



High reproduction, but low biomass: mortality estimates of the copepod *Acartia tonsa* in a hyper-eutrophic estuary

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ABSTRACT: Production, abundance and mortality of the copepod *Acartia tonsa* were studied for a period of 9 d in a hyper-eutrophic estuary, Mariager Fjord, Denmark. The estuary is characterised by oxygen-depleted and often sulphidic bottom water, and a relatively sparse mesozooplankton community with low species diversity. During the study, an intense phytoplankton bloom consisting mainly of the diatom *Skeletonema costatum* developed with chlorophyll *a* concentrations reaching 46 µg l⁻¹. Egg production rate (EPR) in *A. tonsa* ranged from 30 to 65 eggs female⁻¹ d⁻¹, and egg hatching success was >90%, yet the abundance of copepods remained low (1 to 3 nauplii l⁻¹, 0.3 to 1.5 copepodites l⁻¹). Calculated daily copepod mortality ranged from 18% for nauplii, 16% for Copepodite Stage C1, up to 70% for C2 and C3, then declining for older stages. The vertical distribution of copepodites in relation to the depth range of mussel beds suggests strong predation by suspension-feeding *Mytilus edulis* at depths of 5 to 10 m. Moreover, anoxia and the presence of sulphide in deep water, which prevented hatching of *A. tonsa* eggs and decreased the survivorship of older life stages sinking below 20 m depth, further contributed to the low copepod abundance.

KEY WORDS: *Acartia tonsa* · Anoxia · Egg production · Mortality · *Mytilus edulis*

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INTRODUCTION

Mariager Fjord is a highly productive estuary with a maximum depth of 30 m situated on the east coast of Jutland, Denmark (Fig. 1, Table 1). The shallow (<6 m) eastern part of the estuary is long and narrow and functions as a sill, reducing water exchange with the open sea (Kattegat). The water column in the inner, deeper part is permanently stratified by a halocline situated at 10 to 15 m depth (Århus Amt & Nordjyllands Amt 2000, Møller 2005, Bendtsen et al. 2006). Primary production is high (~1000 g C m⁻² yr⁻¹), and the phytoplankton is dominated by diatoms with

shorter blooms of dinoflagellates and autotrophic ciliates (Århus Amt & Nordjyllands Amt 2000, Olesen 2001, Møller 2005). Chlorophyll *a* concentrations reach 70 µg l⁻¹, and sedimentation rates are ~5% of the standing stock of chlorophyll *a* per day (Olesen 2001). This high organic loading results in permanently anoxic bottom water containing sulphide (Fenchel et al. 1995, Fossing & Christensen 1999).

The mesozooplankton community of the estuary is characterised by few species and a low biomass, <20 µg C l⁻¹ for holoplankton, which is 5 to 10 times lower than in the Århus Bugt outside the fjord (Bioconsult 2001). *Acartia tonsa* is the dominant copepod in

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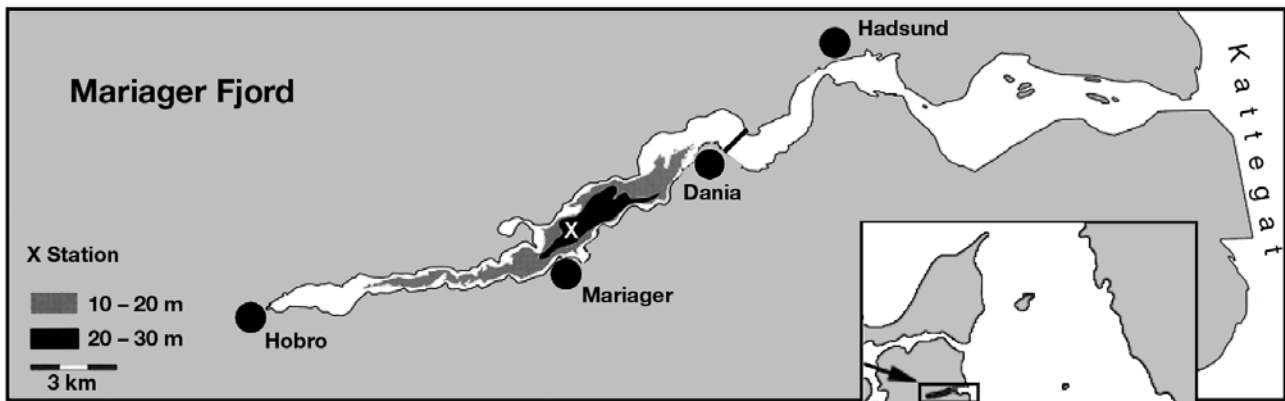


Fig. 1. Map of Mariager Fjord on the east coast of Jutland, Denmark. Sampling station is indicated by X (position: 56° 39.08' N, 9° 58.50' E). The area considered in the present paper is the inner part of the fjord, from Hobro to the line at Dania. See also Table 1 for areas and volumes of the estuary (Århus Amt & Nordjyllands Amt 2000)

the estuary. It may be regarded as an opportunistic species inhabiting brackish ecosystems where conditions are highly variable, temperature is elevated, and hypoxia often occurs (e.g. Roman et al. 1993). Population dynamics of *A. tonsa* were investigated in detail in the shallow (1.7 m) Darss-Zingst estuary, Germany, by Arndt & Schnese (1986). They concluded that mortality by planktivores was the main population-regulating factor, while low salinity and high pH (>9) played a minor role. The *A. tonsa* population in Mariager Fjord is exposed to unlimited food for most of the summer, and predation pressure from pelagic zooplanktivores (fish and jellyfish) is modest (Bio/consult 2001, 2005). However, *A. tonsa* is exposed to predation by suspension-feeding blue mussels *Mytilus edulis* (Davenport et al. 2000, Møller 2005, Nielsen & Maar 2007), which cover extensive areas of the bottom of the estuary. *A. tonsa* has a high reproductive potential, but since mortality in the field is at least as variable as egg production rate (EPR), predation must be included in the population dynamics of the species. The anoxic conditions and presence of sulphide in waters below 15 to 20 m depth may prevent the copepod eggs from hatching and stop recruitment (Nielsen et al. 2006). In addition, pH levels in the surface waters reach up to 9.75 (Hansen 2002), which may be detrimental to copepods (Pedersen & Hansen 2003).

Table 1. Areas and water volumes of different zones of Mariager Fjord (Århus Amt & Nordjyllands Amt 2000)

| Depth (m) | Bottom area (km ²) | Volume (10 ⁶ m ³) |
|-----------|--------------------------------|--|
| 0–10 | 10.5 | 136 |
| 10–15 | 4.3 | 34 |
| 15–20 | 2.1 | 18 |
| 20–30 | 2.5 | 12 |

As part of a 9 d intensive field investigation of pH effects on the planktonic food web, we monitored abundance, depth distribution and a range of life-history parameters for the copepod *Acartia tonsa* in Mariager Fjord. In particular, we were interested in the effect of benthic suspension feeders and how the azoic deeper part of the estuary affects recruitment of the copepod. Detailed data on depth distribution and succession of the plankton are given in T. G. Nielsen et al. (unpubl.), and the structuring potential of pH on the observed distribution pattern are presented (B. W. Hansen et al. unpubl.), where the pH tolerance of proto- and mesozooplankton (including *A. tonsa*) is investigated.

MATERIALS AND METHODS

Abundance. Samples were taken during the day on 16, 18, 20, 22 and 24 August 2005 at a station in the deepest, central part of Mariager Fjord (Fig. 1, Table 1). Water column structure was investigated by CTD casts (ME-profiler, Meerestechnik) measuring temperature, salinity and fluorescence (T. G. Nielsen et al. unpubl.). Based on the water column structure, sampling depths were identified and a 30 l Niskin bottle was used to collect samples at discrete depths (0, 2, 4, 6, 8, 10, 12, 15 and 20 m). The catch was concentrated on a 45 µm sieve and fixed in formaldehyde (5% final concentration). For mesozooplankton, the entire samples were analysed under a dissection microscope (50× magnification) and all copepods were counted and determined to species and stage. Nauplii were not determined to stage. Depth-integrated abundances (ind. m⁻²) were calculated by trapezoidal integration.

Egg production. Egg production experiments were performed every second day. *Acartia tonsa* were col-

lected by gentle oblique tows in the upper 10 m with a plankton net (70 μm) equipped with a large non-filtering cod-end. The catch was transferred to a thermo box, diluted with surface water, and immediately transported to the laboratory. Within 3 h, adult female *Acartia tonsa* were sorted and 4 females were placed in 500 ml polycarbonate bottles (Nalgene) filled with 200 μm screened water collected from 2 m depth at the sampling station (12 replicates). Bottles were incubated without mixing in a thermo constant chamber for 24 h at 17°C and dim light at the ambient light cycle. At the end of the incubations, the condition of females was checked and eggs and nauplii were collected on a 45 μm sieve, washed into Petri dishes, and counted. All eggs were transferred to multi-wells (24), and the number of nauplii hatched was checked after 48 h.

Survival of copepods *in situ*. Since the copepods in the fjord were exposed to high pH above the pycnocline and to sulphide below the pycnocline, an incubation of caged copepods at 5 different depths was performed. Incubation chambers consisted of 200 ml plastic jars with 125 μm mesh in the lids and bottoms. Animals for incubation were collected on 21 August, and 15 females were placed in each chamber. Triplicate chambers were then placed at 2, 10, 12, 15 and 20 m by gently lowering the rig. The time from collection of animals to deployment of the rig was 3 h. After 24 h of exposure, the chambers were retrieved and the survival of females checked.

Sediment traps. To investigate sedimentation and the fate of copepod eggs, 2 rigs with 2 gimbaled sediment traps (7.2 cm inner diameter, 44 cm high) were deployed at 15 m depth in the central part of the fjord close to the sampling station. The traps were filled with GF/F filtered water collected from 15 m depth and deployed each day from 20 to 24 August. After 24 h, the traps were retrieved and the entire contents were poured into 2 l bottles and brought to the laboratory. Samples for chlorophyll *a* and phaeopigments were taken, and the remaining sample (~1.7 l) was filtered through 45 μm mesh, and copepod eggs were counted. Chlorophyll *a* and phaeopigment were analysed on a Turner fluorometer after extraction in 96% ethanol for 24 h (Jespersen & Christoffersen 1987).

Sediment cores. The abundance of copepod eggs in the sediment was investigated by taking triplicate sediment cores (35 cm long, area = 0.002 m²) by SCUBA at 4, 11.5 and 18.5 m depth on 22 August. The cores were frozen (-20°C) until analysis. The top 5 cm of the sediment was treated with the sucrose flotation method (Onbé 1978) to reveal eggs. The rest of the cores were analysed by sieving and inspection of the sample. Observed eggs were measured to confirm that they were *Acartia tonsa* eggs.

Mortality rates. Mortality rates of *Acartia tonsa* were calculated from vertical life tables (Aksnes & Ohman 1996). Stage duration times were adopted from Leandro et al. (2006), who raised *A. tonsa* from eggs to adults at 15 and 18°C. Since stage duration is temperature dependent, the temperature relevant for each stage was determined by calculating the weighted mean depth for that stage and date and then by observing the temperature at that depth. Temperature ranged from 14.9 to 18.7°C, and the stage durations estimated at 18°C were used in all cases except 2, where the durations determined at 15°C were used. Mortality rates (m_i) of nauplii and copepodites were calculated iteratively from the formula (Aksnes & Ohman 1996):

$$\frac{A_i}{A_{i+1}} = \frac{e^{(m_i D_i)-1}}{1 - e^{-m_i D_{i+1}}} \quad (1)$$

where A_i is abundance of the developmental stage i , A_{i+1} is the abundance of the next stage ($i + 1$), m_i is the instantaneous mortality rate (d^{-1}) of stage i , and D followed by subscripts are stage durations of copepodite stages i and $i + 1$. Since the stage duration of adults is infinite, mortality rates of C5 were calculated as:

$$m_5 = \frac{\ln\left(\frac{A_5}{A_{\text{adult}}} + 1\right)}{D_5} \quad (2)$$

The mortality estimates are expressed for each stage, not for pairs of stages. Thus, the mortality of C3 is considered the mortality between moulting to C3 and moulting to C4. Nauplii were not staged, and mortality rates of nauplii were obtained from the vertical life tables using the abundance of all naupliar stages. Mortality rates were only calculated for samples containing >10 individuals of the stage of interest.

RESULTS

Mariager Fjord was strongly stratified during the investigation, with a warm (17 to 20°C), well-mixed surface layer down to 12 to 15 m and cold, deep waters (<5°C) below 20 m (Fig. 2). Salinity ranged from 15 to 16 in the upper mixed layer, to 20 in the deep water. The strong thermocline was located at 15 m and coincided with the weaker halocline. Oxygen was present above 15 m, but declined rapidly at the pycnocline, reaching permanent anoxia at 20 m and below. Sulphide was omnipresent at 20 m and episodically occurred at 15 m (T. G. Nielsen et al. unpubl). There were no major exchanges of water with the Kattegat judging from the CTD profiles.

A bloom of *Skeletonema costatum* during the investigation resulted in exceedingly high chlorophyll con-

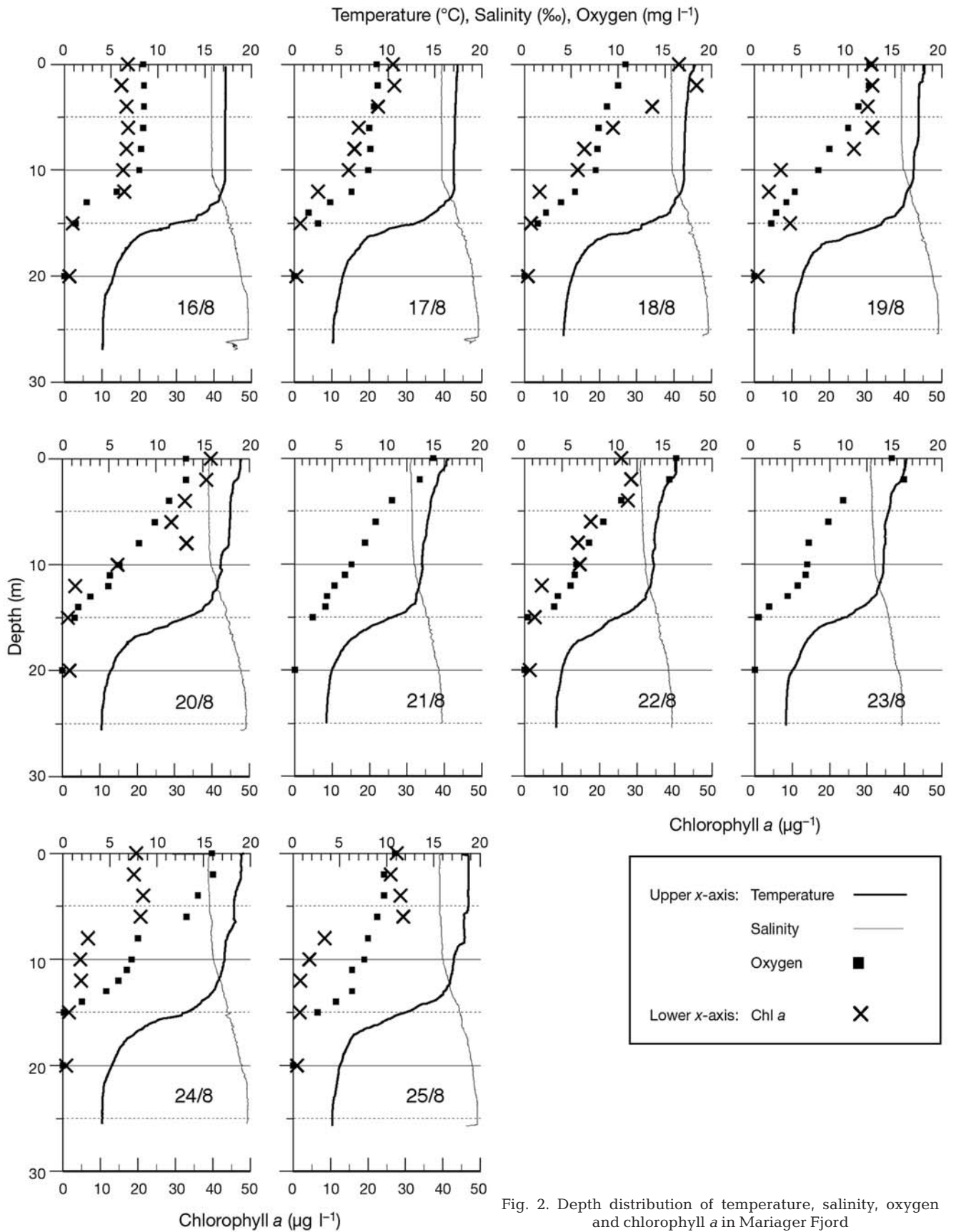


Fig. 2. Depth distribution of temperature, salinity, oxygen and chlorophyll a in Mariager Fjord

centrations. From already high values (15 to 18 $\mu\text{g chl a l}^{-1}$) on the first date, the bloom more than doubled in biomass in 2 d, reaching peak values of 46 $\mu\text{g chl a l}^{-1}$ on 18 August (Fig. 2). Within 6 d, the bloom disappeared, chlorophyll concentrations were back to normal high values and the phytoplankton biomass was dominated by the dinoflagellate *Heterocapsa triquetra*.

Metazoan plankton was dominated by rotifers *Synchaeta* spp. and meroplankton, notably *Balanus* larvae and polychaete metatrochophora larvae (Nielsen et al. unpubl). Among larger zooplankton, the copepod *Acartia tonsa* was the dominant species. Only few occurrences of *Centropages* spp., *Eurytemora affinis*, *Oithona* spp. and harpacticoids were recorded, and together they constituted <5% of the total copepod abundance. *A. tonsa* abundances were still low: integrated values ranged from 27 500 to 93 300 ind. m^{-2} , and 67 to 88% of the individuals were nauplii. Nauplii resided mainly in the upper 5 m, increasing from 2000 to 14 000 ind. m^{-3} over 8 d (Fig. 3). Copepodites also increased over time, but with 1 to 2 orders of magnitude lower abundances than nauplii, and a distribution above 10 m. For later stages, highest abundances were found at depth, around 10 m for C4, 12 to 15 m for C5 and 15 m for adult males and females. Overall, the population of *A. tonsa* grew rapidly during the study: nauplii increased from 22 000 to 62 000 ind. m^{-2} , copepodites from 5000 to 28 000 ind. m^{-2} and adults from 400 to 2900 ind. m^{-2} . The observed reduction in abundance between developmental stages, i.e. between panels in Fig. 3 is still striking, and suggests a strong mortality concurrent with the increase. The changing depth distribution of the various stages points towards an ontogenetic migration or a selective predation on younger copepodite stages.

To investigate reasons for the low abundance of *Acartia tonsa*, several population dynamics parameters were measured. Growth of the copepod population starts with females producing eggs. *A. tonsa* responded to the *Skeletonema costatum* bloom by doubling its egg production (EPR), from already high rates (35 eggs female $^{-1}$ d $^{-1}$) to 62 eggs female $^{-1}$ d $^{-1}$ on 18 August (Table 2). After the peak of the bloom, EPR decreased again and stabilised around 30 eggs female $^{-1}$ d $^{-1}$. The hatching success of produced eggs was always $\geq 90\%$ (Table 2).

The *Acartia tonsa* females were mainly distributed at depths of 10 to 20 m. Survival of adult females caged at 15 m depth was 93 to 100% (Table 3). Thus, the proximity to lethal levels of sulphide at 20 m did not affect survival, even though oxygen levels were lowered also at 15 m (Fig. 2). At the end of the investigation C5 copepodites and adults were relatively abundant at 20 m and females even seemed to be concentrated in the deepest part of the mixed layer.

The vertical flux of eggs declined sharply over time (Table 4), from 9400 to 1700 eggs m^{-2} d $^{-1}$ (equivalent to 17 and 4% of EPR, respectively), which is in accordance with a gradual descent of females over time (Fig. 3). Eggs in the sediment were only present deeper than 5 cm into the sediment (Table 5) and were most abundant in the deepest, anoxic sediment (100 000 eggs m^{-2}).

Mortality of *Acartia tonsa* was high, even though the population as a whole increased. The daily population egg production (number of females \times EPR) was 7000 eggs m^{-2} on 16 August and 56 000 eggs m^{-2} on 20 August, and the population size did not increase nearly that much. Employing the vertical life table approach to estimate mortality, we found a daily mortality of 16 to 18% for nauplii (all developmental stages pooled) and Copepodite Stage C1 (Fig. 4). Stages C2 and C3 experienced 4 times higher mortality, 66% d $^{-1}$, and older juveniles also had quite high mortalities, 43% for C4 and 53% for C5. Data for C4 are uncertain due to low numbers of animals included in the analysis.

Mortality was not equal at all depths, which may be a reason for the relation between water depth and distribution of developmental stages, from nauplii at the surface to adults at 15 m. To investigate the effect of depth on the mortality, each determination of daily mortality associated with a certain stage and date was plotted against the weighted mean depth of that stage on the date for the determination. Weighted mean depth (WMD) was calculated as:

$$\text{WMD} = \frac{\sum n_i d_i}{\sum n_i} \quad (3)$$

where d_i is the mean depth of depth stratum i and n_i is the number of copepods in that stratum. Mortality was highest at depths between 6 and 8 m and for Copepodite Stages C2 and C3, although it varied considerably (Fig. 5). A LOESS smoother (SPSS 13 for Mac OS) was applied to the data showing a mortality peak at 8 m.

DISCUSSION

Egg production

In the Niskin bottle samples, the older stages of *Acartia tonsa* were found at the bottom of the mixed layer, but we were able to collect adult females for egg production measurements (EPR) from the upper 10 m of the water column with a WP-2 net. No night-time sampling was done, but the EPR data provide indirect evidence of vertical migration. The variation in EPR indicates that *A. tonsa* at times left the high food habitat and experienced lower food or other limiting factors

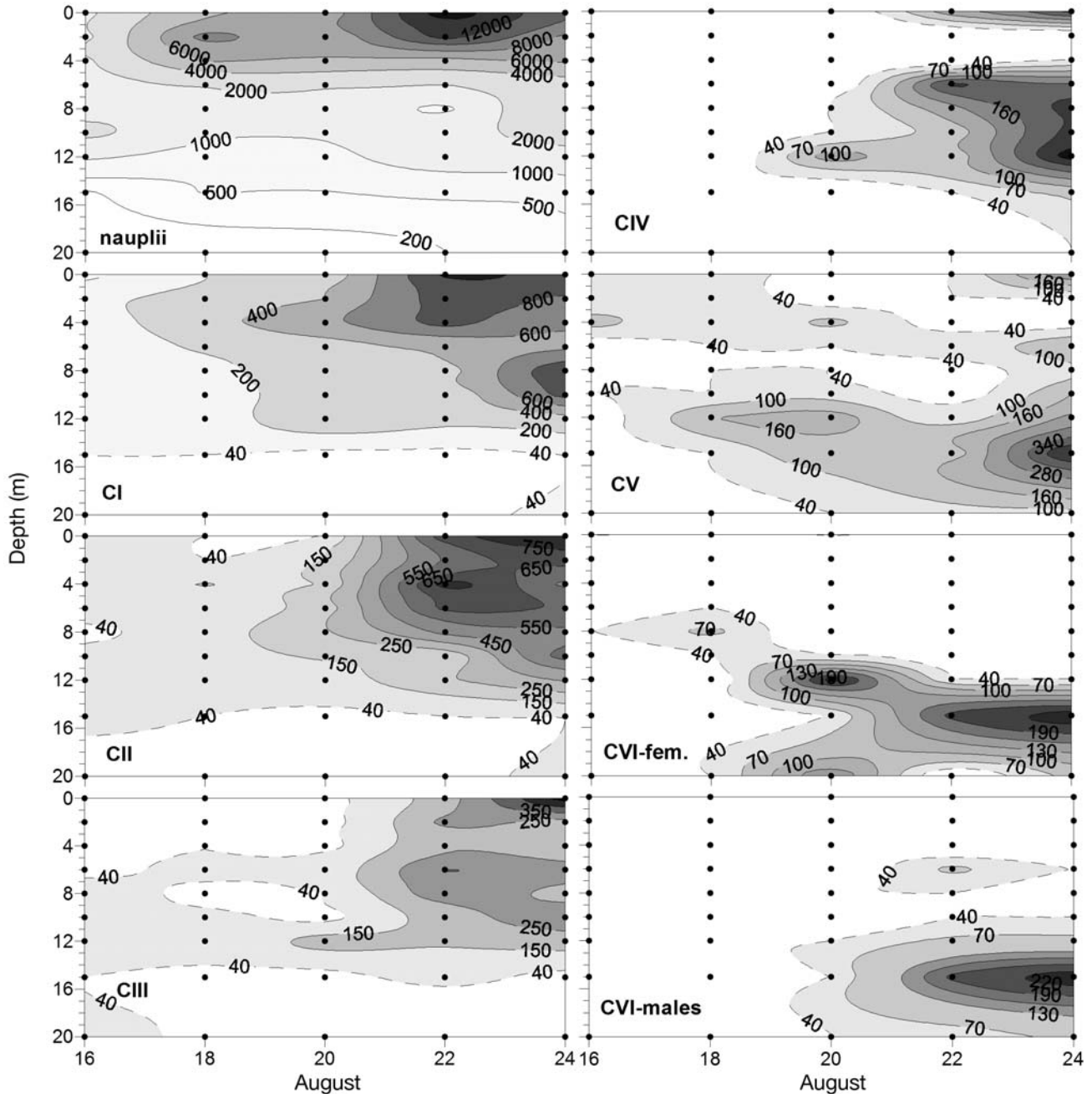


Fig. 3. *Acartia tonsa*. Isopleths of the abundance (ind. m^{-3}) of nauplii (pooled abundance N1 to N6), copepodites and adult males and females during the investigation

(e.g. oxygen, sulphide, temperature). Chlorophyll *a* levels at 10 to 15 m were still 2 to 5 $\mu g\ l^{-1}$ (Fig. 2), which is enough to assure maximal growth (Berggreen et al. 1988, Paffenhöfer & Stearns 1988). The fact that EPR still varied 2-fold over the investigation suggests that females migrated to deeper layers, where food was not saturating, or where other factors reduced EPR. A similar vertical migration has been shown in other locations (Stearns 1986, De Meester & Vyverman 1997).

The high phytoplankton concentrations in Mariager Fjord supported an EPR that ranks among the highest recorded in the field for this species. In East Lagoon, Texas, USA, a maximum EPR of 60 eggs female $^{-1}\ d^{-1}$ was measured (Ambler 1985) and *Acartia tonsa* from Narragansett Bay, Rhode Island, USA, supplied with enriched phytoplankton cultures reached a similar EPR (Durbin et al. 1983). However, since *A. tonsa* for EPR experiments were collected in surface water with

Table 2. *Acartia tonsa*. Egg production rate and hatching success of eggs incubated for 48 h; mean \pm SE, n = 12

| Date in August | Production rate (eggs female ⁻¹ d ⁻¹) | Hatching success (%) |
|----------------|--|----------------------|
| 16 | 35.3 \pm 2.1 | 99 \pm 1 |
| 18 | 61.8 \pm 1.4 | 99 \pm 1 |
| 20 | 43.9 \pm 4.2 | 98 \pm 1 |
| 22 | 28.9 \pm 3.8 | 94 \pm 2 |
| 24 | 33.5 \pm 3.5 | 90 \pm 2 |

Table 3. *Acartia tonsa*. Survival of females in cages placed at different depths in Mariager Fjord, 21 and 22 August, 2005; 15 females were placed in each cage at the start of deployment. n: number of animals recovered; -: lost replicate

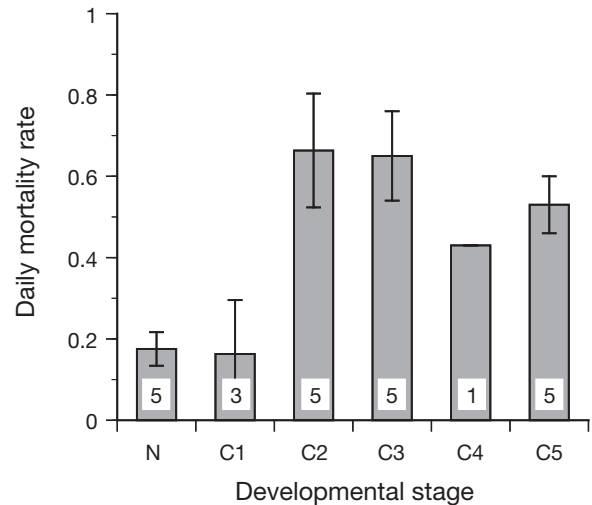
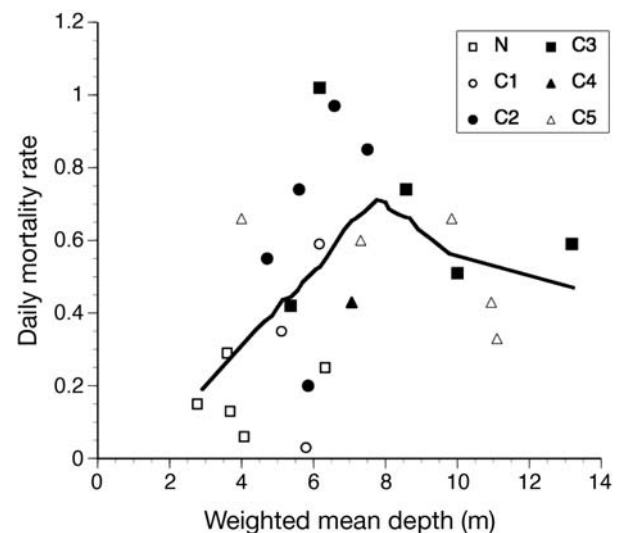
| Depth (m) | Survival (%) | n |
|-----------|--------------|----|
| 2 | 100 | 14 |
| | - | - |
| | - | - |
| 10 | 100 | 15 |
| | 100 | 13 |
| | - | - |
| 12 | 100 | 15 |
| | 100 | 12 |
| | - | - |
| 15 | 100 | 15 |
| | 93 | 15 |
| | 100 | 15 |
| 20 | 0 | 15 |
| | 0 | 14 |
| | 0 | 14 |

Table 4. *Acartia tonsa*. Sedimentation of eggs (no. m⁻² d⁻¹, \pm SD) measured at 15 m depth

| Date in August | Eggs (no. m ⁻² d ⁻¹) |
|----------------|---|
| 20 | 9395 \pm 3560 |
| 21 | 7245 \pm 695 |
| 22 | 3930 \pm 1737 |
| 23 | 2640 \pm 608 |
| 24 | 1719 \pm 868 |

Table 5. *Acartia tonsa*. Eggs (no. m⁻²) in the sediments of Mariager Fjord at 5–35 cm sediment depth. Eggs were collected from 1 core at each of 3 depths (4, 11.5 and 18.5 m), and the cores were divided in 2 sections (0 to 5 cm and 5 to 35 cm below the sediment surface). Egg diameters are mean \pm SD

| Depth (m) | Habitat | Abundance (10 ³ eggs m ⁻²) | Diameter (μ m) |
|-----------|--|---|---------------------|
| 4 | 100% cover of adult <i>Mytilus edulis</i> | 30.0 | 82 \pm 5 |
| 11.5 | Scattered clumps of newly settled <i>M. edulis</i> | 26.3 | 74 \pm 9 |
| 18.5 | Black, sulphide-rich sediment | 102.6 | 84 \pm 4 |

Fig. 4. *Acartia tonsa*. Estimated daily mortality rate (d⁻¹) averaged over the entire investigation (5 determinations) for nauplii (pooled N1 to N6) and copepodite stages. Error bars = SE, n = 1 to 5, indicated at the bottom of the columnsFig. 5. *Acartia tonsa*. Depth dependence of daily mortality rate estimated from single determinations and the weighted mean depth of the stage at that determination. Curve fit is a LOESS curve with 50% of points to fit and an Epanechnikov kernel

high food concentration, while the majority of females were distributed deeper and exposed to less food, the measured EPR was not representative of the entire population.

The superabundant food at the surface may inhibit the feeding of smaller developmental stages. Hansen et al. (1991) showed obstruction of the feeding of *Acartia tonsa* copepodites C2 and C3 by 45 μ m latex beads, and it is possible that the high concentration of *Skele-*

tonema costatum in Mariager Fjord may obstruct the feeding of younger copepodite stages. This could lead to lower ingestion and growth rates of these stages, but probably not to elevated mortality.

Sulphide and pH effects

Elevated pH may affect the copepods and the study of this effect was one of the objectives of our investigation. Experiments showed that egg production ceased at pH 10.0, but at pH 9.5 there were no effects on female survival, EPR, or egg hatching success (B. W. Hansen et al. unpubl.). The highest pH measured *in situ* was 9.75 on a single occasion during the study (T. G. Nielsen et al. unpubl.), so we cannot exclude a negative effect on EPR, but for all other sampling times, pH was <9.5. We did not test for effects on nauplii and copepodites, but the calculated mortality rate of nauplii residing in the upper 5 m of Mariager Fjord suggests no strong detrimental pH effects on nauplii (Fig. 5).

The deep distribution in older stages of *Acartia tonsa* may be dangerous due to the proximity to sulphide-containing deep water. In the cage experiments, survival of adults was 93 to 100% at 15 m depth, where oxygen concentration changed from 2.4 to 0.4 mg l⁻¹ (Fig. 2) during the deployment (Table 3). At 20 m, anoxia prevailed and all animals died. The cold water at 20 m may have caused the death of the copepods, but our experience is that *A. tonsa* is relatively robust to temperature changes. We suggest that the presence of sulphide at 20 m was the ultimate cause of death. In a behavioural study on adult *A. tonsa* (Stalder & Marcus 1997), the copepods did not avoid layers with oxygen concentrations <0.5 ml O₂ l⁻¹ (~0.7 mg O₂ l⁻¹), but sank into them and died after 2 to 3 min. Long-term (24 h) exposure to oxygen concentrations below 1 ml l⁻¹ resulted in gradually decreased survival, and at 0.5 ml l⁻¹ all animals died. In contrast, De Meester & Vyverman (1997) observed migration of *A. tonsa* in a meromictic lake, where the animals were reported to migrate into the anoxic hypolimnion and remain there for >12 h. The hypolimnion also contained sulphide, and the authors speculate that the population may have been genetically adapted to withstand the toxic waters. Our cage experiments show that copepodites and adults that remained close to the bottom of the mixed layer in Mariager Fjord may have suffered some mortality by swimming/sinking down into the anoxic layer.

Copepodite mortality

Predation by suspension-feeding blue mussels *Mytilus edulis* is another source of copepod mortality.

The role of benthic suspension feeders as zooplankton predators has recently been investigated (Nielsen & Maar 2007) and modelled (Maar et al. 2007). These studies showed that the capacity of copepods to avoid predation from filter feeders increases with their body size and escape capability, and decreases with turbulence. The waters of Mariager Fjord are usually stagnant (Bendtsen et al. 2006), and the larger zooplankton is probably able to avoid the siphons of the mussels.

The highest mortality was found in Stages C2 and C3. Ontogenetic shifts in behaviour of younger stages have been found in the congener *Acartia clausi* (Takahashi & Tiselius 2005). Stages C1 to C3 engage much more in filter feeding than older stages, and have long jumps directed horizontally instead of vertically as in later developmental stages. Furthermore, Stages C1 to C3 sink very slowly and do not require a vertical adjustment to maintain position. It is not clear what effect this has on susceptibility to mussel predation, but smaller stages should be more vulnerable. *Mytilus edulis* occur from 1 to 10 m depth in Mariager Fjord, with abundances of 1000 to 8000 ind. m⁻² (Sømod 2000) covering 54% of the bottom in the estuary and with a potential capacity to clear the water column above them several times a day. The depth distribution of C2 and C3 coincides with the optimal depth of *M. edulis*, and a strong predation loss is likely.

Lateral advection of copepodites from the open waters to the shallower areas may be a limiting factor for mussel predation. In a study of vertical mixing in the fjord, Bendtsen et al. (2006) found significant differences in the estimated age of a water parcel when they compared systems where the whole water column is within the mixed layer (shallow solution) and systems where the water depth is deeper than the mixed layer (mixed layer solution). The large discrepancy between the 2 solutions during summer months suggested an important, but not quantified, lateral transport of water near the bottom of the mixed layer. If lateral transport is important, the predation by *M. edulis* may be strong in a certain depth strata *throughout* the fjord.

Despite the likely role of *Mytilus edulis* as the major zooplankton predator, we cannot rule out the effect of fish predation. The most common fish in Mariager Fjord is the 3-spined stickleback *Gasterosteus aculeatus*, but herring *Clupea harengus* and sprat *Sprattus sprattus* are also present (Bio/consult 2005). A large population of visual, selective predators is not sustainable in the fjord because of the low prey biomass. Still, the fact that older and larger copepodite stages migrated to deeper areas of the fjord suggests that predation by fish is also important.

Naupliar mortality

Naupliar mortality averaged $18\% \text{ d}^{-1}$ for all stages combined. Hay et al. (1988) found similar mortalities of *Acartia clausi* nauplii (11 to $38\% \text{ d}^{-1}$), whereas rates were lower for *Calanus finmarchicus* (Ohman et al. 2004) and *Pseudocalanus newmani* nauplii (Ohman & Wood 1996). Since our investigation lasted only 9 d, the mortality estimate for all naupliar stages combined is uncertain because the combined stage duration is 6.5 d. Most nauplii in Mariager Fjord were found at 0 to 5 m depth (Fig. 3), and, since eggs hatch deeper, the nauplii must undertake an extensive vertical ascent to find food. Early-stage *Acartia tonsa* nauplii swim slowly in a jump–sink fashion, with a vertical ascent velocity of approximately 0.1 mm s^{-1} (Titelman & Kiørboe 2003) or 8.6 m d^{-1} if swimming continuously upwards. The non-feeding nauplius has to spend 1 to 2 d constantly swimming to reach the surface waters, where the majority of nauplii were found. The migration is dangerous because of high encounter rates with older conspecifics as well as with other pelagic predators. The pooled naupliar mortality estimate prevents a detailed analysis, but probably the ascent is critical, which would imply a peak in mortality at Stages N1 to N3. In general, naupliar mortality is highest for Stages N1 and N2 (Ohman et al. 2004, Hirst et al. 2007).

Egg mortality

EPR was high in the fjord, and the fate of the eggs is an important population-regulating factor. Egg-hatching success was similar to or higher than previous reports from East Lagoon (Ambler 1985) and La Jolla (Uye & Fleminger 1976). Blooms of *Skeletonema costatum* induce low hatching success (Miralto et al. 1999) and low naupliar survival (Ianora et al. 2004, Nielsen et al. 2006) in several calanoid copepod species, but never in *Acartia tonsa*. There may be large variations in the toxicity of different clones of *S. costatum* on copepod egg-hatching success (Ask et al. 2006), and the toxic effects may show up at older naupliar stages (Carotenuto et al. 2006). Therefore, we cannot rule out toxic effects from *S. costatum* on egg hatching, but we consider it of minor importance compared to other sources of mortality.

There was a large discrepancy between daily integrated EPR (range 7000 to 56 000 eggs $\text{m}^{-2} \text{ d}^{-1}$), and the number of eggs recovered in the sediment traps at 15 m (1700 to 9400 eggs $\text{m}^{-2} \text{ d}^{-1}$). There are several possible reasons for this. It may be due to predation on eggs in the water column and hatching of eggs in the traps. The discrepancy may also indicate an overestimation of integrated EPR, because we used only

females collected from surface waters for incubations. Finally, females were distributed deep and may have spawned their eggs below trap depth. Since the food concentration was high even at 15 m depth, we believe that the EPR is representative and the major reason for the discrepancy is probably the shallow placement of the traps. The possibility of eggs spawned below the traps, unfortunately, prevents the calculation of a full budget for recruitment.

The mortality of copepod eggs in shallow waters such as Mariager Fjord is only partly coupled to predation, while the dynamics of sinking and hatching may be a decisive factor. Tang et al. (1998) showed experimentally that all eggs produced by *Centropages hamatus* and *Temora longicornis* in Long Island Sound (average depth = 20 m) will sink to the bottom before hatching. Corresponding estimates for Mariager Fjord give a sinking velocity of *Acartia tonsa* eggs of 43 m d^{-1} , indicating that they will reach the bottom before hatching. Due to the higher temperature above the thermocline, the eggs will tend to sink faster here than at depth, but hatching time still exceeds the transit time to the colder water or bottom. In a shallow estuary such as Mariager Fjord, with a large part of the spawning population at depth, all eggs must therefore hatch from the bottom sediments and the fate of eggs is tightly coupled to the micro-environment where they settle. Most eggs will sink through the pycnocline and settle at bottoms with overlaying anoxic or sulphidic water. Eggs that sink to sediments where the water is oxygenated are more likely to hatch, but the risk of being buried and surrounded by anoxic/sulphidic pore water is high. If exposure to sulphide occurs at pH 6.5 (typical for pore water) instead of at normal pH 8.2, the effects on the organisms are more detrimental (Vismann 1996, Invidia et al. 2004).

Resuspension is necessary for hatching in areas with sulphidic and anoxic bottom waters, but even oxygenated bottoms will be lethal areas if the eggs are buried in the sediment. No *Acartia tonsa* eggs were found in the upper 5 cm of the sediments, which could indicate rapid hatching of eggs. Below 5 cm, eggs were most abundant in the deeper parts of the fjord with less mobile sediments, albeit not as abundant as reported from other, less enclosed areas. For instance, eggs of *A. bifilosa* were found at abundances of 250 000 m^{-2} in an anoxic fjord in Finland (Viitasalo & Katajisto 1994). As in our study, eggs were fewer at 0 to 3 cm than deeper in the sediment and it was suggested that a major hatching event had taken place in connection to an inflow of oxygen-rich deep water and subsequent resuspension of eggs. Such large inflows of oxygen-rich water are rare in Mariager Fjord, and may occur only every second or third year (Fallesen et al. 2000). Therefore, resuspension and subsequent hatching of

eggs is probably not ecologically important. Egg mortality will always be much higher inside the fjord than outside, and high egg mortality must be a major reason for the small population size in the fjord.

Bottoms with beds of *Mytilus edulis* in the depth interval from 1 to 10 m cover 54% of the estuary, and mussel filtration will also be a significant sink for copepod eggs. Modelled mortality impacts by a mussel bed of ca. 4000 ind. m⁻² (similar to Mariager Fjord) show that under turbulent conditions, the mortality rate of dinoflagellates due to grazing may be as high as 800% d⁻¹. During periods of low mixing, however, re-filtration of bottom water reduces the grazing impact to 36% d⁻¹ (Maar et al. 2007). For copepod eggs, which resemble dinoflagellates in size and lack swimming ability, mortality over a mussel bed will be essentially 100%. The area between the upper limit of anoxic, sulphide-containing water and the lower limit of the mussel beds will be the area where copepod eggs may settle and hatch in Mariager Fjord. This favourable bottom area, with a low abundance of mussels, stretches from approximately 10 to 20 m in depth and covers 33% of the fjord (Fig. 1, Table 1). The deeper part of this zone is often exposed to sulphide leaving the bottom at approximately 10 to 15 m as the main area for successful egg hatching, survival and recruitment to the copepod population. These bottoms harbour low biomasses of benthic suspension feeders and, hence, a lower risk of egg mortality due to predation. They cover 22% of the estuary, usually have aerated sediment surfaces from which eggs can hatch, and the nauplii may migrate up in time to start feeding before starvation occurs. Such a restricted area from which copepod eggs may hatch is rather unique to Mariager Fjord. Outside the estuary, in the Kattegat, the bottoms are not anoxic and all eggs that settle may hatch provided they are not eaten by benthic deposit feeders. In other coastal areas with anoxia at greater depths, most eggs will hatch before reaching the anoxic layer.

CONCLUSIONS

Acartia tonsa in Mariager Fjord is under severe stress from 2 factors, predation by mussels and fish from above and anoxic/sulphidic bottom water from below. The population shows high reproduction and has virtually unlimited food. The vast majority of the eggs, however, sink out of the mixed layer and do not survive until hatching. Predation on eggs by *Mytilus edulis* should be strong, but bottom areas at 10 to 15 m seem to be less harmful, due to a lower abundance of suspension-feeding predators and generally aerated conditions. Among the few survivors that make it to the copepodite stages, mortality is again severe from *M.*

edulis. Copepodite Stages C2 and C3 seem most susceptible to predation, due to their depth distribution exposing them to the mussel beds and to their potentially weak escape responses from the filtering mussels.

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