proportion of males than females 56–62% males to 38–44% females in both non-surfacing and surfacing areas, while in *C. glaucum* the proportion of females to males was similar, ranging between 48–52%, in both non-surfacing and surfacing areas in non-surfacing areas (Figure 4.7).

The percentage of mature cockles in relation to non-surfacing and surfacing beds (Figure 4.7) indicated that there were less mature cockles for both *C. edule* and *C. glaucum* in the buried position of non-surfacing beds.

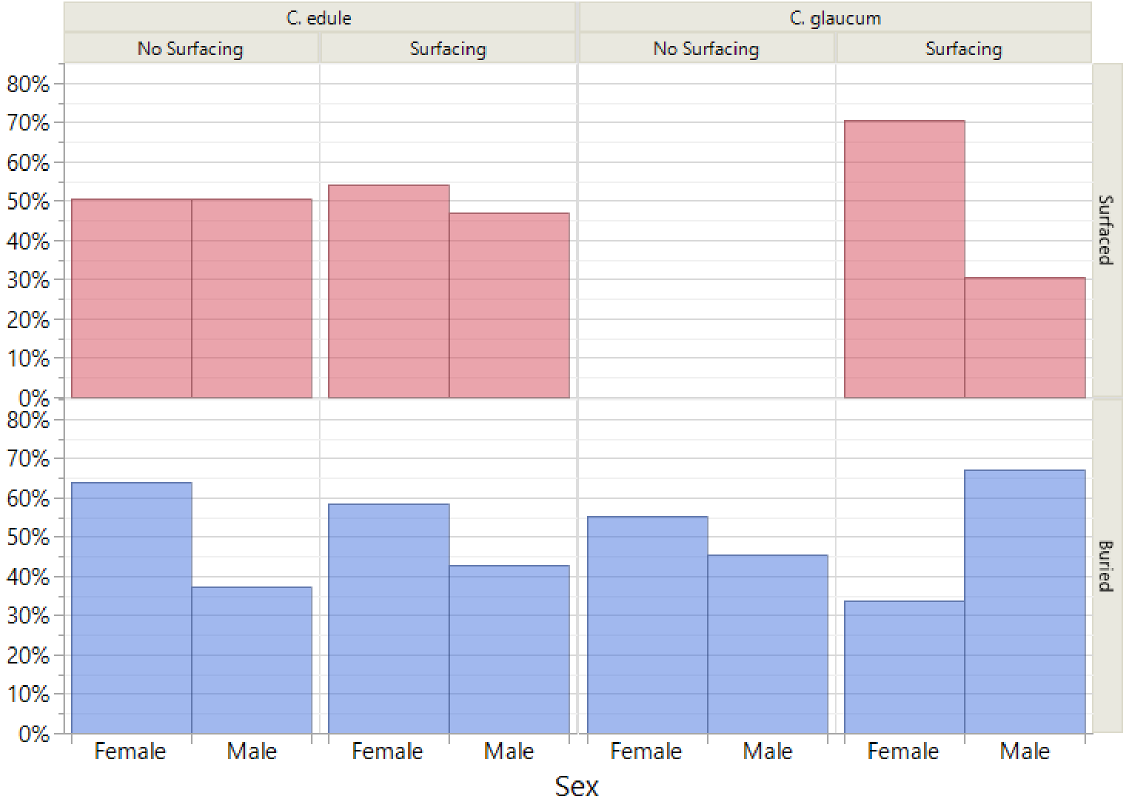

Figure 4.7. Frequency of sex male or female of *C. edule* and *C. glaucum* in non-surfacing and surfacing areas and in the buried and surfaces vertical fractions of the population.

Surfacing areas only: Comparison between surfaced and buried cockles

The comparison between surfaced and buried cockles in surfacing areas showed that (Table 4.3 and Figure 4.8):

The abundance of surfaced cockles (cockles/m^2) was unrelated to the abundance of buried cockles ($r^2 = 0.28$, $p = 0.115$, n = 10).

Vertical fraction did not have a significant effect on shell width and length (log-transformed ANOVA, $F_{(1, 219)} = 0.18$ and 1.86, $p = 0.669$ and $p = 0.174$, respectively), i.e. **body size was similar in surfaced and buried cockles from the same surfacing area.** Species had a significant effect on shell width and length (log-transformed ANOVA, $F_{(1, 219)} = 6.13$ and 20.10, $p = 0.014$ and $p < 0.0001$, respectively), i.e. ***C. edule* were longer than *C. glaucum*** (Table 4.3 and Figure 4.8). Shell width and shell length were not significantly influenced by the interaction of species with the vertical fraction of the population (log-transformed ANOVA, $F_{(1, 218)} = 3.75$ and 3.02, $p = 0.054$ and $p = 0.084$, respectively).

Vertical fraction and species both had a significant effect on shell dry weight (log-transformed ANOVA, $F_{(1, 218)} = 4.22$ and 6.09, $p = 0.041$ and $p = 0.014$, respectively). Shell dry weight was significantly influenced by the interaction of species with the vertical fraction of the population (log-transformed ANOVA, $F_{(1, 218)} = 4.53$, p = 0.034), i.e. **shell dry weight was lighter in surfaced *C. glaucum***

cockles, but similar in surfaced and buried *C. edule* (Table 4.3 and Figure 4.8). Relative to shell size, **shell dry weight was not different according to surfacing status**, **but was affect by species** being heavier in *C. glaucum* (ANCOVA, shell length as covariate, $F_{(1, 218)} = 0.07$, $p = 0.787$ and 24.32, $p < 0.0001$, respectively).

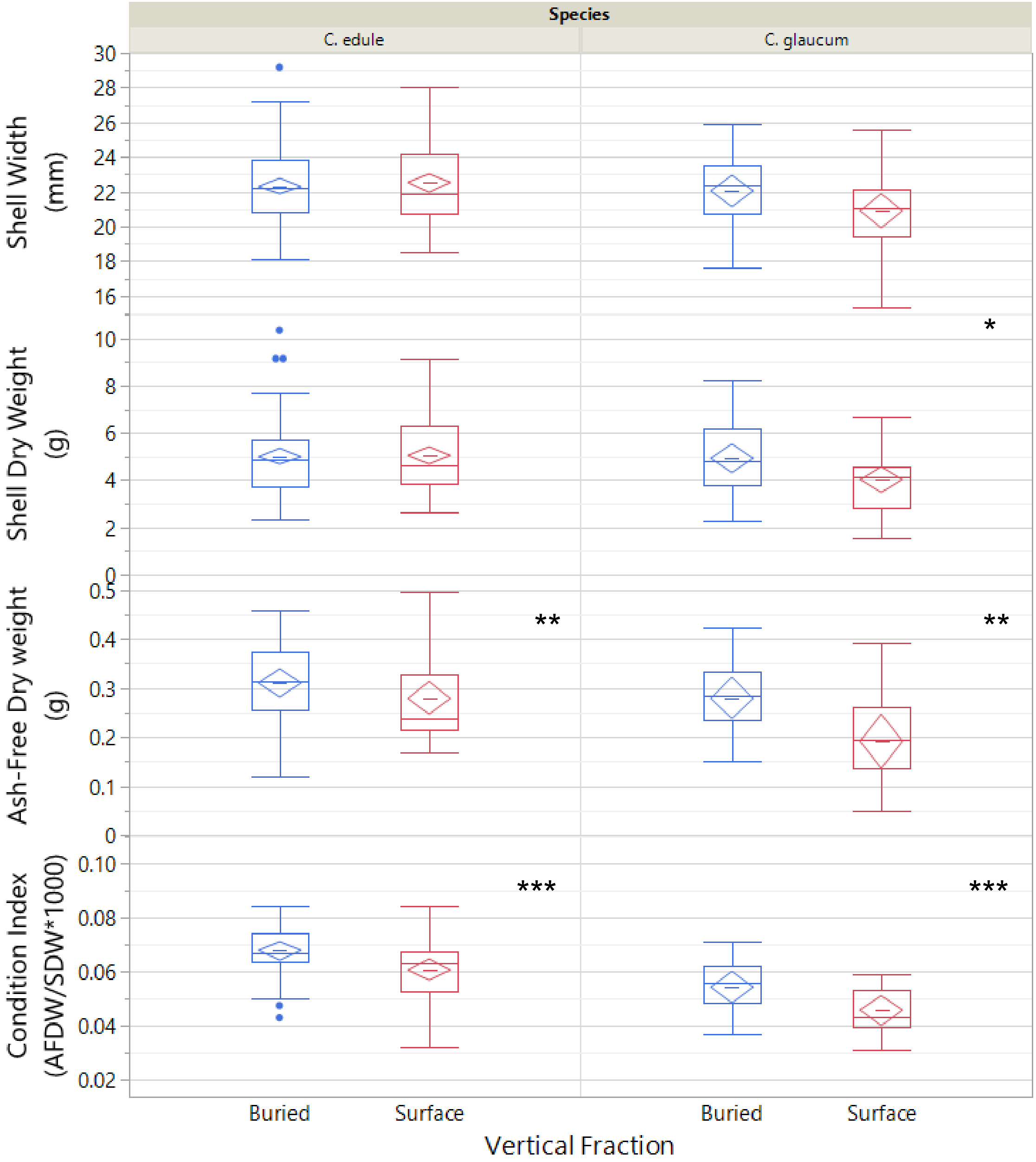

Figure 4.8. Box-plots of shell width, shell dry weight, ash-free dry weight and condition index of surfaced and buried cockles *C. edule* and *C. glaucum* in surfacing areas. Open diamonds are 95% confidence intervals for the mean. Refer to text for statistical significance. * Significant at $p < 0.05$, ** significant at $p < 0.01$, * significant at $p < 0.0001$.**

Table 4.3. Cockle size as shell width (SW, mm) and shell dry weight (SDW, g), ash-free dry weight (AFDW, g) and condition index (CI: AFDW/SDW) of surfaced and buried cockles in surfacing areas. Error is standard error, Min. is minimum and Max. is maximum value. Refer to text for statistical significance.

Species		**SW**			**SL**			**SDW**				**AFDW**			**CI**		
Status	N	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	N	Mean	Min.	Max.	Mean	Min.	Max.
C. edule																	
Buried	95	22.32 ±0.23	18.14	29.18	31.62 ±0.31	23.50	38.53	5.00 ±0.16	2.36	10.34	35	0.310 ±0.014	0.119	0.458	67.78 ±1.64	43.08	84.46
Surfaced	76	22.53 ±0.27	18.55	28.06	31.45 ±0.37	25.32	37.46	5.03 ±0.18	2.66	9.15	33	0.279 ±0.165	0.168	0.497	60.80 ±2.00	32.44	84.11
C. glaucum																	
Buried	28	22.06 ±0.44	17.66	25.91	30.14 ±0.46	25.95	34.88	4.92 ±0.29	2.26	8.26	14	0.279 ±0.019	0.150	0.425	54.35 ±2.67	37.10	71.17
Surfaced	23	20.88 ±0.47	15.31	25.24	28.45 ±0.66	20.45	34.99	4.02 ±0.27	1.58	6.64	13	0.192 ±0.025	0.050	0.392	45.87 ±2.51	31.47	58.95

Both the vertical fraction and species had a significant effect on the amount of soft tissue as indicated by ash-free dry weight (ANOVA, $F_{(1, 92)} = 6.68$ and 8.42, $p = 0.011$ and $p = 0.005$, respectively), i.e. **ash-free dry weight was lower in surfaced cockles and lower in *C. glaucum*** (Table 4.3 and Figure 4.8). Ash-free dry weight was not significantly influenced by the interaction of species with the vertical fraction of the population (log-transformed ANOVA, $F_{(1, 91)} = 1.97$, $p = 0.164$). Relative to shell size, the amount of soft tissue as indicated by ash-free dry weight **was different according to surfacing status and species**, being larger in buried cockles and in *C. edule* (ANCOVA, shell length as covariate, $F_{(1, 91)} = 12.50$, $p = 0.0006$ and 4.92, $p = 0.029$, respectively).

The condition of cockles was significantly affected by both the vertical fraction and species (ANOVA, $F_{(1, 92)} = 12.31$ and 36.61, $p = 0.0007$ and $p < 0.0001$, respectively), i.e. **condition was lower in surfaced cockles and in *C. glaucum*** (Table 4.3 and Figure 4.8). The condition of cockles was not influenced by the interaction of species with the vertical fraction of the population (log-transformed ANOVA, $F_{(1, 91)} = 0.10$, $p = 0.750$).

In surfacing areas, surfaced and buried *C. edule* had a similar age being ca. 80% two years old cockles (Figure 4.5). *C. glaucum* were mainly four years old, but sample size was low and thus results have low certainty (Figure 4.5).

The proportion of females to males in the surfaced and buried fraction of *C. edule* was the same (Figure 4.7), with slightly more females (54–58%) than males (42–46%). In *C. glaucum* on the other hand (Figure 4.7), females (70%) were more prevalent than males (30%) in the surfaced fraction, but the reverse was observed in the buried fraction where males (67%) were more prevalent than females (33%). As with age, sample size for *C. glaucum* was low and thus results have low certainty.

Surfacing and non-surfacing areas: Pathogens

The comparison of number of cockles infected by pathogens between non-surfacing and surfacing areas (the latter with both buried and surfacing cockles) showed that only *C. edule* were infected by *Monorchis parvus* or *Gymnophallus* sp (Table 4.4). In general, buried cockles in both non-surfacing and surfacing areas exhibited more infections for both species than surfaced cockles in surfacing areas.

Regarding *Bonamia* sp. infection, there was significantly more cockles infected in surfacing areas than in non-surfacing areas for both *C. edule* and *C. glaucum* (X^2 (1, N = 253) = 9.23 $p < 0.01$; X^2 (1, N = 134) = 5.68 $p < 0.05$). Buried cockles were more infected in surfacing areas than non-surfacing areas for both *C. edule* and *C. glaucum* (X^2 (1, N = 203) = 13.64 $p < 0.001$; X^2 (1, N = 118) = 6.80 $p < 0.01$). In the surfacing areas only, buried *C. edule* were more infected than surfaced *C. edule*, but the same did not occur in *C. glaucum* (X^2 (1, N = 188) = 16.08 $p < 0.0001$; X^2 (1, N = 77) = 2.42 $p = 0.119$).

A similar trend was found for *Vibrio aestuarianus* infection, where there were significantly more cockles infected in surfacing vs non-surfacing beds for both *C. edule* and *C. glaucum* (X^2 (1, N = 253) = 25.82 $p < 0.0001$; X^2 (1, N = 134) = 13.18 $p < 0.001$). Buried cockles were more infected with *V. aestuarianus* in surfacing areas than in non-surfacing areas for both *C. edule* and *C. glaucum* (X^2 (1, N = 203) = 33.06 $p < 0.0001$; X^2 (1, N = 118) = 9.96 $p < 0.002$). In surfacing areas only, there were more cockles infected in the buried fraction than on the surfaced fraction only for *C. edule*, but not *C. glaucum* (X^2 (1, N = 188) = 8.48 $p < 0.01$; X^2 (1, N = 77) = 1.25 $p = 0.263$).

Table 4.4. Number and percentage of cockle *C. edule* and *C. glaucum* infected by *Bonamia* sp., *Vibrio aestuarianus, Monorchis* parvus, *Gymnophallus choledochus* and *Himasthla* spp. in buried cockles from non-surfacing beds and in both surfaced and buried cockles in surfacing areas.

Species		*Bonamia*		*Vibrio*		*Monorchis*		*Gyn-moph.*		*Hi-masthla*	
Bed types / Status	Total N	N	%	N	%	N	%	N	%	N	%
C. edule	253	25	9.9	164	64.8	6	2.4	2	0.8	5	2
Non-surfacing beds											
Buried	65	1	1.5	25	38.5	1	1.5	0	0.0	0	0.0
Surfacing beds	188	24	12.8	139	73.9	5	2.7	2	1.1	5	2.7
Buried	138	24	17.4	110	79.7	2	1.4	1	0.7	5	3.6
Surfaced	50	0	0.0	29	58.0	3	6.0	1	2.0	0	0.0
C. glaucum	134	5	3.7	17	12.7	0	0.0	0	0.0	2	1.5
Non-surfacing beds											
Buried	57	0	0.0	1	1.8	0	0.0	0	0.0	0	0.0
Surfacing beds	77	5	6.5	16	20.8	0	0.0	0	0.0	2	2.6
Buried	61	5	8.2	11	18.0	0	0.0	0	0.0	1	1.6
Surfaced	16	0	0.0	5	31.3	0	0.0	0	0.0	1	6.3

For *Monorchis* parvus infection, there was no infection in *C. glaucum.* There was no significant difference in infected cockles in surfacing beds vs non-surfacing beds for *C. edule* (X^2 (1, N = 253) = 0.29 *p* = 0.59). The same pattern was observed when comparing the buried fraction of surfacing vs non-surfacing areas (X^2 (1, N = 203) = 0.01 *p* = 0.96). In surfacing areas only, there were not more infected cockles in buried than surfaced *C. edule* (X^2 (1, N = 188) = 2.53 *p* = 0.112).

There was no *Gymnophallus choledochus* infection in *C. glaucum.* Although there was infection in *C. edule,* there was no difference in infection between surfacing vs non-surfacing areas ($X^2_{(1,\ N = 253)}$ = 0.00 *p* = 1). The same applied to buried *C. edule* between surfacing vs non-surfacing areas ($X^2_{(1,\ N = 203)}$ = 0.77 *p* = 0.379). In surfacing areas only, there were not more infected cockles in buried than surfaced *C. edule* ($X^2_{(1,\ N = 188)}$ = 0.50 *p* = 0.479).

Regarding *Himasthla* spp. infection, there was no difference in cockles infected between surfacing and non-surfacing areas for both *C. edule* and *C. glaucum* (X^2 (1, N = 253) = 3.00 *p* = 0.08; X^2 (1, N = 134) = 2.24 *p* = 0.13). However, there was significantly more buried *C. edule* infected in surfacing areas than in non-surfacing areas, but not for *C. glaucum* ($X^2_{(1,\ N = 203)}$ = 3.92 *p* = 0.048; X^2 (1, N = 118) = 1.33 *p* = 0.249). In surfacing areas only, there were not more infected cockles in buried than surfaced cockles, for both *C. edule* and *C. glaucum* ($X^2_{(1,\ N = 188)}$ = 3.14 *p* = 0.076; $X^2_{(1,\ N = 77)}$ = 0.863 *p* = 0.353).

4.5 Conclusions

This study surveyed a cockle bed with surfacing and non-surfacing cockle areas in relative proximity (up to 100 m apart) in August 2019, sampling separately cockles that were surfaced out of the sediment from buried cockles. The aim was to observe potential differences in cockle abundance between surfacing and non-surfacing areas, but also differences in size, age, condition, and pathogen infection rate between surfaced and buried cockles. Even though causation cannot be attributed to these variables, as they may reflect either a cause or a consequence of surfacing, results contribute to the understanding of potential causes and impacts of cockle surfacing in the Limfjorden.

Cockles were larger in surfacing areas than in non-surfacing areas, and also *C. edule* was larger than *C. glaucum*, suggesting higher growth, albeit being slightly younger and density being marginally higher in surfacing areas. Size was similar between surfaced and buried cockles.

The sex ratio of *C. edule* was not found to differ with surfacing, with females being slightly more abundant than males independently of surfacing. However, surfaced *C. glaucum* were mainly females while buried *C. glaucum* were mainly males, but *C. glaucum* results have low certainty due to low sample size. However, buried *C. edule* and *C. glaucum* in non-surfacing areas were less mature than in the surfacing areas, as further discussed in Chapter 6.

Cockle abundance was found to play a role in the surfacing of cockles, albeit not a clear one. Density was similar in surfacing and non-surfacing areas and the abundance of surfaced cockles was not related to the abundance of buried cockles. However, cockle biomass, the volume occupied by cockles in the sediment and the mass of dead shells were all significantly higher in surfacing areas than in non-surfacing areas. In addition, the abundance of surfaced cockles increased significantly with the combined cockle biomass and dead shells mass.

Taken together, these results suggest a potential “crowding” effect on cockle surfacing behaviour. When available space in the sediment for cockles becomes reduced, from cockles themselves, other infauna and dead shells, increased physical contact and disturbance would induce surfacing (Richardson et al., 1993). Nevertheless, the significant variability observed in variables used to assess abundance (e.g. density, biomass or volume), as often occurs in cockle populations, requires a larger sample size to confirm these observations.

Importantly, the condition of cockles was lower in surfacing than in non-surfacing areas and was also lower in surfaced than in buried cockles.

Regarding pathogens, excluding *Bonamia* sp. as it is not a parasite of cockles, only *C. edule* were infected by the parasites taxa identified, *Himasthla spp.*, *M. parvus* and *G. choledochus*, and the bacterium *V. aesturaranius*, while *C. glaucum* was infected only by the first two parasite groups.

Cockle surfacing in the Limfjorden appeared to be associated with higher levels of infection by some of the parasite species observed. Cockles in surfacing areas showed higher infection by *V. aestuaranius* than cockles in non-surfacing areas, and two of the parasite groups were absent from non-surfacing areas, *Himasthla spp.* and *G. choledochus*. However, contrary to the reported higher prevalence of parasite infections in surfaced cockles resulting in reduced ability to re-burrow and higher mortality relative to buried cockles (Jonsson and André, 1992; Desclaux et al., 2002; Blanchet et al., 2003; Thieltges, 2006), surfaced cockles in surfacing areas of the Limfjorden were infected by fewer species and had lower infections than buried cockles by *V. aestuaranius* and *Himasthla spp.*.

Surfaced cockles originate from the buried fraction before surfacing out of the sediment, and thus at the moment of surfacing should have similar parasite prevalence as cockles that remain buried. The three microparasite parasite groups observed were all platyhelminths trematodes with complex and relatively long-life cycles, and at least *M. parvus* and *G. choledochus*.are known to be deleterious and can cause high mortality in cockles (e.g. Longshaw and Malham, 2012; de Montaudoin et al. 2021; See Chapter 6 in this report). *V. aestuaranius* is a bacteria with shorter life-cycle than trematodes and can reach high infection rates and cause high mortality events (de Montaudoin et al. 2021). Therefore, it can be speculated that the lower prevalence of *V. aestuaranius* in surfaced relative to buried cockles may result from high mortatlity of highly infected cockles, with the surviving surfaced cockles being the ones least infected albeit with a lower condition and fitness. However, overall surfacing beds had circa twice as high prevalence of *V. aestuaranius* than non-surfacing beds

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5. The effects of density, distribution, species, and oxygen on the surfacing and burial of the cockles *Cerastoderma edule* and *C. glaucum*

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5.1 Aims

Following the rationale and results of Chapter 4 on the investigation of cockle surfacing behaviour in the Limfjorden, where an *in situ* assessment of differences in abundance, size and condition of surfaced and buried fractions of a cockle population was studied, a laboratory experiment on the role of density and oxygen concentration in the surfacing and burial of *C. edule* and *C. glaucum* was conducted.

The study took into consideration that 1) surfacing can be favoured at high densities by physical disturbance when cockles collide with each other (Richardson et al., 1993); 2) once surfaced, cockle movement and reburial tends to maximize distance between cockles (Richardson et al., 1993) and thus minimize competition for food and space; 3) there is evidence of increased cockle surfacing under low oxygen conditions (Marsden and Bressington, 2009; DLD project, unpublished); and that *C. glaucum* likely has lower burial ability than *C. edule* (Brock, 1979).

The objective of this study was to determine the effects of cockle density, spatial distribution of cockles in the sediment, oxygen concentration on the surfacing and burial activity/ fitness of common cockle *C. edule*. In addition, differences between surfacing and burial activity between the two species of cockles present in European waters, common and lagoon cockle, were also assessed. A set of laboratory experiments were used to evaluate the effect of density, reduced oxygen levels, spatial distribution on surfacing or burrowing activity of *C. edule*, with a fourth experiment assessing differences between *C. edule* and *C. glaucum*.

5.2 Methods

5.2.1 Cockles

Cockles were collected on 25 august 2021 by a diver from non-surfacing sympatric populations of *C. edule* (ca. 80%) and *C. glaucum* (ca. 20%) in Venø Bugt, Limfjorden, Denmark. Based on sampling for parasites and pathogen testing (Chapter 6) little or no infection was found in non-surfacing areas, and thus the burial capacity was considered not to be affected by mechanical impairment of the foot functionality due to parasite infection. As detailed in Chapter 6, although *Vibrio* infected many cockles in the sampling area the previous year, they did not seem moribund nor showed clear signs of disease. Cockles were kept in ambient flowing water and transported to the DTU Aqua laboratory on Mors at the end of the day, sorted by species and placed in holding tanks with upwelling flow of ambient fjord water until the following day.

5.2.2 Experimental set up

A set of laboratory experiments was used to evaluate the effects of density, reduced oxygen levels, spatial distribution on surfacing and/or burrowing activity of *C. edule*, and assessing differences between *C. edule* and *C. glaucum* (Table 4.5). Experiments were a mix of surfacing and burial trials, with the intention to determine surfacing and burial capacity, i.e. inducing surfacing and assessing burial capacity, depending on the variable and the objectives of each experiment (Table 4.5).

Surfacing vs buried cockles were defined as in Figure 5.1.

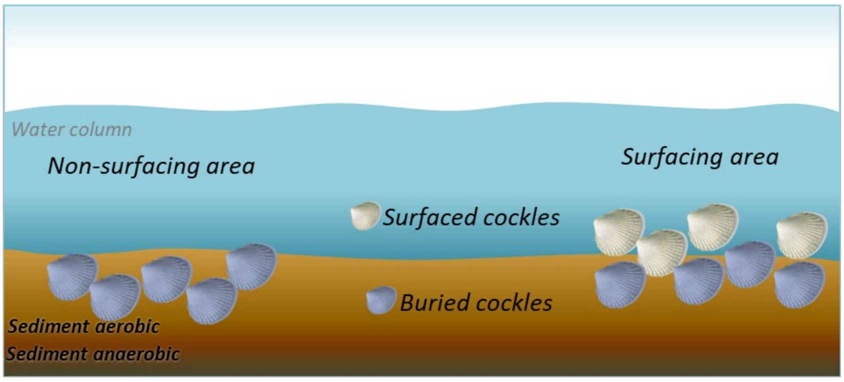

Figure 5.1. Schematic representation of surfacing cockles vs buried cockles in a non-surfacing area vs a surfacing area.

All experiments used plastic containers (18 x 18 cm) filled with 8 cm of sediment overlayed with 10 cm of 1 µm filtered seawater with constant air bubbling to ensure oxygen saturation (Figure 5.2). Clean sand normally used in sand filters was chosen as it provided a homogenous substrate, and since sand is a common habitat of *Cerastoderma* spp. Containers were kept in raceways with flowing water to maintain temperature at ambient fjord level. Cockles were randomly selected and placed in the experimental containers just before the start of the experiments at pre-determined densities and distribution and were placed either on the sediment surface or buried in 3 cm of sediment.

Figure 5.2. Photos of one experimental setup showing containers with cockles and air bubbling. Upon the start of the experiments, surfacing or burial activity was determined by counting the number of surfaced or buried cockles either manually or from images taken with overhanging cameras at variable intervals. Dead cockles were removed when detected and accounted for and were only observed in the distribution experiments (N = 1) and in the oxygen experiment (N = 28). Photo P. Freitas.

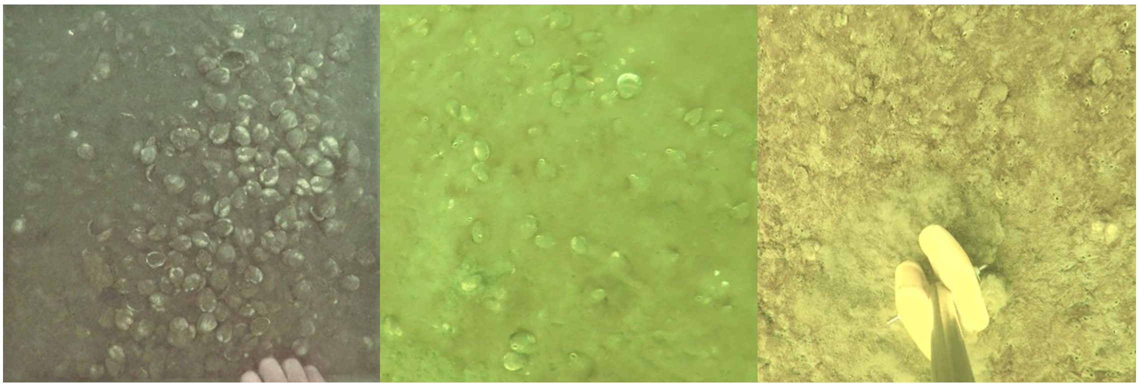

Figure 5.3. Examples of the three different surfacing status in natural wild cockles: Surfaced with all or most of the shell exposed out of the sediment (left), half-surfaced with a large fraction of the shell exposed but still some of the shell remains in the sediment (middle) and fully buried with only the siphons visible (right). Photo Left C. Saurel, middle and right P. Freitas.

The status of cockles was classified as surfaced or buried from regular direct visual observations or from timelapse photographs. Surfaced cockles were those that had more than 25% of the shell out of the sediment and buried cockles were those that had less than 25% of the shell out of the sediment. Such classification arises from observations across the Limfjorden during this project that cockles showed two visually distinct surfacing status (Figure 5.3): rare and uncommon massive surfacing where a significant proportion of the cockle population fully emerges from the sediment and the entire or most of the shell is exposed; a diffuse surfacing where a variable proportion of a cockle shell is partially exposed out of the sediment with a fraction remaining in the sediment. Recent observations of upward movement of *C. edule* under subtidal conditions as a reaction to thermal stress (Zhou et al. 2022), but also surfacing as a temporary reaction to physical disturbance by sediment cover or other cockles (Richardson et al. 1993) supports the classification as surfacing of these two distinct visual categories.

During the experiments, the initial change in status from buried to surfaced or surfaced to burial was referred as "transition period", after the transition period, an equilibrium/adjustment period was defined as a period when the cockles reached a position and no significant change in position occurred over a long period of time and until the end of the experiment.

5.2.3 Cockle density

Two experiments (1 and 2) were carried to determine the effect of density on *C. edule* burial and surfacing behaviour, respectively, based on the more likely inter-cockle disturbance at higher densities (Richardson et al., 1993) with hypotheses that cockles: 1) will have reduced burial activity with increasing density and 2) and will surface more at higher densities compared to lower densities. In the first experiment, cockles were placed on the sediment surface, while on the second experiment cockles were buried in 3 cm of sediment (Table 5.1).

Experiments were done at three densities (6 and 3 replicates per density, respectively) that reflect the range observed in the Limfjorden, while also ensuring cockles occupied an increasing and significant proportion of volume in the sediment and thus increasing likelihood to disturb each other at increasing densities (Table 5.1). Cockles were distributed uniformly on the surface of the sediment and the development of burial or surfacing activity, respectively followed over 22 hours.

Table 5.1. Experimental set up used three densities similar to the range in the wild (6 replicates), increasing the proportion of sediment volume and surface area occupied by cockles. Densities detailed in Table 5.2.

Experiment	Treatment	Species	Start	N	Density	Oxygen	Distribution
1	Density	*C. edule*	Surfaced	6	Low Medium High	Oxic	Uniform
2			Buried	3			
3	Distribution Density	*C. edule*	Buried	3	Low Medium	Oxic	Uniform Aggregated
4	Species	*C. edule* *C. glaucum*	Surfaced	3	Medium	Oxic	Uniform
5	Oxygen Density	*C. edule*	Mix Surfaced/Buried	3	Low Medium High	Anoxic Oxic	Uniform

The volume and area occupied by cockles were estimated by an equivalent ellipsoid and ellipse, respectively, using the dimensions of a mean cockle (2.07 cm of shell width and 2.53 of shell height and length). The % of the volume in the top 3 cm of sediment and surface area occupied by cockles was determined relative to experiment containers (18 x 18 cm). The different densities used in the experiments are detailed in Table 5.2.

Table 5.2. Density experiments used three densities similar to the range in the wild (6 and 3 replicates), increasing the proportion of sediment volume and surface area occupied by cockles. The sediment volume occupied by cockles in the top 3 cm and the % surface area occupied by cockles is shown.

		Cockles			Sediment	
	Density				Volume occupied by Cockles (top 3cm)	Area occupied by cockles
	cockles/m^2	N	cm^3	cm^2	%	%
Low	401	13	104	53	11	17
Medium	1080	35	281	144	29	44
High	2222	72	578	296	60	91

In the low-density treatment, 13 cockles were placed per container (401 cockles/m^2) with 11% of the sediment volume and 17% of the surface area occupied by cockles. In the medium- density treatment 35 cockles were placed per container (1 080 cockles/m^2) with 29% of the sediment volume and 44% of the surface area occupied by cockles. In the high-density treatment, 72 cockles were placed per container (2 222 cockles/m^2) with 60% of the sediment volume and 91% of the surface area occupied by cockles.

5.2.4 Cockle distribution

The effect of spatial distribution (Experiment 3, Table 5.1) on *C. edule* surfacing behaviour – based on the hypothesis that aggregated cockles will move, surface and bury more than uniformly distributed cockles to maximize the distance between them and reduce disturbance (Richardson et al., 1993) – used a 2-factor design (density and distribution) with cockles either aggregated into a clump at the centre or uniformly distributed in the containers (three replicates) at the low and medium densities used in the density experiment (Table 5.1). High density was not used as it did not allow to place cockles in distinct aggregate or uniform distributions.

At the start of the experiment, cockles were buried in sediment and surfacing activity followed regularly over ca. 120 hours. One single cockle died during the experiment in the medium density uniform distribution tank.

5.2.5 Cockle species

Experiment (4, see Table 5.1) looking at differences in *C. edule* and *C. glaucum* surfacing and burial behaviour was based on the hypothesis that *C. glaucum* has a lower burial capacity than *C. edule* (Brock, 1979). Cockles were buried in sediment with a uniform distribution and surfacing activity of the two species followed regularly over 80 hours. Cockles from the two species were placed in separate containers (three replicates) at medium density (Table 5.1).

5.2.6 Oxygen

Experiment (5, see Table 5.1), assessing the effect of reduced oxygen levels on cockle surfacing behaviour – based on the hypothesis that low oxygen hypoxia conditions increase/induce cockle surfacing (Marsden and Bressington, 2009; DLD project, unpublished) – used a 2-factor design (density and oxygen concentration). At the end of the density experiment, once a balance in cockle surfacing and burial activity was reached over 22 hours, an oxygen depletion experiment was started at each of the three densities.

The experiment consisted of two oxygen treatments (low and high = control) at three densities (high, medium and low) where cockles were exposed to a sequence of three periods with different oxygen concentration conditions (Figure 5.4):

1. an initial oxic period with oxygen concentrations above 6 mg/l and oxygen saturation between 80 to 90%;
2. an anoxic period lasting 40 to 48 hours with oxygen concentration below 1.1 mg/l and oxygen saturation below 20%;
3. a final recovery oxic period lasting ca. 43 to 48 hours, with oxygen concentrations lower and more variable than in the initial oxic period, normally above 5.5 mg/l and oxygen saturation above 80%, but with periods of lower oxygen concentrations mainly at high and medium density. Such fluctuations in oxygen concentrations are assumed to result from decomposition of organic matter accumulated in the sediment during the experiments, but also variations in air bubbling.

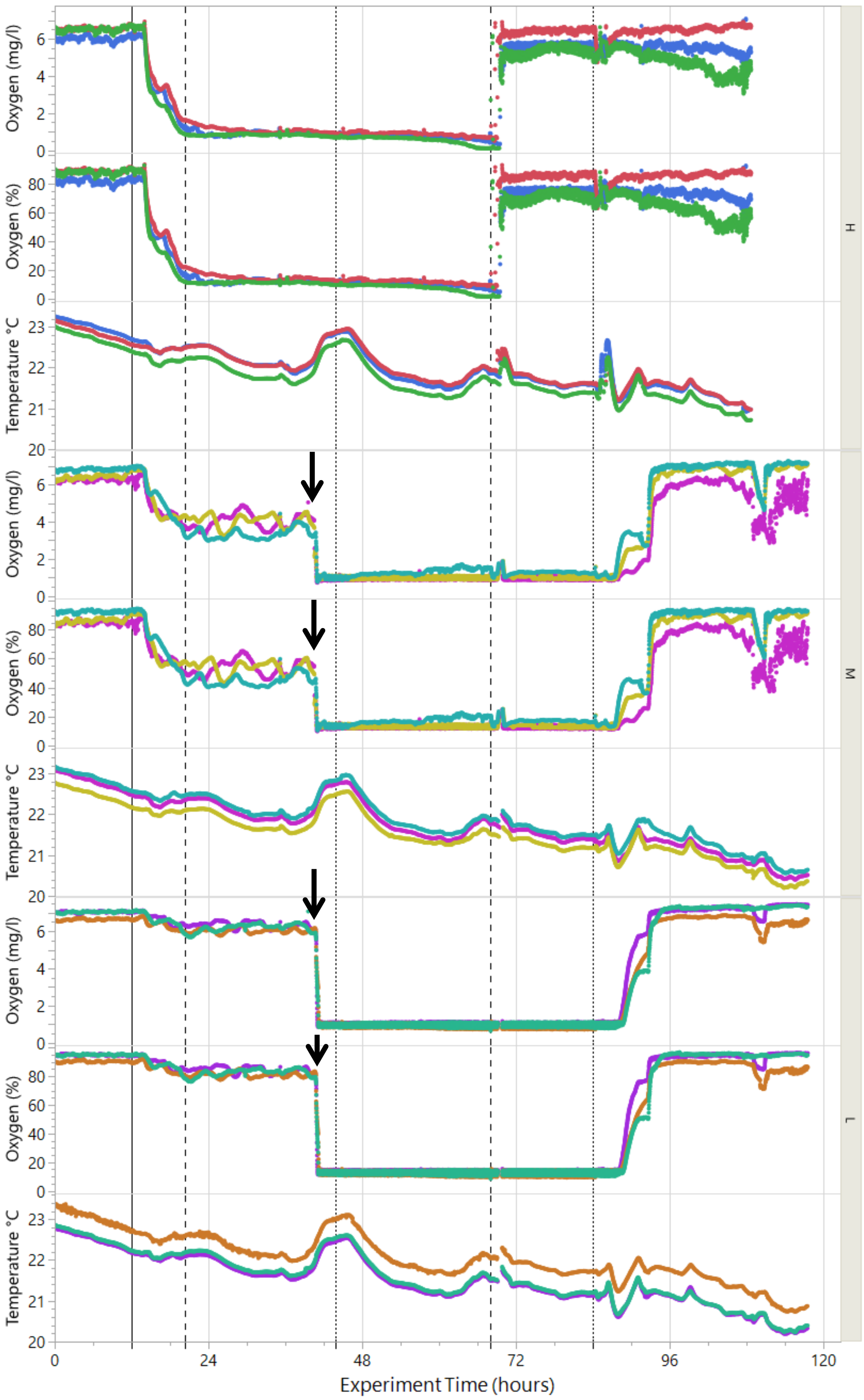

Figure 5.4. Temporal evolution of oxygen concentration (mg/l) and saturation (%) at the three densities; high (H), medium (M) and low (L). Vertical lines: solid line marks the start of oxygen concentration reduction; dashed lines delimit anoxic period for high density; dotted lines delimit anoxic period for medium and low densities. Black arrows indicate the addition of Nitrogen to the tank to reduce oxygen levels in the tanks.

Oxygen levels were lowered to hypoxic levels by stopping aeration and water flow for high density treatment and additionally by adding bubbling of nitrogen for medium and low-density treatments. Note, the use of a closed water circulation system may lead to a decrease of water quality other than a reduction of oxygen levels (e.g. ammonia built up). Once surfacing-burial activity became stable at low-oxygen levels, oxygen levels were brought back to saturation by restarting aeration and water flow. A control set of cockles at each of the three densities (three replicates) was kept at or close to saturation throughout the experiment. A total of 28 cockles died during the oxygen experiment: 1 in each density of the control tanks (0.5 %, 1.0 % and 2.6 %); and 25 cockles in the low oxygen treatment with 2 at medium density (1.9% and 23 cockles in the high-density treatment (10.6%).

Oxygen concentration, saturation levels, and temperature were measured every minute using OxyGuard Commander probes (Figure 5.4) and controlled using the OxyGuard Pacific (OxyGuard International A/S, Birkerød, Denmark) including a relay with solenoid valves that regulated nitrogen gas delivery to wooden air stones to the 2 L tanks, while the 100% saturation was maintained by air bubbling.

5.2.7 Data analysis

Data in each treatment was divided into two periods: a transition period that is the time taken from the start of the experiment for the proportion of buried cockles to reach maximum or minimum values respectively in burial or surfacing experiments; and an equilibrium period when the proportion of buried cockles stabilized with little variation over time.

Hypothesis regarding the effect of density, distribution, species and oxygen on the relative proportion of buried cockles in the surfacing and burial experiments were tested using non-parametric Kruskal-Wallis and Dunn tests. Data could not be tested using repeated measures ANOVA due to low replication and lack of normality and unequal heterogeneity of variances.

5.3 Results

5.3.1 Density experiments

Once equilibrium was reached, density had a significant effect on the proportion of buried cockles in both the burial and surfacing experiments (KW test: *H* = 102.14 and 33.18, *p* < 0.0001 for both). **The proportion of buried cockles was significantly higher at low density and lower at high density in both the burial and surfacing experiments** (Dunn tests: *p* < 0.001 for all; Table 5.3 and Figure 5.5), except between low and medium density in the surfacing experiment (Dunn test: *p* = 0.102).

The duration of the transition period in both the burial and surfacing experiments increased with density, being longest at high density (5 and 7 h) and lowest at low density (3 and 2 h) (Table 5.3 and Figure 5.5).

The proportion of buried cockles at the end of the transition period in both experiments was lowest at high density and highest at low density (Table 5.3 and Figure 5.5). However, differences were only significant in the burial experiment between high density and low and medium densities, but not between low and medium densities (KW test: *H* = 12.19, *p* = 0.002; Dunn test: *p*= 0.003, *p* = 0.044 and *p* = 1.000, respectively), while in the surfacing experiment differences were significant between the three densities (KW: *H* = 6.01, *p* = 0.05, Dunn test: *p* < 0.05 for all).

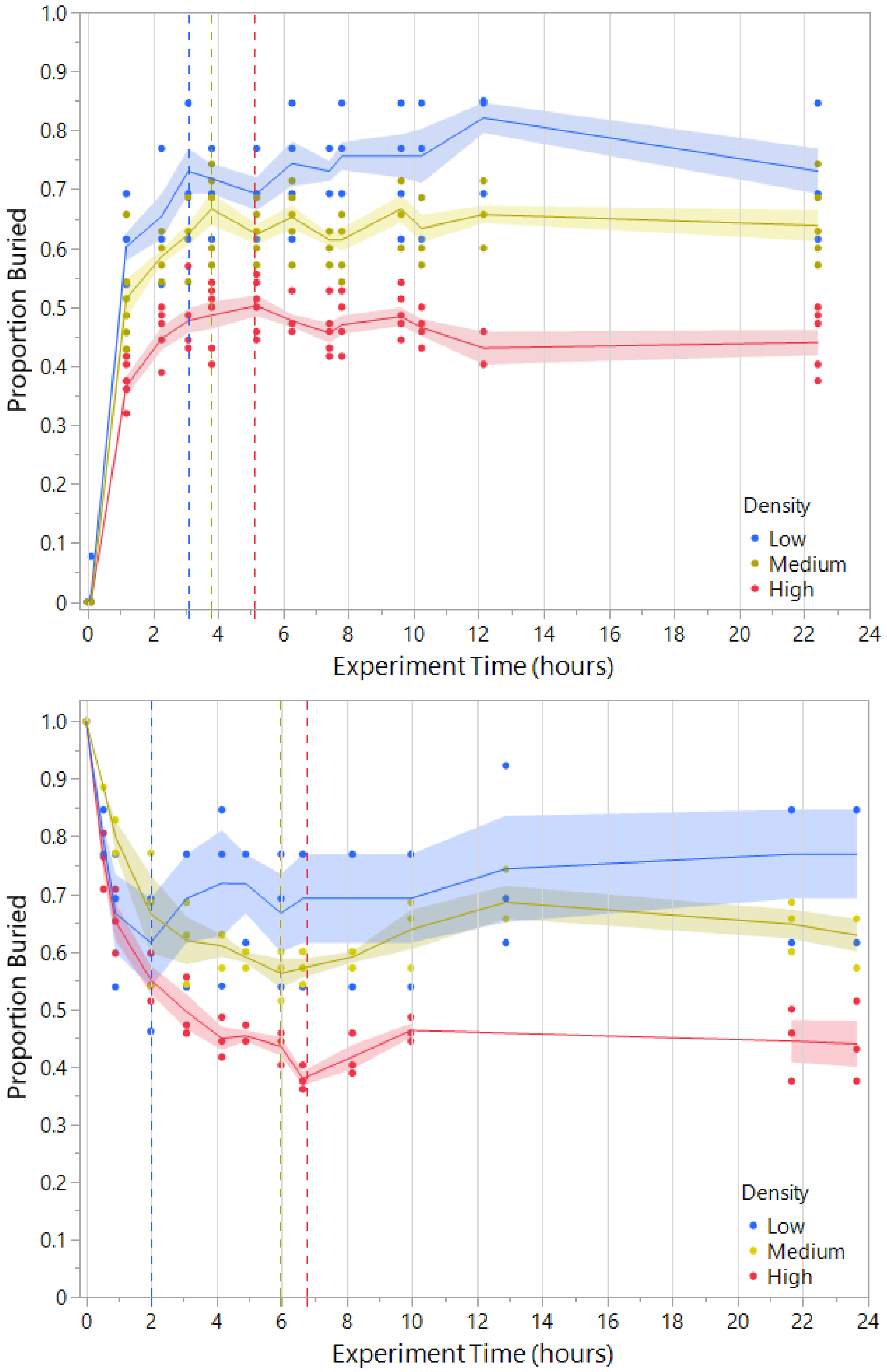

Figure 5.5. Temporal evolution of cockle burial (top) and surfacing activity (bottom) expressed as the proportion of buried cockles. Three different densities were used: High, medium, and low. Vertical dashed lines mark the end of the initial transition periods and the start of the equilibrium periods. See methods for definition of transition and equilibrium periods. Means and standard errors are shown as connecting lines and bands, respectively.

In both experiments, the proportion of buried cockles during the equilibrium period were uncorrelated (Spearman's: $p > 0.05$) or only weakly correlated with time (low density in burial experiment and medium density in surfacing experiment; Spearman's: $\rho = 0.274$, $p = 0.045$ and $\rho = 0.475$, $p = 0.047$, respectively). Therefore, the proportion of buried cockles during the equilibrium period at most showed only small changing trends (Figure 5.5).

Table 5.3. Duration and mean buried proportion (± SE) at the end of transition and during the equilibrium periods. See methods for definition of transition and equilibrium periods. Significant differences marked with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Kruskal-Wallis and Dunn tests).

	Start			Transition		Equilibrium
Experiment	Density	Status	Buried %	Duration (h)	Buried % end	Buried %
Burial	Surfaced	High	0	5	*50.2 ±2.5	***46.3 ±0.7
		Medium	0	4	66.6 ±3.9	***63.8 ±0.7
		Low	0	3	73.1 ±5.4	***74.6 ±1.1
Surfacing	Buried	High	100	7	*38.0 ±1.2	**44.1 ±1.4
		Medium	100	6	*56.2 ±2.5	62.7 ±1.3
		Low	100	2	*61.5 ±7.7	71.5 ±2.1

5.3.2 Distribution experiments

The time elapsed (transition period) for buried cockles to surface was three times longer at medium density (6-7 h) than at low density (2-3 h) but it was similar between uniform and aggregate distributions at each density (Table 5.4 and Figure 5.6), suggesting density and not distribution affected the time taken for cockles to surface.

Table 5.4. Summary of distribution experiment. Duration and mean buried proportion (± SE) at the end of the transition period and during the equilibrium/adjustment period. See methods for definition of transition and equilibrium periods. Significant differences marked with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (non-parametric Kruskal-Wallis).

	Start			Transition		Equilibrium	
Experiment	Density	Status	Buried %	Duration (h)	Buried % end	Buried %	Buried % end
Distribution	Low	Uniform	100	2	61.5 ±7.7	71.5 ±2.1	92.3 ±4.4
		Aggregated		3	69.2 ±4.4	78.3 ±1.2	82.1 ±2.5
	Medium	Uniform	100	6	56.2 ±2.5*	67.4 ±1.3**	82.7 ±1.5
		Aggregated		7	41.9 ±53*	60.9±1.9**	77.1 ±4.4

At the end of the transition period, the proportion of buried cockles was significantly higher in the uniform than in the aggregate distribution at medium density (KW test: $H = 3.86$, $p = 0.0495$), but distribution had no significant effect at low density (KW test: *H* = 0.05, *p* = 0.825; Table 5.4 and Figure 5.6).

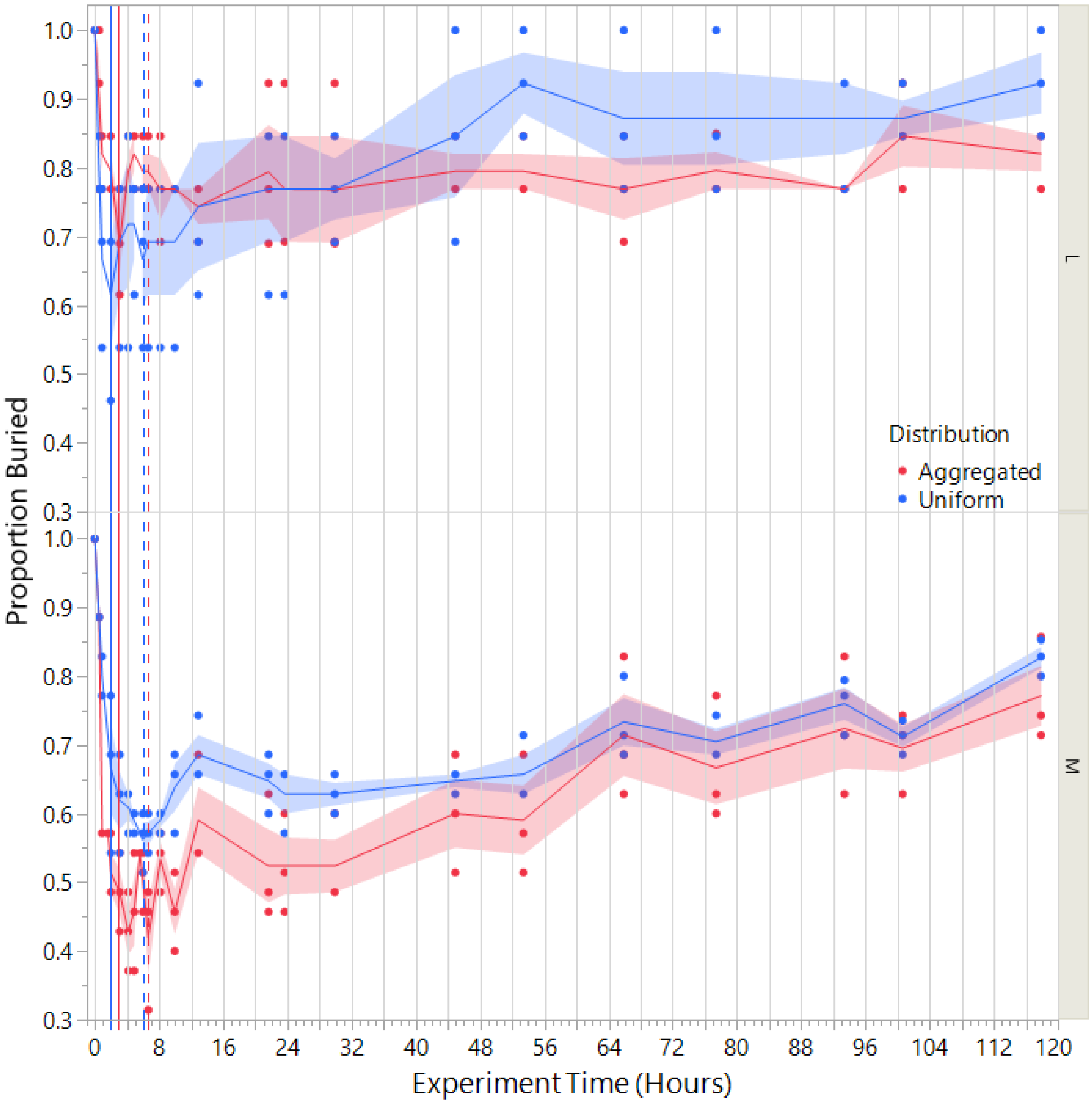

Figure 5.6. Temporal evolution of cockle surfacing activity expressed as the proportion of buried cockles with different initial cockle distribution, aggregated (red dots) or uniform (blue dots) at medium (M) and low (L)densities. Vertical lines mark the end of initial transition periods and the start of the equilibrium periods (full lines: low density; dashed: medium density). See methods for definition of transition and equilibrium periods. Means and standard errors are show as connecting lines and bands, respectively.

During the equilibrium period, the proportion of buried cockles was significantly higher in the uniform than in the aggregated distribution at medium density (KW test: *H* = 12.41, *p* = 0.0004) but not at low density (KW test: *H* = 0.38, *p* = 0.537; Table 5.4 and Figure 5.6).

However, the proportion of buried cockles increased with time until the end of the experiment (Table 5.4 and Figure 5.6; Spearman's correlation: ρ > 0.633, *p* < 0.0001 for all), except for uniform distribution at low density (Spearman's correlation: ρ = 0.048, *p* = 0.731).

At the end of the equilibrium period, the proportion of buried cockles with different distributions was not different at both low or medium density (KW test: H = 0.43, p = 0.513 and H = 2.63, p = 0.105, respectively).

5.3.3 Species differences in burial

The transition period was 4.5 times shorter in *C. edule* than in *C. glaucum*, with the former taking 4h for the proportion of buried cockles to increase to its first maximum while the latter took 18h (Table 5.5 and Figure 5.7).

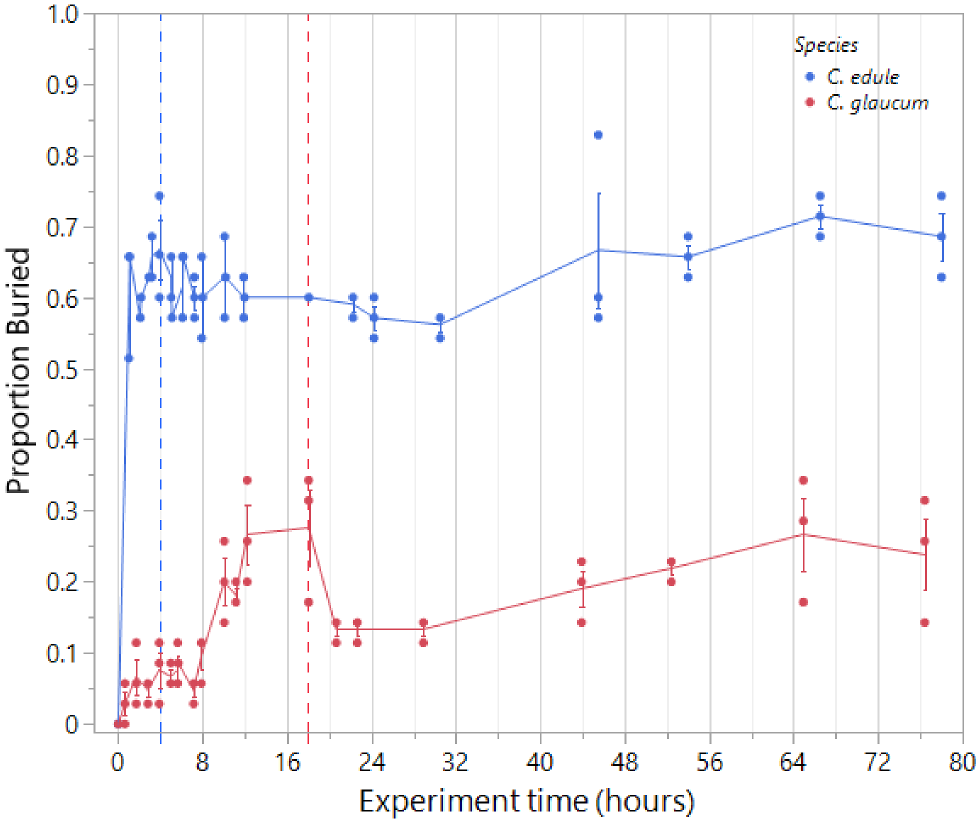

Figure 5.7. Temporal evolution of cockle surfacing activity expressed as the proportion of buried cockles during experiments with the two cockle species at medium density, the common cockle *C. edule* (blue) and the lagoon cockle, *C. glaucum* (red). Vertical lines mark the end of initial transition periods and the start of the equilibrium periods. See methods for definition of transition and equilibrium periods. Means and standard errors are show as connecting lines and bars, respectively.

At the end of the transition period, the proportion of buried cockles was significantly higher, ca. 2.5 times, for *C. edule* than for *C. glaucum* (KW test: H = 3.86, p = 0.0495).

During the equilibrium period, the buried proportion of both species fluctuated, with *C. glaucum* showing a marked initial decrease, but generally increased being significantly positively correlated with time in both species (Spearman's correlation; *C. edule*: ρ = 0.314, p = 0.0489; *C. glaucum*: ρ =0.746, p < 0.001; Figure 5.8).

In the equilibrium period, the buried proportion was significantly higher in *C. edule* than in *C. glaucum*, by ca. 2.5–3 times (KW test: H = 41.12, p < 0.0001) including at the end of the experiment (KW test: H = 3.86, p = 0.0495; Table 5.5 and Figure 5.4).

Table 5.5. Summary of species experiments. Duration and mean buried proportion (± SE) at the end of the transition period and during the equilibrium period. See methods for definition of transition and equilibrium periods. Significance marked with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Kruskal-Wallis).

	Start			Transition		Equilibrium	
Experiment	Density	Species	Buried %	Duration (h)	Buried % at end	Buried %	Buried % at end
Species	Medium	*C. edule*	0	4	66.8 ±4.2*	62.4 ±1.0***	68.6 ±3.3*
		C. glaucum		18	27.6 ±5.3*	18.8 ±1.5***	23.8 ±5.0*

5.3.4 Oxygen

In the initial oxic period, there were significant differences in the proportion of buried cockles between densities in both oxygen treatments (KW test: $H = 18.15$ and 18.48, $p < 0.0001$ and $p = 0.0001$, respectively). The proportion of buried cockles was lower at high density than low density (Dunn tests, $p < 0.0001$), but was not different between the other densities in both oxygen treatments (Dunn tests, $p > 0.068$ for all; Table 5.6 and Figure 5.7). This reflects effect of density on the proportion of buried cockles at the end of the density experiments.

However, at the start of the experiment the proportion of buried cockles was higher in the low than in the high oxygen treatment at all densities (Table 5.6; KW test: $H > 4.65$, $p < 0.031$ for all), i.e. the control high oxygen treatment had a lower proportion of buried cockles by ca. 6 to 11%.

Table 5.6. Summary of oxygen experiment. Mean cockle buried proportion and mean oxygen concentration (± SE) under a sequence of oxygen condition: Oxic at start, anoxic and oxic at the end. For proportion of buried cockles, n = 3; for oxygen concentration measurements, n > 7,000. Significant differences marked with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Kruskal-Wallis.

		Oxic – Start		**Anoxic**			**Oxic - End**		
Density	**Oxygen**	O_2 mg/l	Buried%	Duration (h)	O_2 mg/l	Buried %	Duration (h)	O_2 mg/l	Buried %
High	Low	6.38 ±0.01	**48.1 ±0.7	48	0.84 ±0.01	***16.4 ±3.5	40	5.72 ±0.01	32.2 ±6.9
High	High	>6	**41.2 ±1.2	48	>6	***45.0 ±1.0	40	>6	47.8 ±2.0
Medium	Low	6.53 ±0.01	*67.0 ±1.7	40	1.07 ±0.01	66.8 ±2.3	34	6.40 ±0.1	*84.4 ±2.4
Medium	High	>6	*61.6 ±1.4	40	>6	68.3 ±1.7	34	>6	*76.4 ±1.8
Low	Low	6.91 ±0.1	*82.9 ±2.8	40	0.94 ±0.01	**76.3 ±2.6	34	7.04 ±0.01	89.7 ±1.6
Low	High	>6	*71.8 ±2.9	40	>6	**87.8 ±1.1	34	>6	93.6 ±12.4

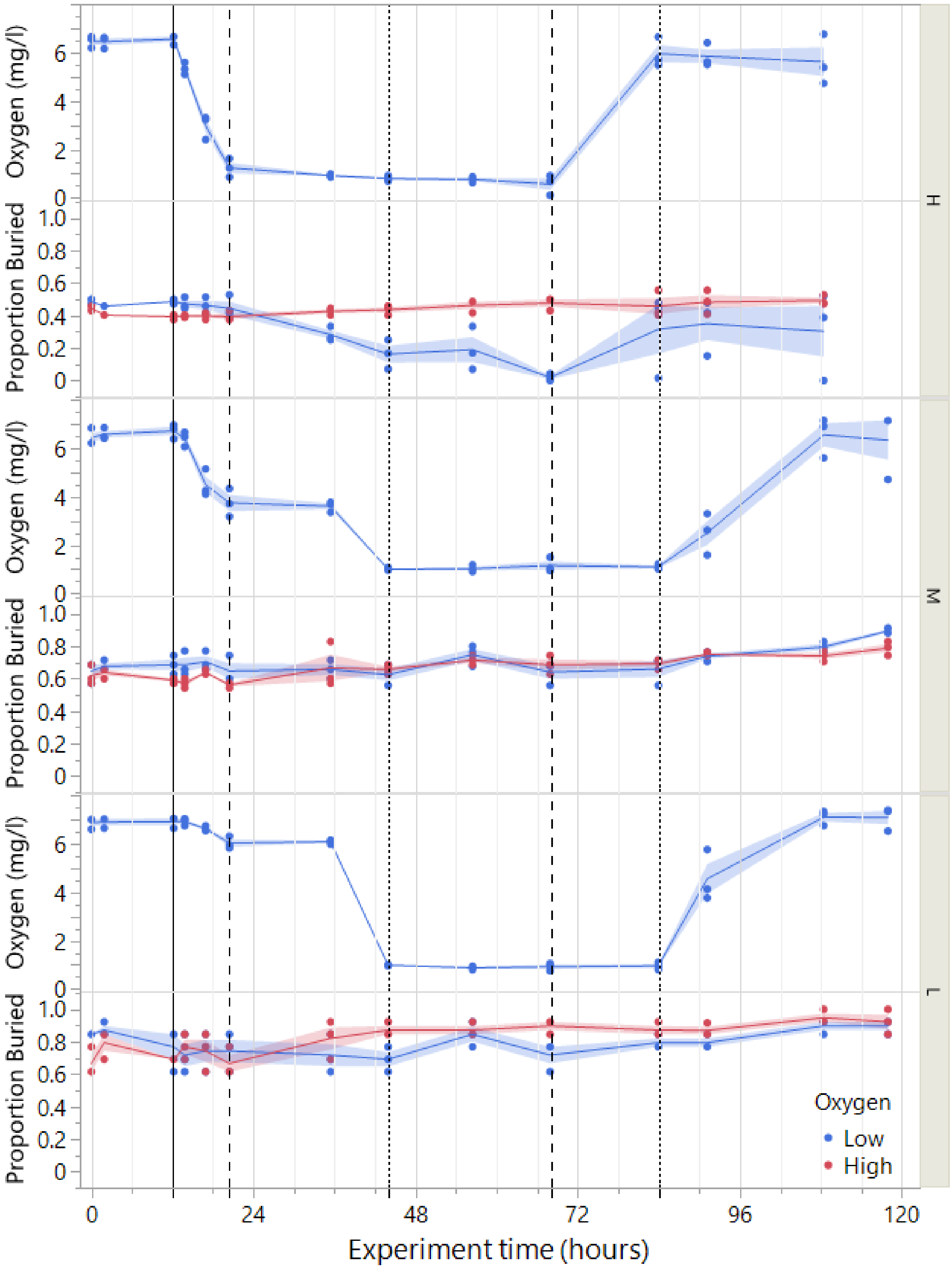

Figure 5.8. Temporal evolution of cockle burial and surfacing activity expressed as the proportion of buried cockles during oxygen experiments. Three different densities were used as in the density experiments: High, medium and low. Vertical solid line marks the start of oxygen concentration reduction; vertical dashed lines delimit anoxic period for high density; vertical dotted lines delimit anoxic period for medium and low densities. Means and standard errors are show as connecting lines and bands, respectively.

During the anoxic period, the proportion of buried cockles was significantly lower in the low than in high oxygen conditions at high and low density (KW test, $H > 11.20$, $p \leq 0.0008$), but not at medium density (KW test, $H = 0.32$, $p = 0.573$; Table 5.6 and Figure 5.7).

In the final recovery oxic period, when oxygen levels were increased in the low oxygen treatment, the proportion of buried cockles was not different from the high oxygen treatment at high and low density (KW test, $H > 2.98$, $p > 0.0843$; Figure 5.7), but was higher at medium density (KW test, $H > 2.98$, $p > 0.0843$).

5.4 Conclusions

Experiments under controlled conditions allowed to monitor and manipulate potential explanatory variables to assess the influence of density, spatial distribution, cockle species and anoxia on the surfacing and/or burial activity of cockles. This approach was based both on the results of the field study reported in Chapter 4, which suggested a crowding effect on surfacing, and also on findings of previous studies indicating effects from density, oxygen and differences between the two cockle species (Brock, 1979; Richardson et al., 1993; Marsden and Bressington, 2009).

Results from the experiments done in this study showed a clear influence on cockle surfacing and/or burial by cockle density, distribution, species and to a lesser extent oxygen conditions. Increased cockle density resulted in increased surfacing and lower burial, after an initial adaptation period when cockles reacted to the initial buried or surfaced conditions of the experiments (Figure 5.5). The proportion of buried cockles ranged between 40–70% from the high to low density treatments, which themselves ranged between the highest (High, ca. 2 000 cockles m^2), above average (medium, ca. 1 000 cockles/m^2) to below average (Low, ca. 400 cockles/m^2) densities observed in the field. These results support the suggestion of a "crowding" effect by Richardson et al. (1993) and the observations from the field study where increased physical contact and disturbance when available space for cockles in the sediment is reduced would induce surfacing.

The impact of distribution of cockles in the sediment, either uniform or aggregated (clumped together), was found to be transitory and short lasting. The proportion of buried cockles increased rapidly into an equilibrium period, albeit with a slow increasing trend suggesting the final equilibrium had not been attained after 120h (Figure 5.6). At the end, the proportion of buried cockles was similar across distributions and densities. Therefore, our results do not confirm the suggestions by Richardson et al. (1993) that cockles would surface and move to maximize distance between them and reduce disturbance.

Similar to the small study by Brock (1979), once exposed on the sediment surface, *C. glaucum* was found to have a lower burial capacity than *C. edule*, with the proportion of buried cockles after ca. 80h being 45% lower in *C. glaucum* (Figure 5.7).

Low oxygen conditions, which are a common occurrence during summer in some parts of the Limfjorden, has been described to induce surfacing in the New Zealand cockle *Austrovenus stutuchburyi* (Marsden and Bressington, 2009). However, our results showed a mixed response in which anoxia could induce rapid surfacing within a few hours (i.e. at high and low density), but also that cockles could withstand anoxia for at least 48h with no increase in surfacing.

Cockle surfacing in the Limfjorden can thus result from a combination of factors, all of which together with the influence of pathogens (e.g. Jonsson and André, 1992; Thomas and Poulin, 1998; Mouritsen, 2002; Desclaux et al., 2002; Blanchet et al., 2003; Tompkins et al., 2004) can induce surfacing or reduce the ability of cockles to bury.

5.5 References

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6. Reproductive cycle and pathogen census

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6.1 Introduction and rationale

Common cockles, *Cerastoderma edule* are known to host a number of pathogens and diseases (e.g. virus, bacteria, *Haplosporidia, Cercozoa, Diagenea*; e.g. Longshaw and Malham, 2012; de Montaudoin et al., 2021), which can have a significant impact on cockle mortality, size, condition/fitness and reproduction, but also in cockle surfacing behaviour and capacity to resist environmental stress (e.g. Jonsson and André, 1992; Thomas and Poulin, 1998; Desclaux et al., 2002; Mouritsen, 2002; Thieltges, 2006; Fermer et al., 2011; Morgan et al., 2012).

Surfacing events have been reported in most of European cockle producing countries (e.g. de Montaudoin et al., 2021 for a review of parasites and diseases in *C.edule*), usually linked to mass mortality events (e.g. Thieltges, 2006; Morgan et al. 2012) and a decline in landings. In most of the cases, pathogens and diseases together with environmental factors are believed to play an important role in the phenomenon. Thus, the cockle fishery in the Limfjorden, which in recent years represents half of the European landings (Eurostat), could be at risk if the occurrence or an outbreak of pathogens and disease would affect cockle stock availability, reproduction, health and resistance to environmental stress. Cockles (both *C. edule* and *C. glaucum*) were sampled in the Limfjorden in connection with investigations of surfacing contra buried cockle populations as well as population structure.

In recent years (2007-2010) census of cockle diseases and pathogens in Europe have been conducted under a European Interreg project entitled COCKLES "Cooperation for the recovery of cockle fisheries and their environmental services in the Atlantic Area", led by Centro Tecnológico del Mar (CETMAR) based in Vigo with partners from the five Member States of the Atlantic Area Programme (Portugal, Spain, France, Ireland and UK).

Only a few studies have looked at pathogens and disease in Denmark (de Montaudoin et al., 2009; de Montaudoin et al., 2021). For instance, significant infestation of cockles by the digenean trematode *Monorchis parvus* has been reported in the Limfjorden[2]. This digenean trematode has the potential to stop reproduction in infested cockles (e.g. Longshaw and Malham, 2012; de Montaudoin et al., 2021). A wide screening of harmful pathogens and diseases is thus important in the Limfjorden and in particular in the Western Limfjorden. This was conducted in parallel to the European COCKLES project, and exchanges between the two projects were conducted via workshops and outputs.

Some of the sampled cockles were screened for pathogens by molecular methods. For molecular methods, it was decided to focus on the bacterium *Vibrio aestuarianus* and the protozoan parasite *Marteilia* sp., as they both are known to cause disease and be highly deleterious to cockles. Furthermore, the screening also included *Bonamia ostreae*, another protozoan parasite that although not

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[2] https://videnskab.dk/miljo-naturvidenskab/mystisk-er-stenalder-fisk-vendt-tilbage-med-klamme-parasitter
Jensen KT og Petersen SV (2013). ”Ikke-nedgravede hjertemuslinger i Limfjorden – snyltere, gonadeudvikling og neoplasi”. Rapport til DSC

affecting cockles causes disease and mortality in the native flat oyster species *Ostrea edulis* in the Limfjorden.

A catalogue of parasites and diseases of the common cockle *Cerastorderma edule* was published in 2021 (de Montaudoin et al 2021). A list of associated parasites and diseases for cockles in Europe is summarized in Figure 6.1. In this Figure, the species identified in the Limfjorden during this project are highlighted in yellow.

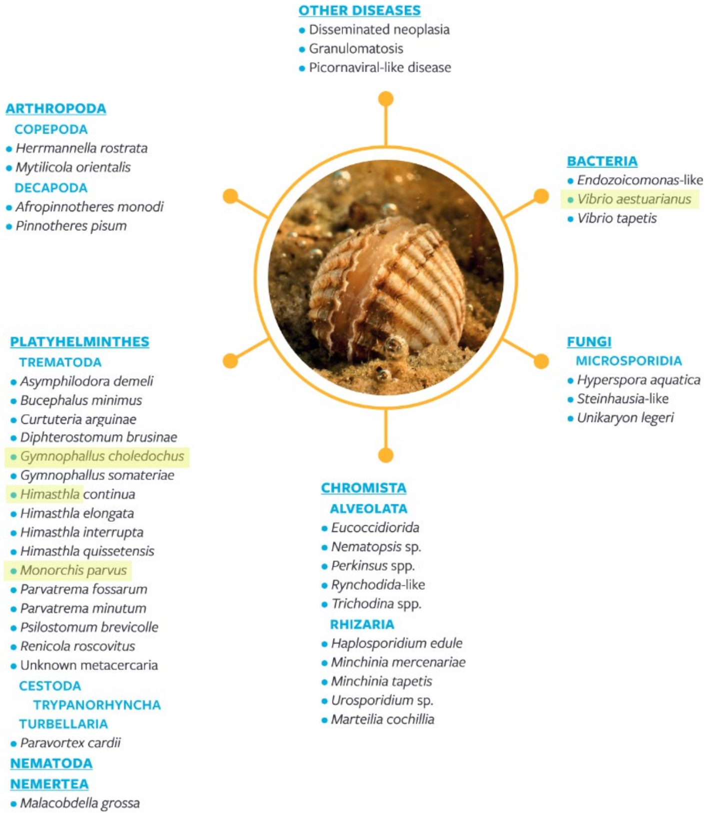

Figure 6.1. Parasites and diseases of the common cockle *Cerastoderma edule* modified from de Montaudoin et al. (2021), in yellow the taxa identified in the Limfjorden during the current study.

The purpose of this study was three-fold: (1) to obtain a baseline assessment of the pathogens and diseases present in cockles of the Limfjorden over the cockle life cycle. (2) to compare the Limfjorden two cockle species reproductive cycle, relative abundance and disease susceptibility and (3) to promote European collaboration with the COCKLES project colleagues.

6.2 Material and methods

6.2.1 Field sampling

Venø Bugt is a microtidal enclosed basin of the Limfjorden of ca. 72 km^2 (Figure 6.2) where in 2018 cockle beds overlapped both a protected Natura2000 area and fished beds adjacent to it. Sites were chosen in the protected Natura2000 to avoid the impact from fishing mortality during the on-year long monitoring. Venø Bugt can support large cockle beds and has provided significant cockle landings since 2017. The cockle bed was located at a depth of ca. 5.5m in muddy sediments, with salinity ranging between 27-32 and monthly temperature ranging between ca. 3-21°C.

To assess the vertical distribution of cockle, samples were collected by divers using quadrat frames (0.25 m^2) in August 2019 and January 2020. Two vertical fractions of the cockle population were sampled separately with a sliding metal plate to guide the depth of sampling into the sediment: A surface fraction, any cockles emerged from the bottom surface and a buried fraction. The definition of surfacing is done in Chapter 4.

The seasonal variability of pathogens and reproductive cycle was assessed on samples taken using a 0.1 m^2 Day-Grab from the DTU Aqua research vessel Egon P. in the south station. Samples were sieved at 1cm to remove sediment. Between 7 and 30 grabs Day-Grabs were conducted per day to estimate the density of cockle bed and patchiness. Live cockles were counted, placed in labelled bags, and brought back to the Danish Shellfish Centre DTU Aqua for dissection and ageing. The detail of sampling dates from August 2019 until January 2021 and number of samples is summarized in Table 6.1. In 2020, monthly samples were taken to study the reproductive cycles of the cockles. Two species of cockles *C. edule* and *C. glaucum* were sampled in the same stations and same Day-Grabs. The proportion of species relative to the other were determined.

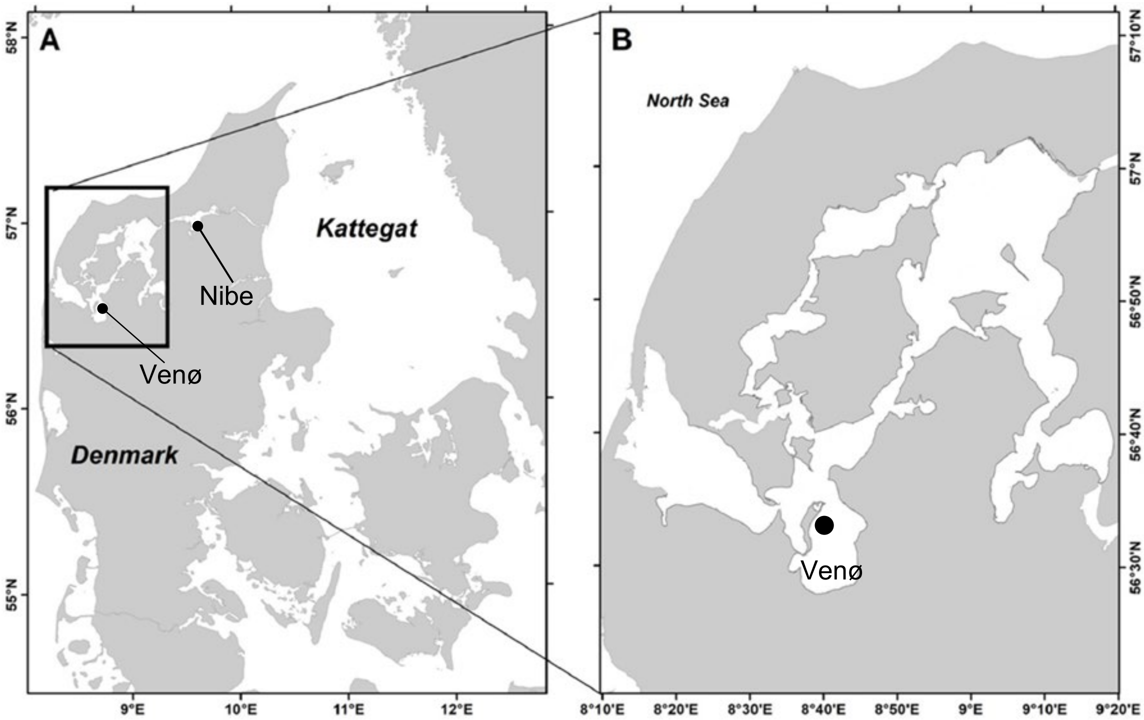

Figure 6.2. Maps of Denmark (A) and the Western part of Limfjorden (B) with selected basin Venø and black dot for sampling stations.

In August 2019 and January 2020, both species were found in the north site, however, mortality of *C. edule* in January 2020 for unknown reasons required the sampling area be moved to the south station ca. 2,000 m south from the North station.

Cockles collected had a size spectrum from 21.2-35.5 mm and an estimated age of 2-5 years. It was not possible to collect cockles under 10 mm and thus, size and age at first maturity in Limfjorden was not done. The density and percentage of *C. edule* relative to *C. glaucum* over the one-year survey period using grab is shown in Figure 6.3. Density varied between 40 to 1 030 cockles/m^2 and the proportion of *C. edule* ranged from 0-98% with an average of 75%.

Table 6.1. Overview of cockle samples per station, month, and year in production area 7, Limfjorden, Denmark. Letter code explanations for sample batch IDs: a = Surfacing, b and c = No surfacing, G = grab; S = Surface, T and B = buried; M = Mid Site, S = South Station. a, b, and c samples were handpicked by a diver, and G samples were sampled with a grab.

Sample Batch ID	Sampling Date	Year	Sampling Station/Site	Species	# of Samples	# of Samples run in qPCR	# of samples for histology
C-7-19-1-a-S	August	2019	North Site	edule + glaucum	50	50	50
C-7-19-1-a-T	August	2019	North Site	edule + glaucum	50	50	50
C-7-19-1-a-B	August	2019	North Site	edule + glaucum	23	23	23
C-7-19-1-b-S	August	2019	North Site	edule + glaucum	13	13	13
C-7-19-1-b-T	August	2019	North Site	edule + glaucum	50	50	50
C-7-19-1-b-B	August	2019	North Site	edule + glaucum	40	40	40
C-7-19-1-c-T	August	2019	North Site	edule + glaucum	50	50	50
C-7-19-1-c-B	August	2019	North Site	edule	1	1	1
C-7-20-1-b-S	January	2020	North Site	edule + glaucum	3	3	3
C-7-20-1-b-T	January	2020	North Site	edule + glaucum	36	36	36
C-7-20-1-c-T	January	2020	North Site	edule + glaucum	52	52	52
C-7-20-1-c-B	January	2020	North Site	edule + glaucum	19	19	19
C-7-20-2-G-M	January	2020	Mid Site	edule	45	45	0
C-7-20-3-G-S	February	2020	South Station	edule + glaucum	60	60	46
C-7-20-4-G-S	April	2020	South Station	edule + glaucum	72	46	46
C-7-20-5-G-S	May	2020	South Station	edule + glaucum	73	46	46
C-7-20-6-G-S	June	2020	South Station	edule + glaucum	40	40	40
C-7-20-7-G-S	July	2020	South Station	edule + glaucum	40	40	40
C-7-20-8-G-S	August	2020	South Station	edule + glaucum	80	46	46
C-7-20-9-G-S	September	2020	South Station	edule + glaucum	46	45	46
C-7-20-10-G-S	October	2020	South Station	edule + glaucum	46	46	46
C-7-20-11-G-S	November	2020	South Station	edule + glaucum	46	46	46
C-7-21-1-G-S	January	2021	South Station	edule + glaucum	46	46	46

6.2.2 Laboratory sampling

Cockles were handled in the laboratory by cutting individuals in half and fixating one half in Davidsons’ fixative (formalin 10 % in sea water) for histology processing. The other half of each cockle were placed individually in 5 ml Eppendorf® tubes filled with 96 % ethanol.

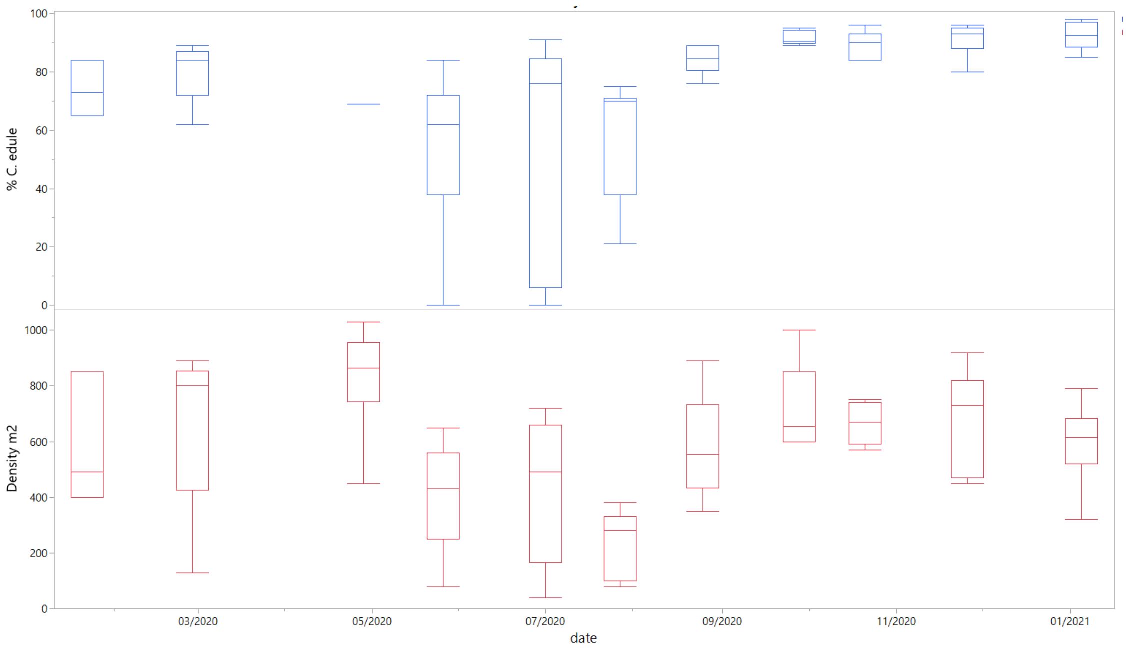

Figure 6.3. Variation of density and % *C. edule* in relation to *C. glaucum* in south station between January 2020-January 2021 collected by grabs.

6.2.2.1 Histology for macroparasites and life cycle

Deshelled, 1/2 cockles of the species *C. edule* and *C. glaucum* were received in Davidson's fixative at the Section for Fish and Shellfish Diseases, DTU Aqua, in Kgs. Lyngby. The methods described in the SOP "Mollusc processing for diagnosis by histology" by EURL for mollusc diseases, 3rd edition (link[3]) were followed. After the fixation step, the tissue samples were dehydrated and infiltrated with paraffin. Thereafter the samples were embedded in blocks of paraffin, and later sectioned in ultrathin sections that were placed on glass slides. The tissue slides were coloured by traditional HE (Hematoxylin and Eosin).

We examined cockles for parasites, sex and gametogenic stage by screening thin tissue layers (3-5 µm). Each histology slide was routinely inspected by using 50-, 100- and 200-times magnification. Macroparasites, other metazoans and protists were identified to lowest taxonomic unit by using reported keys and descriptions (de Montaudoin et al., 2009; Longshaw & Malham, 2013). A few microorganisms were also registered. However, identification of the observed protists requires further studies and approaches by using molecular techniques.

To determine the annual reproductive cycle and spawning time for *C. edule* and *C. glaucum* in the Limfjorden, around 20 specimens of both cockle species sampled monthly from March 2020 to January 2021 were used. The development of gonads (pattern of gametogenesis) was studied by a histological analysis of cockle tissue.

6.2.2.2 Molecular analysis for microparasites and other pathogens

Preliminary processing of cockles for molecular analyses

Deshelled, 1/2 cockles of the species *C. edule* and *C. glaucum* were received in individual 5 ml Eppendorf® tubes filled with a 96 % ethanol solution at the Section for Fish and Shellfish Diseases, DTU Aqua, in Kgs. Lyngby. See Table 6.1 for overview of received samples.

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[3] https://www.eurl-mollusc.eu/content/download/43746/file/Histology_process_3.pdf

Preparing tissue for DNA extraction

Each cockle was removed from the Eppendorf® tube with sterilized tweezers and placed on a sterile Petri dish. From each cockle a total of 25 (± 5) mg of tissue was cut from a mix of gill, mantle and digestive tissues, using sterile scissors. The 25 (± 5) mg sample was transferred to a 2 ml Eppendorf® tube used for lysing. The rest of the cockle was placed back in the 5 ml Eppendorf® tube and stored at 2-8 °C.

Molecular characterization

Genomic DNA extraction from tissue

The IndiMag® Pathogen Kit (Indical Bioscience, Cat. No. SP947257) was used for DNA extraction of samples from August 2019 and January 2020. A 5 mm metal ball was added to each 2 ml Eppendorf® tube with the cockle tissue together with 500 µl 1X PBS (PBS 10X Solution, Fisher BioReagentsTM, Cat. No. YBP3991, diluted with autoclaved Milli-Q water). All samples were tissue-lyzed for 4.5 minute at 25 Hz and then centrifuged at 14,000 g for 2 minutes. 200 µl of supernatant from each sample was run on an IndiMag® 48 machine from Indical Bioscience. The manufacturer’s kit protocol was followed.

The QIAmp® DNA Mini Kit (QIAGEN®, Cat. No. 51304/51306) was used for DNA extraction of samples from February 2020 to January 2021, and the manufacturer’s protocol was followed. The method only deviated from the manufacturer’s protocol for two steps. For the first lysing step, samples were left at 56 °C overnight (up to 14 hours), and for the elution step, elution was done with 130 µl of Buffer AE and with a 5 min incubation before centrifugation.

DNA quality and quantity for each sample were measured by optic density at 260 nm using a NanoDrop™ 2000 Spectrophotometer from Thermo Fisher Scientific. All samples were diluted to appropriate DNA concentrations for respective PCR investigations. All DNA was stored at -20 °C

Quantitative Real Time Polymerase Chain Reactions

All qPCR investigations were performed using an Mx3005P qPCR system from Agilent Technologies®. The qPCR methods used for disease screenings were developed and published by the European Union Reference Laboratory for Molluscs Diseases, Ifremer, Laboratoire de Génétique et Pathologie, in France.

Marteilia sp. & *Bonamia* sp. or *Vibrio aestuarianus* screening: For screening the cockles for the protistan parasites *Marteilia* sp. and *Bonamia* sp. at the same time, a duplex qPCR protocol was used developed by Ifremer (Canier et al. 2020; Link to SOP1[4]). It has been found that *Marteilia cochillia* (pathogenic for cockles) also give a positive result when using this duplex qPCR method developed for *Marteilia refringens* (personal communication: EURL for mollusc diseases, Ifremer). For screening the cockles for the gram-negative pathogenic bacterium *Vibrio aestuarianus* another qPCR protocol, developed by Ifremer, was used (Garcia et al. 2021; Saulnier et al. 2009; Garnier et al. 2008; Nhung et al. 2007; Tison and Seidler 1983; Link to SOP2[5]). Both qPCR protocols were followed without deviations except for the testing of DNA sample concentrations. The qPCR mix for both protocols was prepared using either 2X Brilliant III Ultra-Fast QPCR Master Mix (Agilent Technologies®, Cat. No. 600880) or 2X Luna® Universal Probe qPCR Master Mix (New England Biolabs, Cat. No. M3004S, M3004L). The two master mix products have proven to give comparative results.

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[4] https://www.eurl-mollusc.eu/content/download/118237/file/M.refringens%26BonamiaSp%20_RealTimePCR_editionN%C2%B01.pdf

[5] https://www.eurl-mollusc.eu/content/download/151132/file/Vaestuarianus%20_RealTimePCR_editionN%C2%B04.pdf

6.3 Results

6.3.1 Limfjorden cockle reproductive cycle

Gonad developmental phases were divided into six stages for both males and females: 0) Resting/immature, 1) early gametogenesis, 2) late gametogenesis, 3) mature, 4) spawning, 5) spent (Figure 6.4). This division is a modified version of stages described previously (Martinez-Castro & Vázquez, 2012). There are some uncertainties in identifying the stages as some individuals have gonads showing different stages at the same time. In such cases the most advanced stage was used for stage identification. While it can be difficult to distinguish between some stages, the mature stage (stage 3, Figures 6.4) is usually well defined. The sex of the cockles can usually be determined from the follicle structure (future gonad) early in the maturation process.

The data analysed provide information about spawning periods of the two cockle species – *C. edule* and *C. glaucum*. Both species have only one spawning season. The fraction of mature specimens of *C. edule* does not reach the same high level as *C. glaucum* (57% vs 91% in April). In May, all individuals of both species are either mature or in a spawning process (Figures 6.5 & 6.6). The ongoing spawning is also confirmed by observation of cockle eggs in the intestines of both *C. edule* and *C. glaucum* in mid-May (Figure 6.7). Whereas all *C. edule* has shed their gametes before mid-September, it seems that the spawning period for *C. glaucum* continues throughout September. Furthermore, the rebuilding and maturation of gonads starts later in *C. edule* (mid-October) than in *C. glaucum* (mid-September) as assessed from the pattern in 2020. To what extent this is connected to differences in the larval development of the two species is presently unknown.

The two cockle species have same male/female ratio: 45% for *C. glaucum* and 46% for *C. edule* (Figure 6.8). The fluctuation of male/female ratios throughout the year is somewhat surprising but can probably be ascribed to the small sample sizes and the associated statistical uncertainties.

The results for *C. glaucum* differ slightly from the data from Brock and Wolovic (1994), where the gametogenesis of *C. glaucum* in the Limfjorden, in Nibe area (Figure 6.2) in 1987 indicated that spawning (stage 4) occurred from late June until late September and maturity (stage 3) reached a peak in June in comparison to April/May in our study.

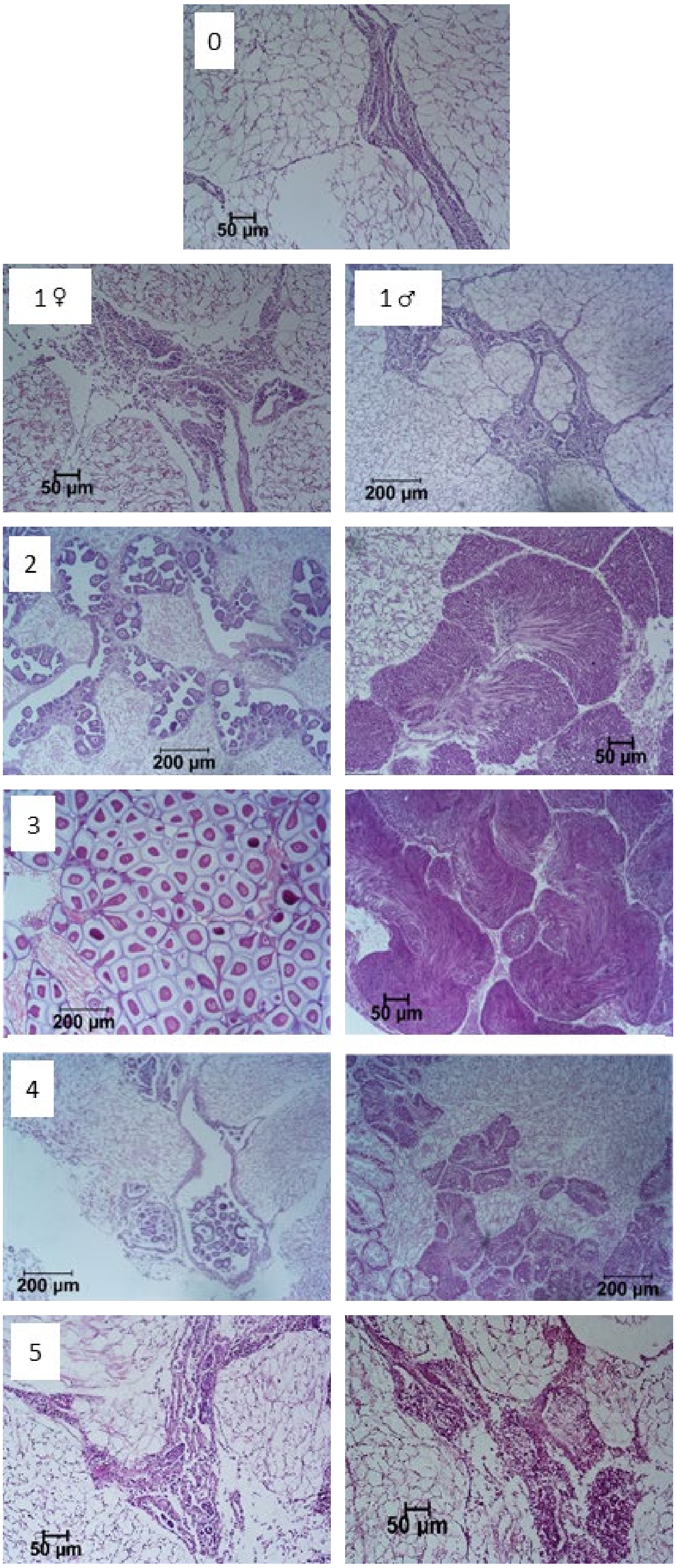

Figure 6.4. Stages in the gametogenesis of *Cerastoderma edule* (Venø). The upper photo shows a follicle in the visceral tissue in an early indeterminable stage; the left column with female stages and the right with male stages (0: indeterminate; 1: early gametogenesis; 2: late gametogenesis; 3: mature; 4: spawning; 5: spent). Identifiers. Indeterminate: 0) C7-20-08-G-S-15. Females: 1) C7-20-09-G-S-20; 2) C7-20-03-G-S-15; 3) C7-20-04-G-S-13; 4) C7-20-06-G-S-3; 5) C7-20-08-G-S-17; Males: 1) C7-20-10-G-S-09; 2) C7-20-03-G-S-1; 3) C7-20-04-G-S-7; 4) C7-20-05-G-S-1; 5) C7-19-1-a-T-38. Photos. K.T. Jensen.

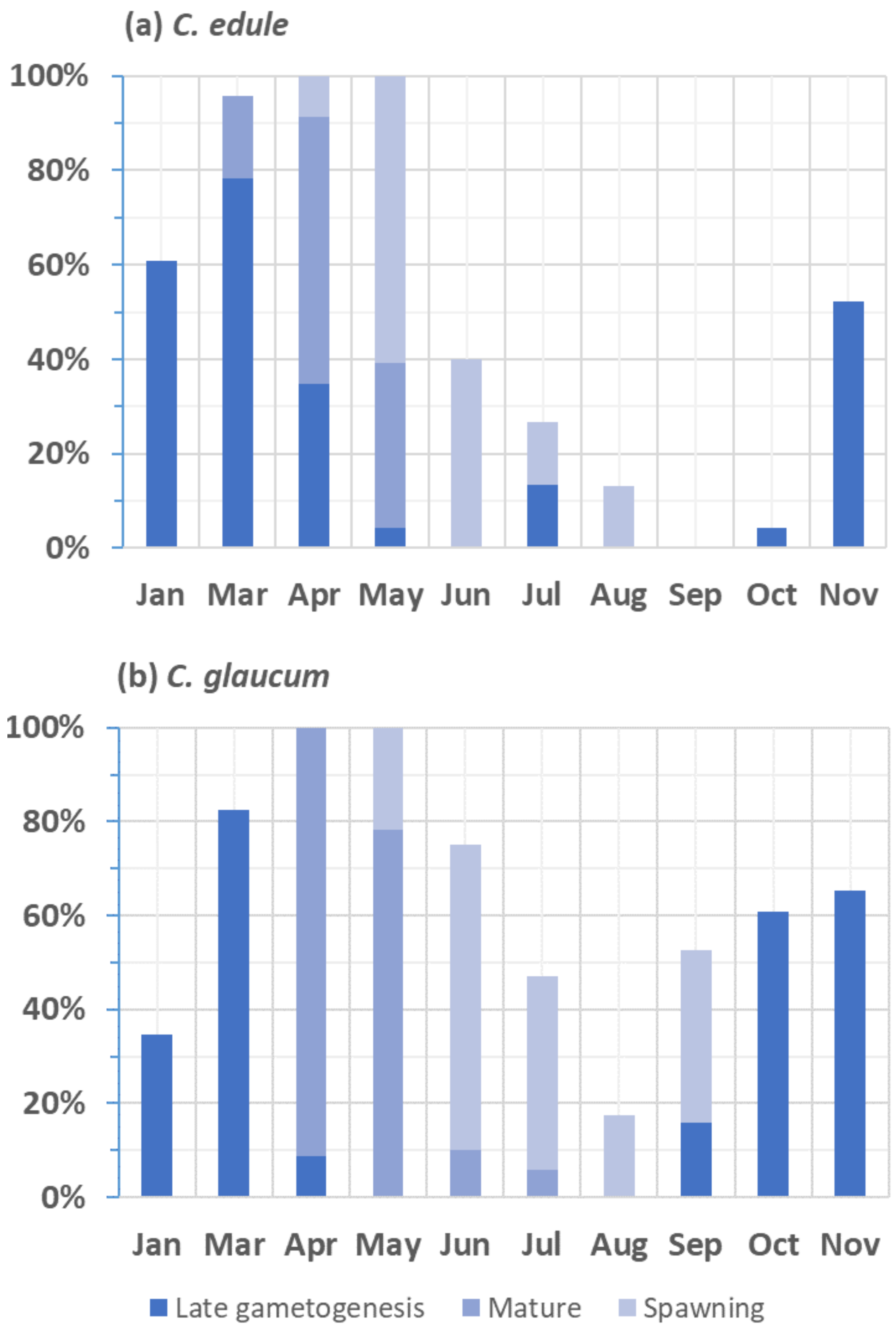

Figure 6.5. Gametogenesis in *C. edule* (a) and *C. glaucum* (b) from Venø, Limfjorden 2020-21. The graphs show the percentage of cockle individuals with gonads in one of three stages (late gametogenesis; mature and spawning) in different months as assessed from histology of tissue samples. The remaining cockles from each date were in various non-mature stages. Males and females of identical stages have been pooled in this presentation. The sample in January was collected in 2021 - all others were collected in 2020 (around the 20th each month). Note that there was no sampling in February and December.

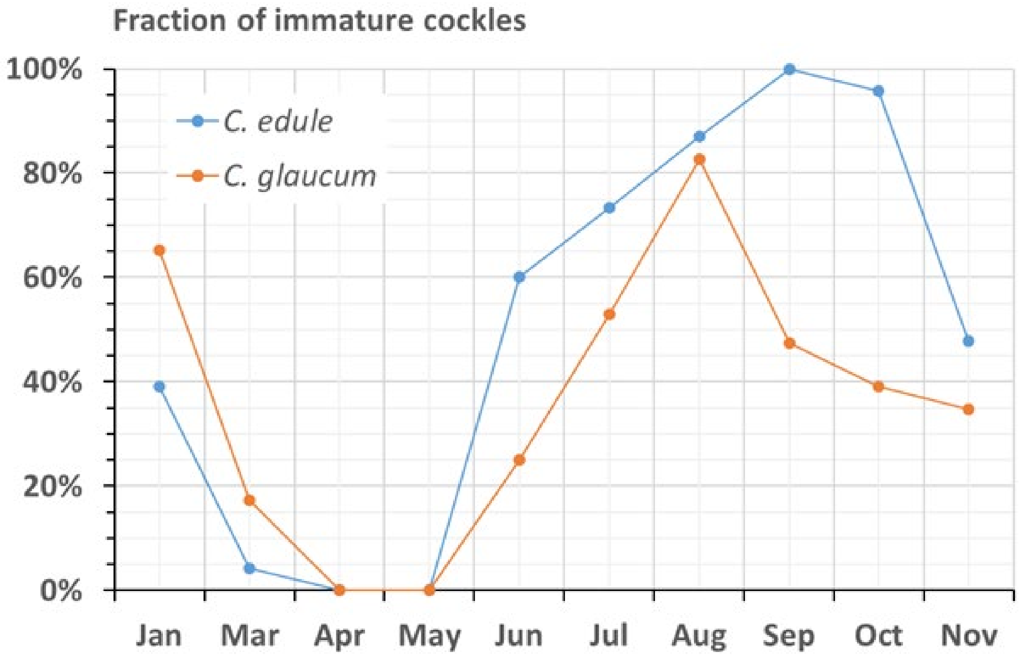

Figure 6.6. Fraction of cockles from Venø during 2020-21 with premature or spent stages.

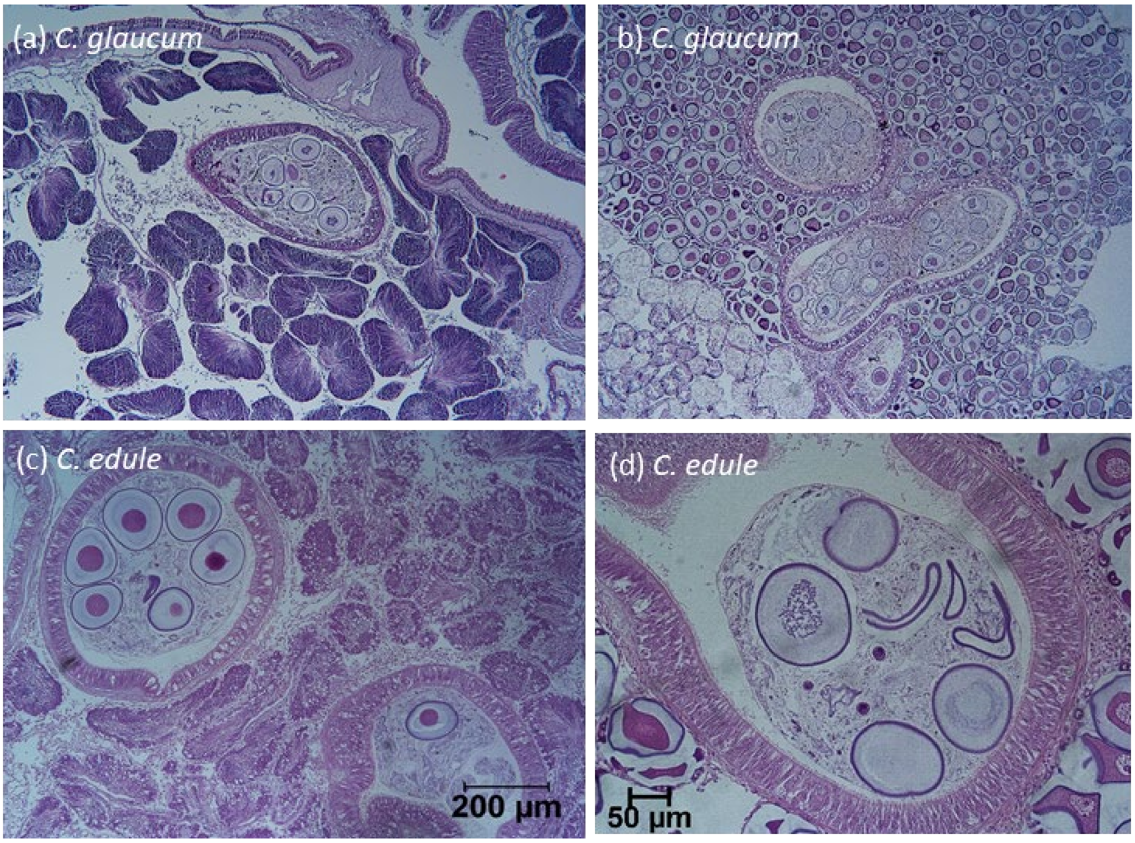

Figure 6.7. Cockles from May 2020 with cockle eggs in their stomach/intestines. An indication that spawning took place on the sampling day. We assume that the eggs entered while the cockles inhaled water. a) male *C. glaucum* (C7-20-05-G-S-44); b) female *C. glaucum* (C7-20-5-G-S_59); c) male *C. edule* (C7-20-05-G-S-01); d) female *C. edule* (C7-20-05-G-S-2). Photos K.T. Jensen.

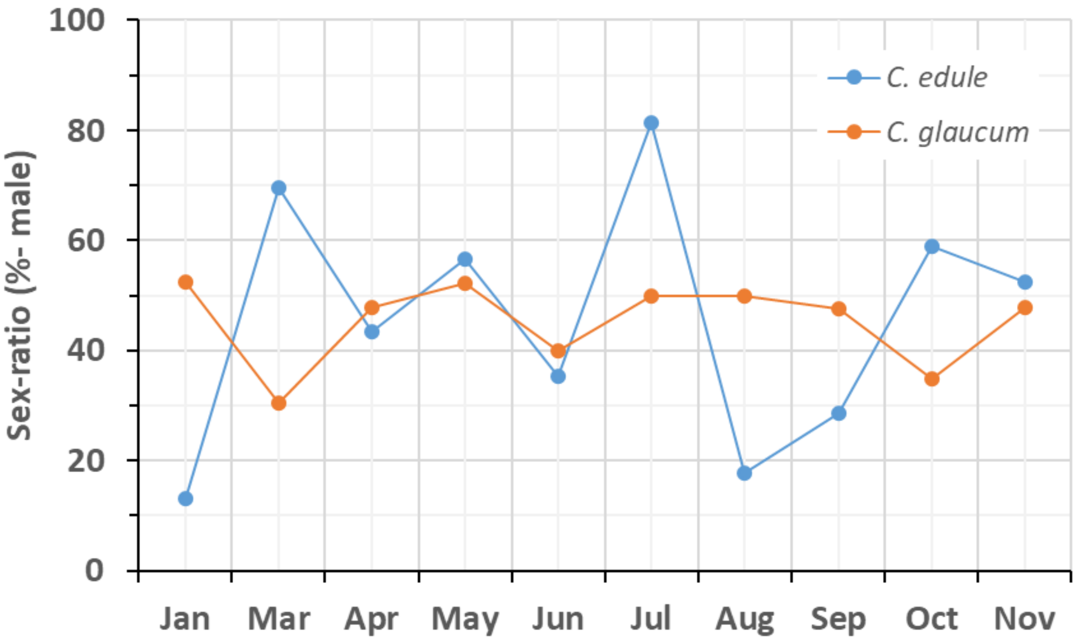

Figure 6.8. Sex-ratio (percentage males) of *C. edule* (blue) and *C. glaucum* (orange) from 18-24 individuals per species each month. The sample in January was collected in 2021 all others were collected in 2020 (around the 20th each month). Note there was no sampling in February and December.

6.3.2 Macroparasite census

Cockles are host to 16-18 macroparasite species – all belonging to the *Trematoda* (Figure 6.9; de Montaudoin et al. 2009, 2021; Bartoli et al., 2000; Longshaw & Malham, 2013). Of these, 4 species use cockles as their first intermediate host, which means that they reproduce asexually in the host and produce various larval stages. Of these 4 species two have been observed in cockles from Danish waters (*Gymnophallus choledochus* and *Monorchis parvus*). A third species *Bucephalus minimus* can be expected to eventually occur in Danish waters in the near future as its final host (*Dicentrarchus labrax*, seabass) has been observed frequently in Danish waters in recent years (https://fiskeatlas.ku.dk/artstekster/Havbars_Fiskeatlas.pdf).

The applied procedure enables identification of the macroparasites using cockles as their first intermediate host whereas those using cockles as their second intermediate host (transport host) not always can be identified to species alone based on the structure and size of encysted metacercariae. Macroparasites using cockles as first intermediate host are detrimental for infected individuals as they typically prevent development and maturation of gonads (gametogenesis). Consequently, high prevalence of such parasites may reduce the reproductive output of a population and thus result in reduced recruitment. In contrast, many of the parasites using cockles as their second intermediate host do less harm to infected individuals.

Only relatively few specimens were infected with macroparasites at the examined sites during the study period (Table 6.2 and Table 6.4). The most important ones were *Monorchis parvus* and *Gymnophallus choledochus* (Figure 6.9 a-e). Among surfacing *C. edule* in August 2019, up to 7.5% was infected with *M. parvus* and 2.5% with *G. choledochus*. Neither of the two species were found among cockles collected monthly in the period from March 2020 to January 2021. And none was observed in *C. glaucum.* Apart from these two species, metacercariae of one or more of the *Himasthla* species (Figure 6.10 f) and *Renicola roscovita* were present in a few cockles (both species of cockles). Typically, with only one or two observed metaceria per cockle.

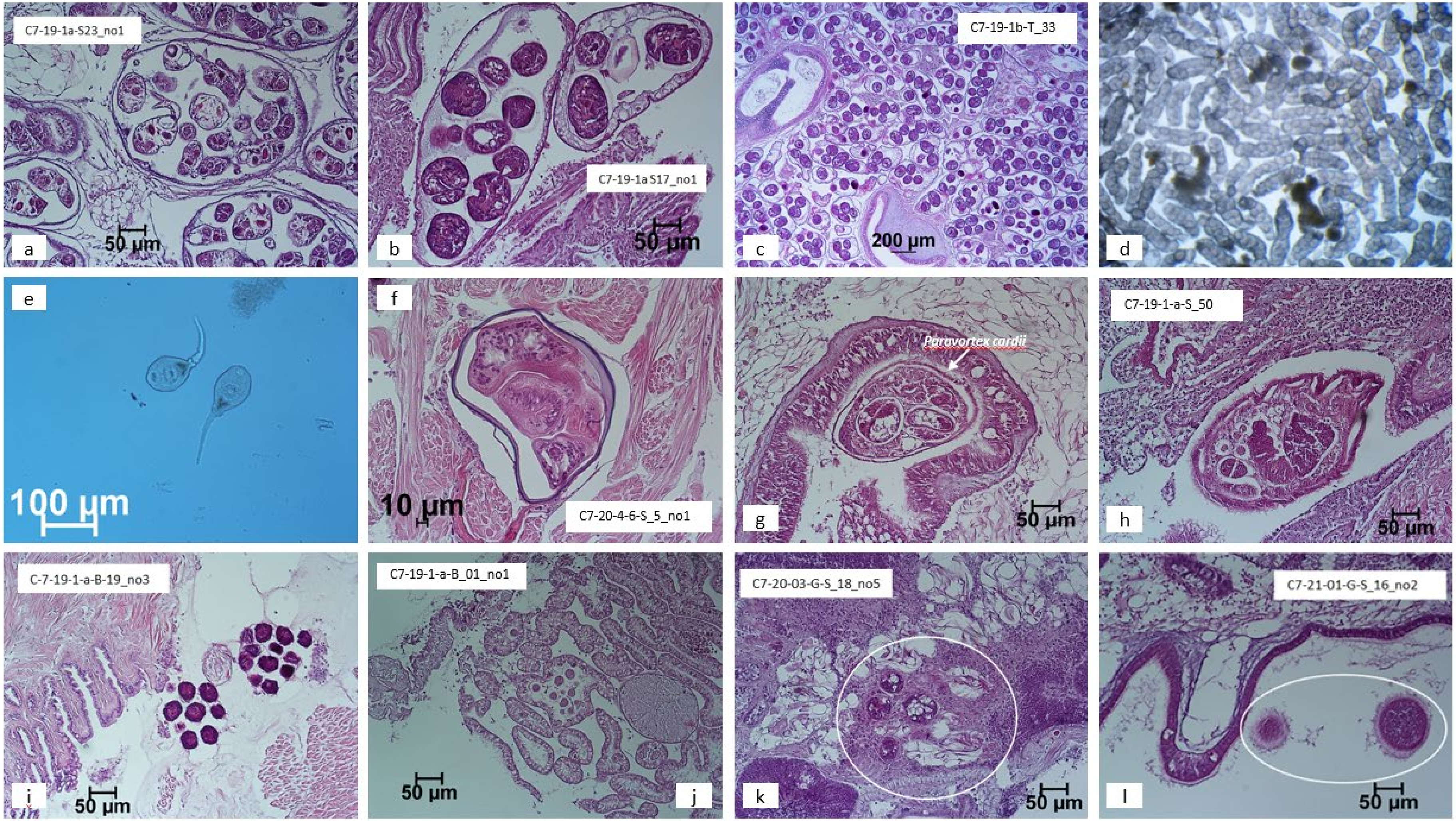

Figure 6.9. Photomicrographs of metazoans and protozoans observed in cockle tissue. A: *Gymnophallus choledochus* sporocysts containing daughter sporocyst and immature cercariae (note the split tail of a cercaria); b & c: *Monorchis parvus* sporocysts with metacercariae in different stages; d: *M. parvus* sporocysts in a cockle – released from the visceral sac after dissection. Each sporocyst contains a number metacercariae; e: *Monorchis parvus* cercariae (Sallingsund, July 2018); f: *Himasthla* metacercaria; g: the turbellarian *Paravortex cardii* inside the intestine of a cockle; h: *Paravortex cardii* infected with *Urosporidium*; i: Supposed to be eggsacs from the copepod *Hermannella rostrata*j; j: Spores of the protist *Pseudoklossia*; k: Unknown protist – *Perkinsus*?; l: the ciliate *Trichodina.* Photos K.T. Jensen.

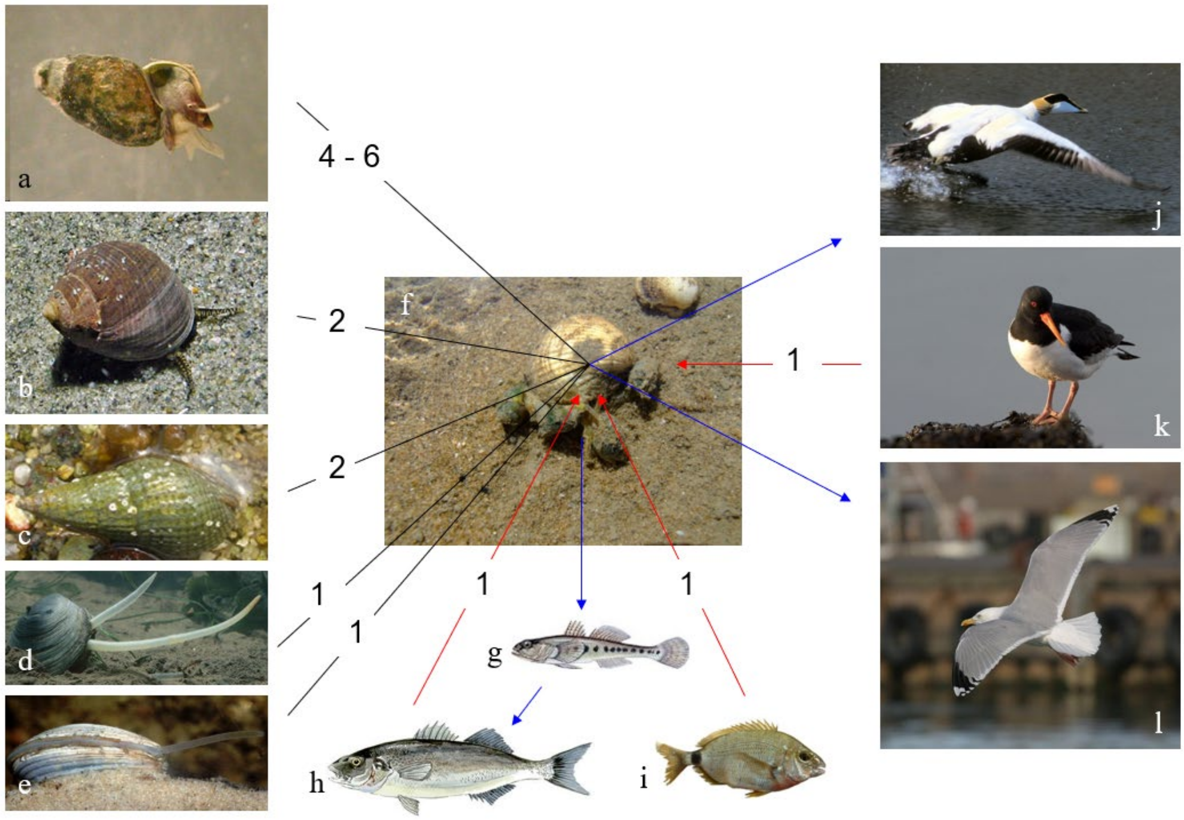

Figure 6.10. Network of host species involved in life cycles of trematodes using cockles (*Cerastoderma edule*) as their host. Each black line indicates a transmission route for 1 or more trematode species using cockles as their second intermediate host (transport host) – link from first intermediate hosts to cockle. Red lines indicate transmission of species using cockles as their first intermediate host – link from their final host to cockle. Blue lines indicate transmission from cockles to the next upstream host. Numbers: number of trematode species. The choice of first intermediate host is mostly limited to one species or to a group of sibling species. Regarding the second intermediate host, more species within the same taxonomic class can be hosts. Regarding the final host many alternative species usually exists to fish and bird parasites. Feeding biology typically determines if a species is host to a specific trematode species as the transmission of many trematodes capitalize on trophic connections. In addition to the trematode species indicated, 3 more species using cockles as their host are known from Southern Europe. a: *Hydrobia ulvae*; b: *Littorina littorea*; c: *Nassarius reticulatus*; d: *Macoma balthica*; e: *Scrobicularia plana*; f: *Cerastoderma edule*; g: *Pomatoschistus minutus*; h: *Dicentrarchus labrax*; i: *Diplodus sargus*; j: *Somateria mollissima*; k: *Haematopus ostralegus*; l: *Larus argentatus*. Photos no copyright from internet.

We did also observe the commensalistic turbellaria *Paravortex cardii* in the stomach/intestine of many cockles of both species Figure 6.9 g). Egg sacs from the commensalistic copepod - *Hermannella rostrata* - was also observed in a few cockles (Figure 6.9 i). A few protists species were also observed but their identity cannot be determined solely based on morphology and tissue localization (Figure 6.9 j-i). Neither of these protists are considered to be significant disease agents and deleterious to cockles.

The *Himasthla* species (*H. elongata, H. continua and H. interrupta*) and *R. roscovita* are generally widespread in cockles from various shallow water areas. Cockle-eating waterbirds such as seagull, oystercatcher and common eider can be final hosts for these trematodes. While the common periwinkle (*Littorina littorea*) is first intermediate host to *H. elongata and R. roscovita,* mudsnails (*Hydrobia ulvae, H. ventrosa and H. neglecta*) are first intermediate host to the other two *Himasthla*-species. Generally, *Himasthla* metacercariae are in foot tissue except *H. interrupta* that is more common in mantle tissue (de Montaudouin et al., 2009). *R. roscovita* metacercariae are typically located in the palp tissue. We have previously found all these trematode species in snails from the Limfjorden (own observations, K.T. Jensen). The intensity and prevalence of these trematodes in cockles depends on the presence and density of their snail and bird hosts. Generally, their impact on infected cockles is modest but infected cockles have reduced growth rate (Wegeberg & Jensen, 2003) and suffer higher mortality than non-infected if exposed to anoxic conditions (Wegeberg & Jensen, 1999). However, no host effect is expected at the low intensities observed in the present study. At shallow depths (< 1m) in the Limfjorden, cockles may accommodate hundreds of metacercariae – some older specimens even thousands at places where both the first intermediate host (snails) and the final host (waterbirds) are present (own observations, K.T. Jensen).

Table 6.2. Metazoans and protists observed in cockles (*C. edule and C. glaucum)* collected in Venø Bugt, Limfjorden (2019-2021). Some of the species cannot be identified with certainty based on histology - molecular techniques or macroscopic observations are needed for verification (see comments).

	Species	1. int. Host	2. int. Host	Final host	Comments
Trematoda – Digenea	*Monorchis parvus*	*C. edule, C. glaucum*	*C. edule, C. glaucum*	Fish: *Diplodus*-species	• Life cycle in the Limfjorden is different as the reported final hosts are missing • in gonads and visceral mass • no offspring production; mass mortality reported
	Gymnophallus choledochus	*C. edule*	*C.edule* Polychaetes	Waterbirds: seagull, oystercatcher, eiders	• life cycle with summer (polychaetes) and winter (cockles) generations • in gonads and visceral mass • no offspring production; mass mortality reported
	Himasthla elongata *H. continua,* *H. interrupta*	*Littorina littorea* Mudsnails	Bivalves	Waterbirds: seagull, oystercatcher, eiders	• typically in foot tissue (*H.e.* and *H.c*) and in mantle margin (*H.i.*) • minor sublethal effects (growth reduction, reduced tolerance of anoxia)
	Renicola roscovita	*Littorina littorea*	Bivalves	Waterbirds: seagull, oystercatcher, eiders	• typically in the palps • no effects on cockles reported
Copepoda	*Hermannella rostrata*	In bivalves: Direct life cycle with free-living larvae			• indirect evidence for occurrence (egg sacs); • located around mantle and gills; • no pathology demonstrated
Turbellaria	*Paravortex cardii*	In cockles: Whole life in the host; horizontal transmission			• in the gut/intestine • no pathology
Rhizaria - Haplosporida	*Urosporidium* (?)	*Paravortex cardii in cockles*			• hyperparasite of *Paravortex cardii*
Alveolata - Eucoccidiorida	'*Pseudoklossia*' (?)	*C. edule and C. glaucum*			• molecular confirmation necessary • In nephridium tissue
Alveolata - Perkinsea	*Perkinsus* sp. *(?)*	*C. edule (C. glaucum?)*			• molecular confirmation necessary • connective tissue of labial palps, gills mantle and visceral mass

Gymnophallus choledochus has a spring-summer and autumn-winter life cycle (Loos-Frank, 1969) and cockles can be both first and second intermediate host. Various waterbirds can be final host (seagull, oystercatcher, common eider). During the spring-summer cycle cercariae (the dispersal stage) are released from infected cockles. They may infect various polychaetes such as nereidae and nepthyidae, where they are in tissue around the bristles in the parapodia as metacercariae (own observations, K.T. Jensen). In birds eating such worms, the metacercariae hatch and develop to adults that mates in the digestive system of the host. Their eggs are expelled with bird droppings and larvae emerging from the eggs infect cockles. In the autumn-winter cycle, the dispersive stage is skipped and instead the larvae develop directly to metacercariae. If such cockles are eaten by a bird, the metacercaria develops to an adult that find a mate in the bird host.

Infected cockles are generally filled by larval stages, and they have become a machinery for producing *G. choledochus* larvae (and no cockle offspring). In other words, infected cockles are not part of the reproducing stock. Infected cockles from intertidal flats exposed to high summer temperatures may exhibit surfacing (Thieltges, 2006). However, generally only a minor fraction of cockle populations is infected by this species. Nonetheless, we have observed up to 25 % of a cockle population from Norsminde fjord infected with *G. choledochus* (own observations, K.T. Jensen). So, the species is potentially a harmful agent. In the Limfjorden, there could be a risk for incidences of mass occurrence of infected cockles at shallow water sites where birds have access to cockles.

Monorchis parvus was discovered in cockles in 2012 (August and November) where 20% of the cockles from sites in the Sallingsund area were infected.[6] This was the first observation of *M. parvus* in cockles from Danish waters. In the present study, we found a maximum prevalence of 7.5% (August 2019). As this parasite like *G. choledochus* uses cockles (both cockle species can be host to the parasite) as its first intermediate host, infected specimens cannot reproduce. Given that the prevalence of this species previously has reached 20% in the Limfjorden[7], we consider it as one of the important agents that could impact the size of the reproducing stock of cockles and thus should be monitored.

The life cycle of *M. parv*us is described as a two-host system (cockle to fish) with fish belonging to the family Sparidae such as *Diplodus sargus* (Sorthalen) *D. vulgaris* (Tobåndet havrude) and *D. annularis* as final hosts (Bartoli et al., 2000). Members of this fish group have only occasionally been observed in Danish waters and not in the Limfjorden at all.[8] Our observations of infected cockles from Limfjorden supports a two-host system as the infected cockles were filled with wormlike sporocysts containing metacercariae (Figure 6.9 b-d) ready for being ingested by a fish host. However, to our knowledge we do not have a fish species in the Limfjorden that can handle and eat cockles within the infected size spectrum. Preliminary studies of various fish caught near the study site did not reveal any specimens infected with *M. parvus* neither any eating cockles. Therefore, the presence of *M. parvus* in cockles in the Limfjorden is an enigma.[9]

To clarify the life cycle of *M. parvus* in Limfjorden, we examined the possibility for alternative patterns to the reported two host life cycle. There could be a possibility for a three-host system with a second intermediate host involved because cercariae (the dispersal stage) have been observed in *M. parvus*-infected cockles from UK and Mediterranean. In the present study, we found infected cockles with cercariae in July 2018, Sallingsund 4-5 m's depth (Figure 6.9 e), so a three hosts life cycle with an unknown second intermediate host cannot be excluded. However, it appears to be a minor transmission route if at all, as only few cercariae have been observed among dense stocks of sporocysts with metacercariae.

Inspired by observation of dying cockles with exposed flesh and surrounded by small gobies (*Pomatoschistus microps*) we hypothesized that the small wormy sporocysts (Figure 6.9 d) released from disintegrating cockle flesh could be bait to small fish such as *P. microps*. In this study, we did pilot experiments to test this by feeding such sporocysts to juvenile gobies. After 1 week we found maturing adults of *M. parvus* in the fish intestine and some of these survived at least 20 days post infection. Although we did not observe fully mature *M. parvus* specimens, we consider this as an obvious dispersal mechanism for *M. parvus* in the Limfjorden. We expect that there is a critical threshold for *M. parvu*s

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[6] https://videnskab.dk/miljo-naturvidenskab/mystisk-er-stenalder-fisk-vendt-tilbage-med-klamme-parasitter
Jensen KT og Petersen SV (2013). ”Ikke-nedgravede hjertemuslinger i Limfjorden – snyltere, gonadeudvikling og neoplasi”. Rapport til DSC

[7] ibid

[8] https://fiskeatlas.ku.dk/artstekster/Sorthale_Fiskeatlas.pdf

[9] https://videnskab.dk/miljo-naturvidenskab/mystisk-er-stenalder-fisk-vendt-tilbage-med-klamme-parasitter

larvae in cockles. Ultimately, infected cockles will die from their load of *M. parvus* larvae and then they may become accessible to fish.

Though prevalences of *M. parv*us observed in the present study were low, this parasite has a potential for mass occurrence in cockle populations and further studies are required to identify factors controlling its dynamics.

Table 6.3. Overview of disease positive animals per month and year in production area 7, the Limfjorden, Denmark. Letter code for sample batch IDs: a = Surfacing, b and c = Non surfacing, G = grab; S = Surface, T and B = buried; M = Mid Site, S = South Station. a, b, and c samples were handpicked by a diver, and G samples were sampled with a grab.

Sample Batch ID	**Sampling Month**	**Sampling Year**	**Bed type/position**	**# of Samples run in qPCR**	***Marteilia refringens* positives**	***Bonamia* sp. positives**	***Vibrio aestuarianus* positives**
C-7-19-1-a-S	August	2019	Surfacing - Surface	50	0	0	27 (54 %)ˣ
C-7-19-1-a-T	August	2019	Surfacing - Buried	50	0	0	33 (66 %)ˣ
C-7-19-1-a-B	August	2019	Surfacing - Buried	23	0	0	19 (83 %)ˣ
C-7-19-1-a total	*August*	*2019*	*Surfacing - Buried*	*73*	*0*	*0*	*52 (71%)*
C-7-19-1-b-S	August	2019	Non surfacing - Surface	13	0	0	7 (54 %)ˣ
C-7-19-1-b-T	August	2019	Non surfacing - Buried	50	0	0	35 (70 %)ˣ
C-7-19-1-b-B	August	2019	Non surfacing - Buried	40	0	30 (75 %)*	39 (98 %)ˣ
C-7-19-1-c-T	August	2019	Non surfacing - Buried	50	0	0	25 (50 %)ˣ
C-7-19-1-c-B	August	2019	Non surfacing - Buried	1	0	0	1 (100 %)ˣ
C7-19-1-c total	*August*	*2019*	*Non surfacing - Buried*	*141*	*0*	*30 (21%)*	*133 (94%)*
C-7-20-1-b-S	January	2020	Non surfacing - Surface	3	0	0	0
C-7-20-1-b-T	January	2020	Non surfacing - Buried	36	0	0	0
C-7-20-1-c-T	January	2020	Non surfacing - Buried	52	0	0	0
C-7-20-1-c-B	January	2020	Non surfacing - Buried	19	0	0	0
C-7-20-1-c-total	*January*	*2020*	*Non surfacing* - Buried	*107*	*0*	*0*	*0*
C-7-20-2-G-M	January	2020	Mix in grab	45	0	0	0
C-7-20-3-G-S	February	2020	Mix in grab	60	0	1 (2 %)*	0
C-7-20-4-G-S	April	2020	Mix in grab	46	0	0	0
C-7-20-5-G-S	May	2020	Mix in grab	46	0	0	0
C-7-20-6-G-S	June	2020	Mix in grab	40	0	0	29 (73 %)ˣ
C-7-20-7-G-S	July	2020	Mix in grab	40	0	1 (3 %)*	7 (18 %)ˣ
C-7-20-8-G-S	August	2020	Mix in grab	46	0	0	26 (57 %)ˣ
C-7-20-9-G-S	September	2020	Mix in grab	45	0	0	1 (2 %)ˣ
C-7-20-10-G-S	October	2020	Mix in grab	46	0	0	1 (2 %)ˣ
C-7-20-11-G-S	November	2020	Mix in grab	46	0	0	0
C-7-21-1-G-S	January	2021	Mix in grab	46	0	0	0

* Extremely weakly positive ˣ Positive

6.3.3 Microparasites and other pathogens

Findings from the molecular disease screening of *C. edule* and *C. glaucum* individuals from the Limfjorden Denmark proved both very interesting and surprising, while also revealing some predicted results. An overview of the results can be seen in Table 6.3. Most of the cockles were found to be without any of the three pathogens chosen for the screening. Not a single cockle tested positive for *Marteilia refringens* (and thereby also *Marteilia cochillia*), which complies with past testing for the pathogen in European flat oysters, *Ostrea edulis*. *M. refringens* has yet to be observed in any tested molluscs species sampled from Danish waters.

No pathogens were found in cockles collected between November and May, except for one single cockle that was found extremely weakly positive of *Bonamia* sp. collected in February 2020 (2%). Another single cockle from July 2020 (3%) as well as 30 cockles from August 2019 (75%) also tested positive, although also extremely weakly, for *Bonamia* sp. *Bonamia* sp. has not been reported in cockles before, but other haplosporidian species have been observed in European cockle beds (Azevedo et al., 2003; Albuixech-Martí et al., 2020). Only specimen of *C. edule* were infected by *Bonamia sp.*

Other haplosporidian species in European cockles have been observed all year round, which could explain the finding of *Bonamia* sp. in a Limfjorden cockle in February as well as higher prevalence in the warmer summer months, where most diseases and pathogens tend to be more prominent. It was not possible in this study to determine any correlating factor to the finding of 30 *Bonamia* sp. positive cockles in the Limfjorden collected in August 2019, all in buried cockles in a non-surfacing area (Table 6.3).

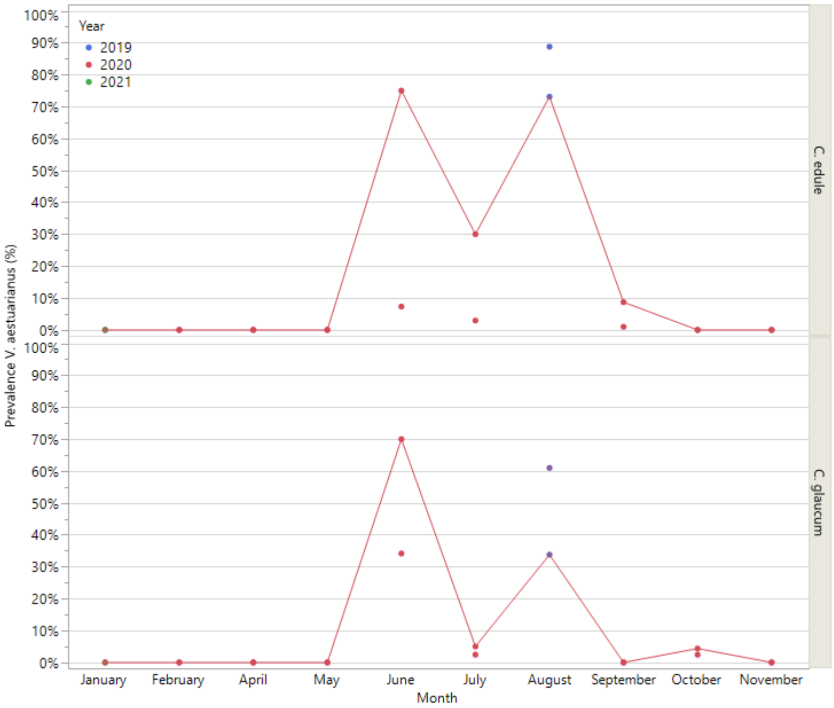

Figure 6.11. ***Vibrio aestuarianus*** **prevalence in** ***Cerastoderma*** **spp. in Venø Bredning in Limfjorden between August 2019 and January 2021 in both surfacing and non-surfacing areas and in buried and surfaced cockles.**

The cockles were also tested for the bacterium *Vibrio aestuarianus*. In August 2019, June 2020, and August 2020 the prevalence of the bacteria was found to be very high with more than 50% of screened individuals from each batch testing positive. The prevalence was less high in July 2020 with only 18% of individuals testing positive. A single cockle tested positive in both the batches from the fall months of September and October 2020. No cockles tested positive from batches collected between November and May. *V. aestuarianus* is prevalent only in summer months both in 2019 and 2020 (Figure 6.11), a season when *V. aestuarianus* has been described to cause high mortality (e.g review by de Montaudoin et al., 2021).

6.3.4 Surfacing behaviour of cockles: possible role of parasites and sex

High numbers of cockles have regularly been reported laying on the sediment surface in different areas in the Limfjorden by fishermen. Such individuals have been considered as moribund, but causative factors for the surfacing behaviour have not yet been identified (See Chapters 4 and 5, this report). As surface-dwelling cockles have been part of the cockle fishery in the Limfjorden, an explanation for this behaviour is required. Considering the need for a sustainable fishery, it is also important to clarify if surfacing cockles are a decaying fraction of the population or if they are a viable part. We have tested two hypotheses in this respect:

1. surfacing is caused by a parasite or a pathogen through either host manipulation to promote transmission to a final host or because tissue damage inflicted by the parasite/pathogen may impair burrowing ability.
2. previous observations have suggested that surfacing in cockles is associated with release of gametes during spawning[10].

One first attempt to test these hypotheses would be to inspect cockles from the sediment surface and cockles established in their normal bottom-dwelling position during an episode with mass occurrence of surfacing cockles. Such episodes were discovered near Venø in August 2019. Cockles from different sediment layers were collected (see methods in Chapter 4 and section 6.2.1) examined for parasites and gamete stages (section 6.2.2.1, this chapter).

Three groups of macroparasites were found in *C. edule* (Tables 6.2 and 6.4): *Himasthla* spp., *Monorchis parvus* and *Gymnophallus choledochus.* Of these only *Himasthla* spp. occurred in *C. glaucum*. Their characteristics, life-cycles and host effects are described in section 6.5. *Himasthla* spp. metacercariae were observed in very low numbers and only in a few individuals of both species. With their very low intensity and frequency in cockles from the studied site they do not have impact on the behaviour of cockles. The two other macroparasites have significant impact on their individual host as described above. We have also observed a low number of few protists in the examined cockles.

The highest prevalence of *M. parvus* infected cockles (7.5%) were observed in surfaced cockles exposed on the sediment surface at '*Surfacing sites*' and all *M. parvus* infected *C. edule* were on the surface (Table 6.4). However, on '*Non-surfacing*' sites, the few infected *C. edule* were buried. For the two *C. edule* specimens infected with *G. choledochus*, one was at the surface and one buried.

We identified each cockle's sex and gametogenic stage (Section 6.3.2.1) to test if these traits could be involved with surfacing. As most cockles already have shed their gametes in August, relatively few individuals were mature or spawners (stages 3 and 4), which are the stages that might trigger surfacing if any stage at all.

There is no remarkable difference in the frequency of males and females of *C. glaucum* between different positions (surface, buried) (Figure 6.12). As seen on Figure 6.13, the pattern of frequencies of mature cockles with position differs in the two cockle species: the frequency increases with depth (surface-buried) in *C. glaucum* and stays equal with depth in *C. edule.* However, considering the minor differences there is no support for any strong relationship between matureness and surfacing.

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[10] Jensen KT og Petersen SV (2013). "Ikke-nedgravede hjertemuslinger i Limfjorden – snyltere, gonadeudvikling og neoplasi". Rapport til DSC

Table 6.4. Trematoda in *Cerastoderma edule* sampled in August 2019 (Limfjorden) at sites with surfacing cockles. Surface: on the surface; Buried: in the sediment; prevalence: percentage of infected; in brackets: number of infected divided by sample size.

	Trematod Prevalence (%)		
Species	**Surface**	**Buried**	**Identifier**
Monorchis parvus			
-Surfacing	7.5% (3/40)		C7-19-1-a-S13 (edu); C7-19-1-a-S17 (edu); C7-19-1-a-S31 (edu)
-Not-surfacing		2.4% (3/126)	C7-19-1-b-T-33 (edu); C7-19-1-b-B40 (edu) C7-19-1-c-T-26 (edu)
Gymmnophallus choledochus			
-Surfacing	2.5% (1/40)		C7-19-1-a-S23 (edu)
-Non-surfacing		1.3% (1/75)	C7-19-1-b-T48 (edu)
Identifier-series	C7-19-1-a-S	C7-19-1-a-T	
	C7-19-1-b-S	C7-19-1-b-T	
	C7-20-1-b-S	C7-19-1-c-T	
		C7-20-1-b-T	
		C7-20-1-c-T	
		C-7-19-1-a-B	
		C-7-19-1-b-B	
		C-7-19-1-c-B	
		C-7-20-1-c-B	

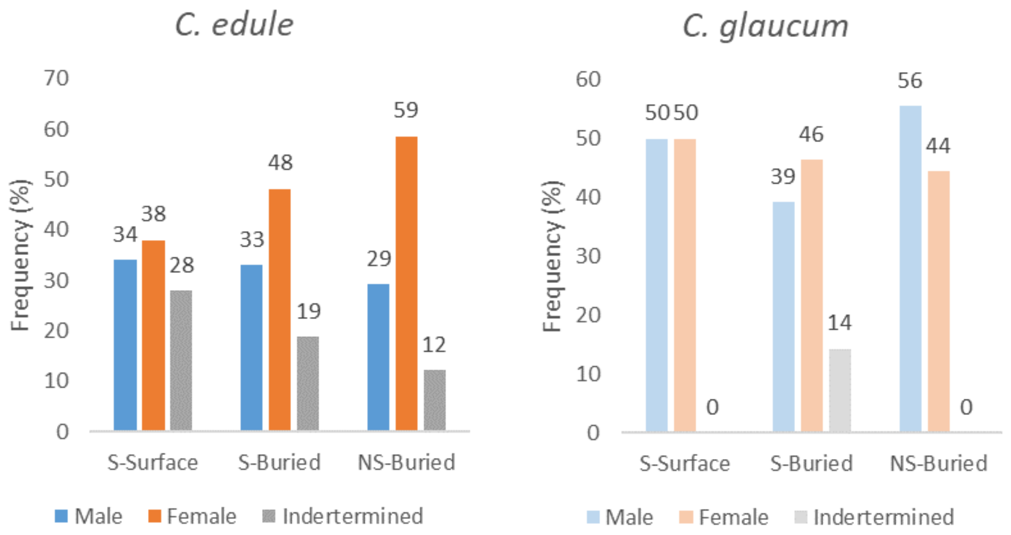

Figure 6.12. Sex-composition of *C. edule* and *C. glaucum* populations from the different positions: surface and buried in August 2019 in Venø from surfacing (S) and non-surfacing (NS) beds.

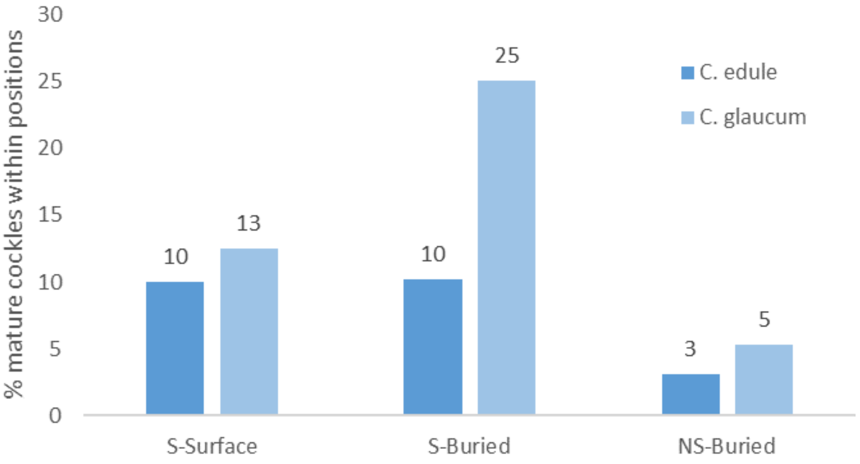

Figure 6.13. Frequency (%) of mature cockles (stages 3 and 4) of *C. edule* (dark blue) and *C. glaucum* (light blue) in populations from the different positions (surface, buried) from surfacing (S) and non-surfacing (NS) natural beds in Venø August 2019. The number of *C. edule* and *C. glaucum* individuals at the 2 positions were S-surface 5 and 2; S-buried 14 and 15 and NS-buried 2 and 3 respectively.

6.3.5 Co-occurrence of pathogens

In a total of 676 specimen of *C. edule* and 577 specimen of *C. glaucum* analysed both for macroparasites and disease, only a few specimens showed co-occurrence of parasite/disease. In Figure 6.14, focusing on the August 2019 dataset, there were only a few specimens of *C. edule* cockles that had infection with *Vibrio aestuarianus* as well as either by *Gymnophallus choledochus, Monorchis parvus*, or *Himasthla* spp.. Only 1 immature *C. edule* cockle from August 2019 accumulated *M. parvus* with *Himasthla spp.*, *Bonamia* sp. and *V. aestuarianus.* In general, specimens of *C. edule* were more infected by macroparasites and disease (Figure 6.14). In August 2019, 93% of the *C. edule* contaminated with *Bonamia* sp. also had *V. aestuarianus*. In 2020, no co-occurrence with *Bonamia* sp. Was observed. Out of the 17 cockles infected in July and August 2020 with *Himasthla spp.*, only 2 also had *V. aestuarianus*.

Figure 6.14. Scatter plot data co-occurrence of macroparasites and *Bonamia* sp. and *Vibrio aestuarianus* on individual cockles *C. edule* (e) N = 226 and *C. glaucum* (g) N = 51 in populations from the different positions: surface and buried from non-surfacing and surfacing natural beds in Venø August 2019. Blue dots for *C. edule* and red dots for *C. glaucum*. Yes: Indicates presence of the pathogen; and No: Indicates absence of the pathogen in the cockles.

6.4 Conclusions and perspectives

Both cockle species *Cerastoderma edule* and *C. glaucum* were sampled monthly over a 1-year period for pathogen census and reproductive cycle.

Macroparasites

Pathogen census of macroparasites revealed that only two species of trematode macroparasites (*Gymnophallus choledochus* and *Monorchis parvus*) were present in *C. edule* but none was present in *C. glaucum*. Both trematode species were found up to 7.5% and 2.5% in surfacing cockles. Both species may be potentially harmful agents and their prevalence should be monitored. There were a few *Himasthla* spp. and *Renicol roscovita* present in both species. The low density observed in cockles are not expected to influence the cockle population.

Given the low numbers of infected cockles with macroparasites and together with earlier observations[11], there is not much support for their role in promoting surfacing in cockles. However, interactions with environmental factors such as oxygen depletion in the bottom water or high temperatures might still trigger surfacing of infected cockles as seen in Chapter 5, this report. Furthermore, we have reasons to expect that *M. parvus* infected cockles will die when the mass of *M. parvus* larvae has reached a critical level. When this point is reached, cockles may end on the surface of the sea bottom. This may be a mechanism to secure transmission to their final fish host (gobiids attracted by worm baits in the cockle). There have also been reports that *G. choledochus* infected cockles on the bottom surface under certain circumstances (Thieltges, 2006), but due to the low parasite prevalence in the Venø/Limfjorden cockles, parasites were not responsible for this particular surfacing event, unless the high infection specimens died out leaving a population with lower prevalence, but still higher than buried cockles.

Microparasites and other pathogens

Regarding pathogen screening using a molecular approach, not a single cockle tested positive for *Marteilia refringens* (and thereby also *Marteilia cochillia*). There were only a few *C. edule* over the yearly sampling and one specific sample in August 2019 with *Bonamia* sp. Investigations of other haplosporidian spp. in cockles show that body size can be a determining factor for infection rate, as older hosts are more likely to accumulate pathogens over time. This is due to their longer duration of exposure as well as their larger filtration capacity, and thus a larger pathogen intake (Albuixech-Martí et al., 2020; Lafferty & Kuri, 2009; Mouritsen et al., 2003; Breitburg et al., 2015). During the summer months, the greater reproductive output of larger individuals might make the cockles less capable of clearing infections due to more energy invested in reproductive measures (Taskinen & Saarinen, 1999). As all cockles testing positive for *Bonamia* sp. were extremely weakly positive, it is not believed that the pathogen is causing serious disease in the affected individuals, but that the cockles may have simply acquired the pathogen most likely through feeding. However, it does raise the question if cockles can thereby be unknown transmitters of *Bonamia* sp. and act as reservoir if moved around within an area also resident to European flat oysters, which is the case in the Limfjorden.

The lack of *V. aestuarianus* positive cockles in the colder winter months, while finding a high prevalence in the summer months, correlates with other findings of the bacterium, which has been associated with mortality events in cockles as well as Pacific oysters during the summer months (Garcia et al. 2021; Nguyen et al, 2019; EFSA Panel on Animal Health and welfare (AHAW), 2010). Despite significant levels of infection in the positive individuals, none of the cockles tested seemed moribund or

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[11] Jensen KT og Petersen SV (2013). ”Ikke-nedgravede hjertemuslinger i Limfjorden – snyltere, gonadeudvikling og neoplasi”. Rapport til DSC

showed clear signs of disease. This could be explained by the bacterial strain perhaps not being of a particular virulent nature. Since no bacterial isolations on agar plates were performed, and the detection protocol did not permit discrimination of virulent vs non-virulent strains of *V. aestuarianus*, it cannot be determined if that is the case, or if the cockles in the Limfjorden simply have a high tolerance towards *V. aestuarianus*.

Although both *Bonamia* sp. and *V. aestuarianu*s pathogens were found in the tested cockles, none of the two pathogens presently seem to be the cause of serious disease in the cockle beds of the Limfjorden. Though, *V. aestuarianus* could potentially cause serious mortalities in the future if combined with other biotic or abiotic stressors such as eutrophication and pollutants from freshwater discharges, temperature increases, decrease in dissolved oxygen, other pathogens (bacteria, virus, parasites) (Garcia et al. 2021; Lupo et al. 2019, 2020; Travers et al. 2017).

Reproductive cycle

C. glaucum reached a high level of maturity (97% in April) before *C. edule and* had a longer spawning period through to September. Both species were either mature or in a spawning process in May. The rebuilding and maturation of gonads starts later in *C. edule* (mid-October) than in *C. glaucum* (mid-September) as assessed in 2020. The two species had similar male/female ratio of around 45% and was similar in surfacing and non-surfacing areas. Results suggest there were more mature *C. glaucum* than *C. edule* in surfacing area on the buried fraction and that both species were more mature than in non-surfacing areas. Only a few mature specimens were recorded in the buried fraction of non-surfacing beds.

The level of pathogen occurrence in the Venø area of the Limfjorden over a one-year survey revealed that both species of cockles were healthy without signs of poor condition or being moribund. Although in August 2019 when surfacing events occurred, more pathogens were found, there were still no signs of potential mass mortalities and neither of the examined individual factors (parasites, matureness, gender) can explain the observed surfacing events in August at Venø. In cases where mass occurrence of cockles happens, it is probably triggered by a combination of environmental factors and individual traits (see Chapters 4 and 5, this report).

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7. Fishing efficiency

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7.1 Rationale

Cockles (*Cerastoderma edule* and *Cerastoderma glaucum*) are fished in the Limfjorden as a by-catch of the blue mussel (*Mytilus edulis*) fishery. However, the blue mussel fishery uses a surface dredge not expected to significantly dig into the sediment and thus cockles are not expected to be significantly caught by the surface dredge, except during episodic emergence to the sediment surface.

Albeit a by-catch, almost if not all the entire cockles catch in the Limfjorden comes from targeted fishing of cockles at different locations than blue mussels (Chapter 2, this report). This suggests a normal practice by the fishery to fish non-surfacing cockles when found by the fishermen's acoustic techniques and prospection dredging, and thus that the mussel surface dredge can capture a significant proportion of buried cockles.

This chapter presents results from DTU Aqua fishing trials to determine the efficiency of the Limfjorden blue mussel surface dredge to fish cockles, assessing its relationship with surfaced and buried cockles.

7.2 Methods

7.2.1 Fishing efficiency

Two approaches can be used to estimate fishing efficiency: a pairwise disturbed (fished) vs control (non-fished) design on single dredge tracks; and a catch-based approach that allows to assess fishing efficiency on areas dredged multiple times. The pairwise approach determines the real instantaneous fishing efficiency of the surface mussel dredge as it actually fishes a cockle population, i.e. inside relative to outside a dredge track, and ignores areas with no cockles. It thus can be seen as more accurate and only affected by meter-scale spatial variability in abundance. The catch-based approach integrates areas with variable abundance over the entire fished track, both with and without cockles, and reflects fishing efficiency of entire fished tracks being affected by variability from tens to hundred meters. The two approaches produce different estimates of fishing efficiency and catch-based estimates are likely closer to fishermen perception of the mussel dredge cockle fishing efficiency. Due to the high sampling resolution required for the catch-based approach to produce accurate estimates, this approach was not used in this study.

Fishing trials used a pairwise disturbed (fished) vs control (non-fished) design on single dredge tracks (Appendix 3). Fishing efficiency is defined as the percentage decrease in cockle abundance between each pairwise fished and non-fished samples, i.e. the proportion of the cockles removed by dredge fishing in a fished track relative to the immediately adjacent non-fished area.

7.2.2 Fishing trials

Fishing trials were performed in August 2019 in Venø Bugt fishing area 7 ("Muslingeområde" 7, Figure 7.1) to determine the cockle fishing efficiency with a commercial mussel dredge (Appendix1) provided by the blue mussel Limfjorden fishery through Foreningen Muslingeerhvervet (FME).
Trials were carried on a non-fished cockle bed in a Natura 2000 area at ca. 5.5 m depth, but contiguous to fished cockle beds by fishery in November 2018 (Freitas et al., 2021). Four test fishing tracks

were fished with (n=2) and without (n=2) an inner net inside the dredge (Appendix 1), as both practices occur in the fishery.

Figure 7.1. The location of fishing efficiency trial site (white area) in Venø Bugt, production area 7. Yellow lines delimit Natura 2000 area. Image from Google Earth.

Adjacent pairwise samples were collected inside and outside fished tracks by divers using quadrat frames (0.25 m^2) from Two vertical fractions of the cockle population were sampled separately: Surface fraction, any cockles emerged from the bottom surface and buried fraction, cockles buried and covered by sediment.

7.2.3 Surfacing definition

The cockle population were considered as surfacing or non-surfacing based on analysis of video images and on cockle density in the surface fraction of non-fished samples (Surfacing >28 cockles/m^2; see Chapter 4, this report for definition).

7.2.4 Cockle population

Prospection surveys in May and July 2019 (Day grab 0.1 m^2) indicated the cockle bed occupied an area of 190 x 90 m, ca. 12 000 m^2 and had a range of abundance and size (Table 7.1). Of the total cockle population, the common cockle (*C. edule*) constituted 85.7% (±5.2%, N = 17) and the lagoon cockle (*C. glaucum*) constituted 14.3% (±5.2%, N = 17) (identification according to morphological identifiers, Parada et al., 2018). Cockle size (shell width) averaged 21.8 mm (±2.2 mm, N = 474), while fresh weight averaged 10.5 g (±3.1g, N = 474).

Table 7.1. Cockle density and biomass in fished and non-fished areas. 'SE' is standard error, 'Min.' is minimum and Max. is maximum value. *Tracks 1 and 2 where fished without an inner net.

				Density (cockles/m^2)				**Biomass (g/m^2)**			
	Track	N	Net	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.
Non-Fished	*1	3	N	816	135	560	1016	7087	701	5744	8108
	*2	3	N	873	210	504	1232	7136	1576	4384	9844
	4	5	Y	758	74.2	512	980	6674	635	4988	8908
	5	6	Y	326	58.1	152	520	3936	731	1920	6820
	All	17		636	74.3	152	1232	5862	532	1920	9844
Fished	*1	3	N	659	19.6	620	684	5791	186	5464	6108
	*2	3	N	535	172	192	724	4291	1299	1692	5592
	4	5	Y	547	63.2	432	768	4964	529	3648	6444
	5	6	Y	170	39.1	44	248	2100	494	452	3124
	All	17		432	59.7	44	768	3980	467	452	6444

7.2.5 Statistical analysis

Fishing effects on cockle density and biomass were tested using paired t-test after evaluation of normality of data and homogeneity of variance. The use of inner net and cockle positioning (surfacing or not) were tested using ANOVA multivariate analysis after evaluation of normality of data and homogeneity of variance. Morphometric such as size (shell height, length and width) and density, biomass, or total fresh weight effects were tested using non-parametric Spearman's correlation.

7.3 Results and discussion

7.3.1 Fishing Efficiency

Fishing with the mussel dredge had a significant effect on the density and biomass of the cockle bed, being significantly lower in fished than in non-fished areas by 204.5 cockles/m^2 (paired *t*-test, p = 0.008, n = 17) and 1 881.9 g/m^2 (paired *t*-test, p = 0.005, n = 17) respectively.

The cockle catch per dredge track was on average only 54.7 ±32 cockles/m^2, (SE, n = 4). Cockle catch rate was thus only 8.7% of cockle density in non-fished areas of the bed. This is the result of areas with no cockles being included in the dredge tracks and would be close to a catch-based estimate of fishing efficiency and close to the fishermen's perception of the mussel dredge fishing efficiency (See methods). The pairwise approach used in this study, only sampled areas with cockles where the dredge actually fished a cockle population and ignores areas with no cockles.

The cockle fishing efficiency of the mussel surface dredge estimated from all pairwise fished-non-fished samples was 28.5 ±8.5% (SE, n = 17; Table 7.2).

Table 7.2. Cockle fishing efficiency of the Limfjorden mussel surface dredge from pairwise fished and non-fished samples in surfacing and non-surfacing areas in each fished tracks, and for tracks fished with (4 and 5) and without (1 and 2) an inner net in the dredge. SE is standard error; Min. is minimum and Max.is maximum value. *Tracks 1 and 2 were fished without an inner net and tracks 4 and 5 were fished with an inner net.

			Fishing efficiency - %			
Track	Net	N	Mean	SE	Min.	Max.
*1	N	3	14.9	13.3	-10.7	33.9
*2	N	3	22.0	35.0	-36.5	84.4
4	Y	5	25.0	11.6	-18.8	44.0
5	Y	6	41.5	14.9	3.1	90.0
No net (1+2)	N	6	18.5	16.8	-36.5	84.4
Net (4+5)	Y	11	34.0	9.6	-18.8	90.0
Surfaced	Y+N	6	40.8	16.0	-10.7	90.0
Non-surfaced	Y+N	11	21.8	9.8	-36.5	71.1
All	Y+N	17	28.5	8.5	-36.5	90.0

7.3.2 Impact of surfacing, inner nets, cockle abundance and size on cockle fishing efficiency

Fishing efficiency in surfacing areas of 40.8 ±16.0% (SE, n = 6) was almost double than in non-surfacing areas of 21.8 ±9.8% (SE, n = 11; Table 7.2). Similarly, fishing efficiency when fishing with an inner net of 34.0 ±9.6% (SE, n = 6) was also almost double than when fishing without an inner net of 18.5 ±16.8% (SE, n = 11; Table 7.2),

However, neither surfacing status nor the use of an inner net had a statistically significant effect on fishing efficiency (Table 3). However, considering the large variability observed in fishing efficiency estimates, a larger number of replicates would be necessary to test more robustly the significance of these results.

Table 7.3. Analyses of variance of the effects of cockle surfacing and use of inner net in fishing gear on fishing efficiency.

	df	**SS**	**MS**	***F***	***p***
Surfacing	1	577.4	577.4	0.440	0.519
Inner net	1	1504.1	1504.1	1.147	0.304
*Surfacing * Inner net*	1	165.6	165.6	0.126	0.728
Error	13	17043	1311		
Total	16	19487			

The mussel surface dredge does not require the surfacing of cockles out of the sediment to capture cockles. In areas with cockles buried in the sediment and no surfacing cockles, 21.8 ±9.8% (SE, n = 11; Table 7.2) of cockles were fished. Even in surfacing areas if all surfaced cockles were fished, surfaced cockles accounted for only 18.5 ±21.8% (SE, *n* = 6) of cockles removed by fishing, with buried cockles accounting for the remainder 81.5%.

Fishing efficiency was not affected by the abundance of cockles in the bed or by shell size and weight. Fishing efficiency was uncorrelated with cockle density, biomass, size (shell height, length and width) or total fresh weight (non-parametric Spearman's correlation: $\rho < 0.329$ and $p > 0.198$, for all).

7.4 Conclusions

Fishing with a mussel dredge, either with or without an inner net, resulted in a significant reduction in cockle bed density and biomass with an average catch per dredge track of 54.7 ±32 cockles/m^2.

The overall fishing efficiency was estimated at 28.5 ±8.5%, which included surfacing and non-surfacing areas. Fishing efficiency was not significantly affected by the density of the cockle bed.

The mussel surface dredge was able to fish buried cockles and thus does not require the surfacing of cockles out of the sediment to significantly capture cockles. Even in surfacing areas buried cockles made up most of the cockle catch.

The cockle fishing efficiency of the mussel dredge showed significant variability and a future trial, both increasing sample size and also evaluating catch-based fishing efficiency when dredging multiple times the same area, as done by the fishery, would be beneficial to refine the results and increase the robustness of conclusions presented here.

7.5 References

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7.6 Appendixes

The Limfjorden mussel surface dredge

Surface dredges used by the blue mussel fishery in the Limfjorden show adaptations and variations (pers. communication, FME), from the shape, size and angle of the spoiler to the use of an inner net attached to the upper net panel. The DTU Aqua fishing efficiency calibration used the mussel dredge from the fishing boat T229 “Linde Kirsten”, Captain Karl Bækhøj from Nykøbing Mors, made available by FME, and is assumed to represent the dredges used by the mussel fishery to fish cockles (Figure A7.1).

The dredge had a total weight of 174 kg, a mouth opening of 144 x 36 cm (inner dimensions), with a 31 cm wide V-shaped spoiler at a 130°angle from the mouth frame. The length of the bag was 170 cm (from the bottom mouth bar), with 80mm net openings behind the spoiler, followed by a net with 40 mm openings on the upper side and metal rings of 30 x 20 mm diameter on the bottom side. An inner net with 35mm openings can be attached inside to the upper side of the bag.

Figure A7.1. The mussel dredge used for the cockle fishing efficiency calibration. The inner net is on the lower right image. Photos P. Freitas.

8. Recommendation to management

Current management: By-catch of the blue mussel fishery

The current management practice where cockle fishery is managed as a by-catch fishery precludes a direct management of the cockle populations and its fishery in the Limfjorden. A shift in management of cockle fishing in the Limfjorden to an independent and autonomous fishery separate from the blue mussel fishery would allow to take into account cockle biology, variations in cockle abundance and cockle fishing practices in the Limfjorden. As a non-independent fishery, cockle fishing in the Limfjorden is currently tied to fishing practices, management and harvest control rules of the blue mussel fishery (e.g. TAC, daily cockle landing limits), which may be inefficient to the fishery at best or detrimental at worst for exploited cockle populations. For instance:

- As cockles and blue mussels must be landed together but are often fished in different and distant locations that require sailing additional 28 to 60 km before landing.
- Daily landing limits can hamper efficient fishing of both cockles and mussels.
    - e.g. of abundant cockle beds if the required 51% catch of blue mussels cannot be met.
    - e.g of blue mussels if low quality, low meat blue mussels are fished to allow landing 49% of catch as more valuable cockles. A situation that often occurs in autumn months (FME).
- Blue mussel TAC imposes a catch limit on cockles that is unrelated to cockle population abundance. Thereby cockle landing limits may be a too high or too low fishing effort for cockle stocks depending on the blue mussel TAC or daily quota.

The present study has gathered new knowledge and information on the Limfjorden cockle populations and cockle fishery that can be taken into consideration for providing recommendations and advice for the implementation of an independent cockle fishery in the Limfjorden.

Conclusions from this study

The area Kås Bredning ("muslingerområde” 9) is fundamental for the fishery, and a high dependence on a single area poses a risk to the future stability and sustainability of the Limfjorden cockle fishery. The current Limfjorden cockle fishery thus has been heavily and increasingly dependent on Kås Bredning. However, the relative stability of cockle landings in the Limfjorden over 2015-2020 fishing seasons have been maintained in the last three fishing seasons by a significant change in the spatial structure of fishing: i) landings from Kås Bredning have doubled in the three fishing seasons 2017–2020 and remained relatively stable; ii) the potential impact on exploitation rates is unknown; iii) the contribution from secondary fishing areas has decreased. Variations in the cockle stocks in Kås Bredning, either from recruitment failure or natural and fishing mortality, may compromise the sustainability of the Limfjorden cockle fishery if other secondary areas cannot provide additional landings, with the risk of significant economic and social impacts on fishermen and other industry stakeholders.

Regular monitoring programs are essential to react to annual changes in stock size and structure due to variations in natural mortality and recruitment, particularly in short lived species with variable recruitment such as cockles. Furthermore, regular stock assessment is required to set knowledge-based sustainable quotas and other management regulations. Cost efficient methods for regular population assessment thus need to be developed and implemented. The cockle stock estimation from suction dredge survey was clearly significantly underestimated and it is recommended to develop another cost-efficient survey approach to assess cockle populations. This is being addressed in the follow-up project COCKLE II (j.nr. 33113-B-20-172) funded by the European Maritime and Fisheries Fund (EMFF) and the Danish Ministry for Food, Agriculture and Fisheries ("Ministeriet for Fødevarer, Landbrug og Fiskeri") program "Hav- og fiskeriudvikling".

As an infaunal species normally buried in the top 5 cm of the bottom sediment, cockles were not expected to be significantly available to the blue mussel fishery surface dredge, except during episodic emergence of cockles to the sediment surface. However, from test fishing performed by DTU Aqua, the commercial mussel surface dredge catches a significant fraction of non-surfaced cockles estimated to an efficiency of 28.5 ±8.5% (SE, n=11) and it is a normal practice of the fishery to fish non-surfacing cockles when found by the fishermen's prospection. Therefore, the emergence of cockles from the sediment is not required for the mussel surface dredge to significantly fish cockles. Thus, and in view of the spatial offset in the mussel and cockle fishing, cockles can be described as a co-target species in the current blue mussel fishery, which can be viewed as a co-target fishery on two separate species. The results support the need for a management practice where cockle fishing is managed as an independent fishery in the Limfjorden.

Considerations and recommendations on short term management:

1. Until a long-term management plan of a new independent cockle fishery is implemented, short-term management actions should ensure the stability of the fishery, minimizing economic and social impacts on fishermen and industry stakeholders, while also considering the long-term sustainability of cockle stocks and fishery.
2. Regular pre-fishing assessments of cockle population and stocks biomass, and recruitment dynamics are required to advise setting cockle specific harvest control rules (e.g. TAC) before each fishing season.
3. This requires cost-efficient survey methods adapted to the infaunal and subtidal habitats, and highly clumped distribution of cockles in the Limfjorden and allocation of funding for regular surveys.
4. Currently, blue mussel fishery uses Black Box data to link fishing areas and tracks with catches. It is not possible to fully and accurately separate between blue mussel and cockle fishing events, and thus to assess cockle fishing patterns and effort. It is thus recommended to make sure from logbook notes that a separation of the fisheries in the Black Box data and Elog can be established to provide important information to the fishery management.

Considerations and recommendations on long term management

DTU Aqua recommends that a future management of an independent Limfjorden cockle fishery should consider the following among other elements:

1. Implementation of a long-term overall harvest and management strategy for a viable and sustainable fishery in coordination with relevant stakeholders.
2. Harvest strategies, control rules and limits should be defined in advance and based on biological data: e.g. TAC per fishing season; definition of minimum landing size for cockles; protection of areas with high abundance of non-harvestable cockles or important broodstock populations; definition of fishing restrictions; target fishing of high-density areas to efficiently fulfil catch limits with the least fishing effort, cost and ecosystem impact.
3. Regular pre-fishing surveys to provide data-based scientific advice to support setting of annual harvest strategy and catch limits (e.g. TAC for cockles) in reaction to annual changes in landings, stock size and structure due to variations in natural mortality and recruitment.
4. Establishment of long-term fishery independent data series of cockle abundance at least in Kås Bredning, but also of population structure and recruitment dynamics as important supplementary information.

5. In a separate cockle fishery from the mussel fishery, information from Black Box and logbooks can provide data specific to cockle fishing, and thus improve the quality of data to be included in cockle fishery analysis and advice to management, thus is advised to be mandatory.
6. The use of catch or landings per unit effort (CPUE/LPUE) in sessile species, such as cockles, as fishery dependent indices of relative abundance has severe limitations and should not guide long-term management and harvest decisions.
7. The Kås Bredning fishing area is highly important to the industry and management should ensure it is sustainably exploited with regular stock assessment of cockle populations, monitoring of total and harvestable cockle biomass, recruitment dynamics and evaluate reproductive connectivity to understand and manage its singular high cockle abundance and population renewal.
8. The resilience of the fishery to common natural variations in cockle population biomass would benefit if the high dependence on Kås Bredning can be reduced. However, management must consider that higher contribution of secondary areas to landings may not be possible in certain seasons, while new non-fished areas are only available in Natura 2000 areas (e.g. Venø Bugt or Nissum Bredning).
9. Ecological carrying capacity of the Limfjorden to identify food supply of the cockles, as well as monitoring of pathogens is also recommended to follow the risks associated to climate change, invasive species and potential collapse of the cockle population.

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