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Status of the Pacific Oyster *Crassostrea gigas* (Thunberg, 1793) in the western Limfjord, Denmark – Five years of population development

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Abstract

The Pacific oyster, *Crassostrea gigas*, was introduced into the Netherlands in 1964 for aquaculture purposes and has since spread extensively in Northern European waters. Eight locations in the western part of the Limfjord, Denmark, first sampled in 2006 were revisited in 2011, to determine how the population of *C. gigas* has changed. Densities were lower at all but two locations. No differences in average shell lengths or condition indices were detected. No changes in the number or distribution of shell size classes were observed. These similarities suggest there is a single population that has not expanded in terms of geographic distribution. While reproduction does occur, conditions for population growth appear to be suboptimal. The species has become established in western Limfjord but abundance is low and densities are much lower than those considered harmful to the ecosystem. At present, the *C. gigas* population is not a cause for concern in the Limfjord ecosystem.

Key words: invasive bivalve, winter mortality, abundance, population development, distribution

Introduction

The Pacific Oyster *Crassostrea gigas* (Thunberg, 1793) is native to Japan and the Pacific Coasts of Asia between latitudes ~30°N and ~48°N (Arakawa 1990). *Crassostrea gigas* was first introduced to Europe as a bivalve aquaculture species in 1964 due to a severe decline in the populations of the native European oyster *Ostrea edulis* Linnaeus, 1758 (Shatkin et al. 1997; Reise et al. 1999). Spat of *C. gigas* was first imported to the Oosterschelde, The Netherlands, from British Columbia, Canada, and a successful trial to grow the species commercially was carried out (Drinkwaard 1999). The low water temperatures in the Oosterschelde estuary were deemed to be unfavorable for reproduction; thus, subsequent import for aquaculture was allowed (Shatkin et al. 1997; Drinkwaard 1999; Wehrmann et al. 2000). However, *C. gigas* did reproduce in the Dutch estuaries and, by the early 1980s, extensive spat falls were observed (Drinkwaard 1999).

Warm summers and mild winters may have aided the dispersal of *C. gigas* in northern Europe (Nehls et al. 2006; Troost 2010). Furthermore, a lack of predators and a high competitive ability have contributed to the establishment of this invasive bivalve (Nehls et al. 2006; Schmidt et al. 2008; Troost, 2010).

In the Limfjord, Denmark, *C. gigas* was introduced in 1972 for aquaculture purposes but culture efforts have since been abandoned (Jensen and Knudsen 2005). In coastal systems, it is nearly impossible to control an ongoing invasion regardless of whether the non-native species is benign or has a negative impact on the system (Reise et al. 2006). *C. gigas* has established reproducing populations in Scandinavia (Wränge et al. 2010), and there is concern that the dispersal of *C. gigas* in the Limfjord may have a...
negative impact on populations of the native blue mussel *Mytilus edulis* Linnaeus, 1958 and presumably also on *Ostrea edulis*; both represent important fisheries resources in the estuary (Dolmer and Frandsen 2002; Wrange et al. 2010).

A population survey of *C. gigas* was conducted at eight locations in the western part of the Limfjord during the summer (late July – late September) of 2006, and sampling was repeated during June - August 2011 to determine how the population had changed. Specifically, we compared estimates of abundance, shell size, and condition between the two sampling periods.

**Methods**

**Study area**

The Limfjord is a long and shallow sound that separates the northern part of Jutland from the mainland; it is approximately 170 km long with a 1,000 km coastline, and an average depth of 4.3 m (Naturstyrelsen 2009; Miljøcenter Aalborg 2007). The system is connected to the North Sea in the west and to the Kattegat in the east (Figure 1). An easterly current, combined with fresh-water inputs from the surrounding catchment area, results in a highly saline environment (salinity 32) in the westerly part of the sound, where the North Sea water enters the system at Thyborøn Channel, and a lower salinity (salinity 18) in the eastern part of the sound (Dolmer 2000; Miljøcenter Aalborg 2007). The current patterns have not changed significantly since 1875 (Naturstyrelsen 2009).

Water temperatures range from 2–3°C in winter to 15–17°C in summer (Wiles et al. 2006). Limfjorden is considered a eutrophic system due to high nutrient loading from the surrounding catchment area. Parts of the sound are subject to recurring oxygen depletions, although there are large inter-annual differences in duration and the area affected (Dolmer et al. 2009). The annual primary production in the western part of the Limfjord is approximately 90–100g C m⁻² (Limfjordsövervågningen 2005).

**Sample collection**

Eight locations (Table 1) in the western part of the Limfjord were surveyed in 2006 and 2011 (Figure 1). An additional station, Fur, was added in 2011 to determine whether *C. gigas* had spread further east in the estuary.

At each location, five transect lines (four at Hjortholm) were placed perpendicular to the shoreline 20 m apart. The starting point was the high water mark, which was marked by bands of deposited seaweed. GPS positions were recorded at start and end of each transect line, and water depth was measured in 5 m intervals along the line. Sampling at water depths below 0.6 m was done in waders with aquascopes (Strand et al. 2012). At depths between 0.6 and 1 m, sampling was done by SCUBA divers. At Agger Tange, Ronland, Lysen Bredning and Vile Vig, the sampling in 2011 was done to a depth of 0.6 m. At Klosterbugten, Harrevig, Hjortholm and Dråby Vig, three of the five transects were done to a depth of 1.0 m (Table 1). At locations where three long and two short transects were made; the average length refers to the three long transect lines. Ronland in 2006 was not surveyed by transect, and no attempt to collect individuals for calculating densities was done. Some specimens were collected to determine condition from this site.

Every *C. gigas* found within 1 meter on either side of the transect line was collected and shell length (SL) and distance from shore were recorded. Only individuals > 50 mm SL were included to minimize detection bias. Living individuals were retained for further processing and dead individuals were discarded. The shell length of dead individuals was measured in 2011 only. To be included, the left and right valves had to be connected, indicating a recent death (1–2 years). No consideration was given to the extent of epiphytic growth on empty shells.

In 2011, the south eastern coast of Fur (Figure 1) was searched for *C. gigas*. This was done with waders and aquascope at five sites along the shoreline. Locations were chosen based on availability, and 20–30 m of the sea floor along the coast was randomly scanned to a water depth of approximately 1 m.

Each living *C. gigas* retained was provided with a unique number, placed in individual bags, and stored frozen at -18°C before further treatment. After thawing, the shell length was measured, and the flesh and shell was separated. The flesh was dried at 105°C until constant shell-free dry weight (SFDW) was reached. Shells were washed and cleaned, then dried until they reached constant weight (Shell DW). Condition (C) for each individual was calculated as:

\[
C = \left(\frac{\text{SFDW}}{\text{SDW}}\right) \times 1,000
\]

for shell free dry weight (SFDW in g) and shell dry weight (SDW in g) (Brown and Hartwick 1988;
Status of *Crassostrea gigas* in the western Limfjord, Denmark

**Figure 1.** Overview of the study area in the western part of the Limfjord. Dråby Vig, Klosterbugten, Harrevig, Lysen Bredning, Vile Vig, Hjortholm, Agger Tange, Rønland, Fur. Solid circles mark presence in both years, open circle means not found in 2011 and not sampled in 2006, open square means found in 2011 and not sampled in 2006, and the solid square means found in 2006 and not found in 2011. Red circles show locations of anecdotal reports of *C. gigas.*

**Table 1.** All sampled locations presented with regional designations. Corresponding coordinates for the start point of the first transect at each location are noted, as are the average transect length, and the water depth at the end of transect. Coordinates for Fur designate the first site of survey (only in 2011). Number of specimens presents all living *C. gigas* found along the transect lines at each location. Density averages m⁻² for each location are presented with standard deviations. Statistical significance between 2006 and 2011 is marked with an ‘s’ (Mann-Whitney test: p<0.05), non-significance is marked ‘ns’. Arrows mark an increase (up) or decrease (down) in density. Densities are stated for the long transects where those were applied in 2011. No density data were collected for Rønland in 2006 or for Fur.

<table>
<thead>
<tr>
<th>Location</th>
<th>Coordinates</th>
<th>Avg. transect length ± sd (m)</th>
<th>Water depth (m)</th>
<th>No. specimens</th>
<th>Density (m⁻²)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klosterbugten, Mors</td>
<td>56.791634  8.860580</td>
<td>128.0 ± 7.6</td>
<td>1.0</td>
<td>52</td>
<td>153</td>
<td>0.02±0.04 0.12±0.12</td>
</tr>
<tr>
<td>Dråby Vig, Mors</td>
<td>56.868258  8.828974</td>
<td>258.0 ± 2.9</td>
<td>1.0</td>
<td>78</td>
<td>94</td>
<td>0.04±0.01 0.06±0.01</td>
</tr>
<tr>
<td>Harrevig, Salling</td>
<td>56.709785  8.897354</td>
<td>187.0 ± 7.6</td>
<td>1.0</td>
<td>67</td>
<td>28</td>
<td>0.18±0.04 0.02±0.02</td>
</tr>
<tr>
<td>Lysen Bredning, Salling</td>
<td>56.686789 8.840281</td>
<td>47.0 ± 3.0</td>
<td>0.6</td>
<td>35</td>
<td>1</td>
<td>0.02±0.02 0.00±0.01</td>
</tr>
<tr>
<td>Vile Vig, Salling</td>
<td>56.710474  8.866136</td>
<td>26.0 ± 4.2</td>
<td>0.6</td>
<td>30</td>
<td>0</td>
<td>0.06±0.06 0.00±0.00</td>
</tr>
<tr>
<td>Hjortholm, Salling</td>
<td>56.696486  8.846435</td>
<td>82.0 ± 12.6</td>
<td>1.0</td>
<td>52</td>
<td>1</td>
<td>0.04±0.02 0.002±0.04</td>
</tr>
<tr>
<td>Agger Tange, Thyboron</td>
<td>56.722358 8.251669</td>
<td>10.0 ± 1.9</td>
<td>0.6</td>
<td>47</td>
<td>20</td>
<td>3.12±1.37 0.20±0.13</td>
</tr>
<tr>
<td>Rønland, Thyboron</td>
<td>56.672144  8.213861</td>
<td>83.0 ± 5.3</td>
<td>0.6</td>
<td>n.a.</td>
<td>38</td>
<td>n/a 0.04±0.06</td>
</tr>
<tr>
<td>S-E coast of Fur</td>
<td>56.805729  9.022294</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Statistical analyses

All statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, California). Level of significance was $\alpha = 0.05$ for all tests. Abundances were compared with a two-way ANOVA test, followed by Sidak’s multiple comparisons test. Abundance data were transformed using square-root ($x + 0.5$). Shell lengths and condition data were analysed through two-way ANOVAs and Sidak’s multiple comparisons tests.

Differences between shell-length frequencies between years and between winter and summer 2011 were detected by means of Kolmogorov-Smirnov tests.

Modes, assumed to correspond to cohorts or age classes, were determined using Bhattacharya’s Method in the FiSAT II software (FAO, Rome). This method visually separates normally distributed groups from a mixed group, by using natural logarithms on ratios of frequencies, overlapping distributions can be identified (Goonetilleke and Sivasubramaniam 1987).

Results

The analysis showed that the interaction term, the year, and the location had significant effects on abundance of *C. gigas* (Table 2A). Overall, the abundance *C. gigas* declined between 2006 and 2011, with Agger Tange having the highest decline in abundance (Table 1).

For the average shell lengths (± SD), only the interaction term and the location were significant (Table 2B). Between the years, there was a significant difference seen at Dråby Vig, Harrevig, Klosterbugten, Agger Tange, and Rønland, but these were not consistent. The shell lengths at Dråby Vig, Klosterbugten, and Agger Tange decreased between 2006 and 2011; while at Harrevig and Rønland, shell length has increased (Figure 2A).

The average condition (± SD) of *C. gigas* showed significant effects of the interaction term, the year, and the site (Table 2C). The only significantly different locations were Dråby Vig and Harrevig, which both show decreased condition from 2006 to 2011 (Figure 2B). No specimens were found at Vile Vig or Lysen Bredning in 2011.

Based on Kolmogorov-Smirnov tests, the shell length-frequencies between 2006 and 2011 were significantly different ($p \leq 0.01$), with more small animals collected in 2006 (Figure 3).
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Discussion

By revisiting the same sampling stations after five years, it is clear that C. gigas remains present in the western part of the Limfjord; however, abundance has decreased at most locations. The stochastic nature of the changes in population densities suggests physical conditions (e.g., salinity) may be limiting the dispersal of C. gigas in the Limfjord. It may well be that C. gigas has not had enough time to have been able to establish colonies further east than Mors. If this is the case, regular visits to the east of the study area will be of great value in determining the long term dispersal rate of which the oyster is moving through the Limfjorden ecosystem.

The two winters preceding 2011 were harsh, with average air temperatures of -1.5°C and -1.3°C in 2009–2010 and 2010–2011, respectively (with a normal 30-year average of 0.5°C (DMI 2010; DMI 2011)). Crassostrea gigas generally occurs in areas with a sea surface temperature between -1.9°C and 19.8°C in the coldest month of the year (Carrasco and Barón 2010). Few live specimens were collected in the shallow water close to the shore, and the size frequency analysis shows that the dead specimens tend to be larger than the live oysters. Crassostrea gigas has been shown to have a decreased mortality with increased water depth in Scandinavia, which may well explain the observed pattern (Strand et al. 2012). The specimens located in shallow waters would be exposed to the mechanical stress caused by ice, as well as the cold air temperatures. Mortalities during the winters 2009/10 and 2010/11 at Agger Tange were found to be 17% and 13%, respectively (MW Holm, unpublished data). Predators or wave action potentially can remove shells, and result in some uncertainty and underestimations in the data. Reports of mortalities during harsh winters from the Wadden Sea are much higher than found at Agger Tange; with mortalities ranging from 34 to 90% (Reise 1998; Bütter et al. 2011). The Limfjord seems to be a more protected environment, which could be due to the protection land-fast ice would afford, and although the harsh winters have an effect, it seems not to be a major regulator of the population.

Dredging samples in the Limfjord have revealed few to no specimens in waters > 3 m deep (P Dolmer, unpublished data), although the species has been observed at depths > 40 m deep elsewhere (Minchin and Gollasch 2008). Thus the species has yet to spread to the deeper waters of the Limfjord. All locations are accessible to

Figure 3. The number of cohorts and their size vs. frequency distribution, of the collected C. gigas: total live 2006 (A), total live 2011 (B), and total dead 2011 (C). Data for mean lengths, standard deviations, and number of individuals in the groups is shown in Table 3.

Overwinter mortality in 2011 was highly size selective (P < 0.01) with large animals (>125 mm SL) largely disappearing from the population.

In 2006, three apparent cohorts were observed in living animals, while in 2011 (living animals) only one cohort could be identified. There were two clear modes in the length distribution of dead animals collected in 2011 (Table 3, Figure 3).
Table 2. ANOVA tables for A) Abundance, B) Shell lengths, and C) Condition.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F (DFn, DFd)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>2.104</td>
<td>6</td>
<td>0.3507</td>
<td>F (6, 56) = 34.10</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Site</td>
<td>3.548</td>
<td>6</td>
<td>0.5914</td>
<td>F (6, 56) = 57.51</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Year</td>
<td>0.4360</td>
<td>1</td>
<td>0.4360</td>
<td>F (1, 56) = 42.40</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>0.5758</td>
<td>56</td>
<td>0.01028</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>66025</td>
<td>7</td>
<td>9432</td>
<td>F (7, 826) = 25.29</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Site</td>
<td>74899</td>
<td>7</td>
<td>10700</td>
<td>F (7, 826) = 28.69</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Year</td>
<td>364.0</td>
<td>1</td>
<td>364.0</td>
<td>F (1, 826) = 0.9760</td>
<td>P = 0.3235</td>
</tr>
<tr>
<td>Residual</td>
<td>308056</td>
<td>826</td>
<td>372.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>76112</td>
<td>7</td>
<td>10873</td>
<td>F (7, 572) = 14.76</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Site</td>
<td>22284</td>
<td>7</td>
<td>3183</td>
<td>F (7, 572) = 4.320</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Year</td>
<td>18437</td>
<td>1</td>
<td>18437</td>
<td>F (1, 572) = 25.02</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>421470</td>
<td>572</td>
<td>736.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Data for the total and the regionally grouped cohort analysis, using Bhattacharya’s Method in the FiSAT II software. Shown are the numbers of age groups in each year, the mean length of each group, standard deviation, number of individuals belonging to each group, and the Separation Index (S.I.). A S.I. > 2 indicates a reliable separation of the size classes. No dead specimens were collected in 2006.

A Total live 2006 Total live 2011

<table>
<thead>
<tr>
<th>Size group</th>
<th>Mean (mm)</th>
<th>SD</th>
<th>Individuals</th>
<th>S.I.</th>
<th>Mean (mm)</th>
<th>SD</th>
<th>Individuals</th>
<th>S.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55.1</td>
<td>9.9</td>
<td>264</td>
<td>n.a.</td>
<td>86.8</td>
<td>19.2</td>
<td>330</td>
<td>n.a.</td>
</tr>
<tr>
<td>2</td>
<td>92.3</td>
<td>11.8</td>
<td>201</td>
<td>3.44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>124.8</td>
<td>11.1</td>
<td>45</td>
<td>2.84</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

B Total dead 2006 Total dead 2011

<table>
<thead>
<tr>
<th>Size group</th>
<th>Mean (mm)</th>
<th>SD</th>
<th>Individuals</th>
<th>S.I.</th>
<th>Mean (mm)</th>
<th>SD</th>
<th>Individuals</th>
<th>S.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>69.5</td>
<td>10.6</td>
<td>45</td>
<td>n.a.</td>
</tr>
<tr>
<td>2</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>120.1</td>
<td>15.0</td>
<td>163</td>
<td>3.95</td>
</tr>
</tbody>
</table>

larvae that are carried with the currents, sometimes for weeks, before they settle as spat (Quale 1988; Wehrmann et al. 2000). The transport of larvae from one location to the others likely takes place, probably through a stepping stone process. Reproduction at Agger Tange was detected in 2004, 2007 and 2008, and spawning oysters were observed when sampled for this study (MW Holm, unpubl. data). However, the rate at which the settled larvae mature into reproducing individuals is currently unknown. Since reproduction appear to be continuous, and under the proper environmental circumstances can result in a massive settlement of juvenile oysters, monitoring of the recruitment would appear to be warranted. Winters with heavy ice formation (and perhaps frequent storms) can greatly reduce the population size, and result in a temporary setback for local populations; however, but the high fecundity of C. gigas gives the population high potential for expansion during warm summers (Büttger et al. 2011). Locations like Fur, where no C. gigas were found, could easily be populated by spawn released at near-by locations.

That the Pacific oyster is still in the establishment phase (sensu Reise et al. 2006) in the Limfjord is a hypothesis supported by the pattern between sites, years, and interaction terms seen in the data, and relatively low numbers of small specimens in 2011. In the Limfjord, the species is subjected to colder water temperatures and a different environment than its natural habitat,
and has not yet fully adapted to conditions in the Limfjord ecosystem. However, *C. gigas* is not situated at its northernmost limit in Denmark, as it is reproducing and settling successfully in Norway and Sweden (Wrange et al. 2010). The density data shows that the abundance is very low, with less than one specimen per m², yet spawning individuals were observed in 2011 at both sampling times, and during the sampling in 2006. The species has so far failed to become truly invasive, i.e., a threat to the Limfjord. Nevertheless, it has successfully colonized the Limfjord, albeit at low abundance. Several mild winters accompanied by warm summers could change this state. A combination of large reproduction events; ideal conditions for settlement, recruitment, and growth; and several years with low overwinter mortalities could trigger a rapid population expansion. The main limitation for the spread of *C. gigas*, however, is unlikely to be only due to climatic conditions. It has been present in the Limfjord for 40 years without experiencing the same expansion and growth that has been seen in the Wadden Sea. This difference in success may be linked to the tidal effects: tidal range in the Limfjord is about 0.2 m compared to 2 m in the Wadden Sea (Dolmer and Frandsen 2002; Diederich 2006). Thus, at the shallow-water Limfjord stations, the supply of phytoplankton typically replenished (presumably from offshore) by tidal mixing and transport may be reduced, resulting in localized food limitation.

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