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Spawning habitat and egg mortality in capelin, *Mallotus villosus* (Müller) – responses to extreme abiotic conditions

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ABSTRACT

The oceanic Barents Sea capelin spawns demersally in deeper waters along the Finnmark and Murman coasts in March and April. The spawning habitat contrasts that of many fjord populations – e.g. the Balsfjord capelin – which spawn intertidally on the beaches in April and May. Capelin eggs adhere to the substratum (coarse sand) and hatch after approximately 150 day-degrees. However, eggs which develop on the beaches are periodically exposed to air and extreme variations in both temperature and salinity. The abiotic challenges which meet the eggs of beach spawning capelin differ markedly from those spawned under relatively stable microclimatic conditions in deeper waters. In this study, we report on the abiotic *in situ* conditions which characterize the spawning beach of the Balsfjord capelin during an incubation period. Data provided from laboratory experiments show the effects of an extreme microclimate on the mortality and osmoregulatory capacity of eggs.

Keywords: Capelin, *Mallotus villosus*, mortality, osmoregulation, spawning habitat, microclimate,

INTRODUCTION

The oceanic Barents Sea capelin spawns demersally in the deeper waters along the Finnmark and Murman coasts in March and April (Jangaard, 1974; Gjøsæter, 1998). The spawning habitat contrasts that of the Norwegian fjord populations – e.g. the Balsfjord capelin – which spawn intertidally on the beaches in April and May. The Newfoundland capelin has been shown to differentiate in spawning mode directed by abiotic factors such as temperature and substrate (Carscadden et al., 1989; Nakashima & Taggart, 2002; Nakashima & Wheeler, 2002). Thus, the spawning mode is not genetically predetermined (Dodson et al., 1991; Nakashima & Wheeler, 2002). The difference in spawning habitat suggests that eggs laid on the substratum may experience differences in temperature and salinity and hence in mortality and hatching success. Eggs spawned at the beach may experience daily temperature fluctuations of 25°C (Davenport et al.,1979), which differ markedly from those spawned under relatively stable microclimatic conditions in deeper waters of the Barents Sea.

No biological antifreeze activity has been found in the yolk and embryos (K. Præbel, unpublished.), even though experiments have shown that capelin eggs tolerate exposure to temperatures at -5.2°C for up to 6 hours without the survival rate was affected (Davenport & Stene, 1986). The eggs presumably survive these temperatures by a chorion that prevent ice penetration whereby supercooling of the yolk and embryo is possible (Davenport et al., 1979; Davenport & Stene, 1986).

Salinity (1.7-34‰), does not seem to affect the survival and hatching success of cultured eggs from capelin with either spawning mode (Davenport & Stene, 1986; Davenport, 1989). Eggs from several other marine teleosts have been shown to maintain the osmotic concentration in the yolk (346-395 mOsm depending on species), when incubated in both hypoosmotic and hyperosmotic mediums (Kjørsvik

et al., 1984; Lønning et al., 1988). However, none of the studied species were exposed to rapid changes in the osmolality of the external medium, as does the capelin eggs when spawned intertidally. In view of the fact the chorion of capelin eggs has twice the water permeability compared with that of plaice eggs (demersal) (Davenport & Vahl, 1983), it is interesting how and if the yolk can maintain its osmotic concentration during rapid changes in osmolality of the external medium.
In this study we presents data that show the microclimate at a typical intertidal spawning site. Furthermore we have investigated how salinity and temperature affects the survival and hatching success of eggs from the Barents Sea capelin. Lastly we determine the total and yolk osmolality of capelin eggs from the Barents Sea when exposed to similar conditions as found on the spawning site in Balsfjord.

MATERIALS AND METHODS

Measurement of abiotic factors

Temperature, salinity and pressure loggers (DST-200, Star-Oddi, Reykjavik, Iceland) were deployed at a known capelin spawning site in Balsfjord, Northern Norway. The loggers were attached to a 6 mm nylon rope with a total length of 80 m, running from the shoreline to a 10 kg anchor placed below the low-tide mark. One logger was placed below the low tide mark (the reference-station) and two loggers were placed directly on the spawning ground; i.e. on the gravel and 4 cm within the gravel (sub-surface). The reference and surface loggers were protected from direct sunlight by a white plastic tubing (5 cm diameter) with the openings pointing towards the tide-flow, while the sub-surface logger was uncovered. The loggers recorded the temperature, salinity and pressure every 35 minutes in the period from 7 April to 29 May 2004.

Temperature and salinity measurements have an accuracy of ± 0.1°C and ± 0.75‰ respectively (Star-Oddi specifications).

At retrieval the loggers were placed in a water/ice-bath made of distilled water for 12 hours. After downloading data the logged temperature values were corrected with the values obtained in the water/ice-bath (± 0.1°C). The temperature data given here is the corrected raw-data and the mean temperature for a 24 hour period. The salinity data are presented as raw-data for the reference and sub-surface loggers and an extract of 48 hours tidal cycles for the logger on the surface of the spawning ground.

During low-tide periods the logger on the surface of the spawning ground was exposed to air, meaning that no salinity measurements were obtained. Samples of capillary water surrounding capelin eggs were obtained by placing fertilized eggs from to Barents Sea capelin (see below), on the spawning ground and then collecting water samples at low tide by a capillary micropipette. The salinity was then determined as described below.

Animal collection

Sexually mature Barents Sea capelin (*Mallotus villosus*) were caught by pelagic trawl from R/V Jan Mayen at 71°20N, 24°45E in March and transported live to the marine research station in Tromsø, Northern Norway. The fish were kept in 2x2 meters fibreglass tanks in running seawater with ambient temperature and salinity and with ambient light regime (70°N).

Fertilization of eggs and experimental design

The capelin spawning behaviour was observed in the holding tanks on 15 April. Then approximately 50 specimens were moved to the experimental facility. Eggs from 16

females were dry-fertilized with sperm from 20 males and the eggs were placed in 34‰ seawater at 4°C for 16 hours.

Egg used in the study of simulated tidal cycles effect on the osmolality were placed in beakers each containing 4 litres of 34‰ seawater. The beakers were kept in a refrigerator at 5°C and constant light regime.

To study the effect of salinity and temperature on the survival rate and hatching success about 75 fertilized eggs were placed in beakers each containing 1 litre of 51‰, 34‰, 17‰ and 3.4‰ seawater. The beakers were then placed in refrigerators at 5 and 0 ± 0.5°C at constant light. The low salinities were obtained by diluting 34‰ seawater with distilled water and the 51‰ water was made by adding Instant Ocean Seasalt (Instant Ocean) to 34‰ seawater. About 75 fertilized eggs were placed in the aquarium at ambient salinity and temperature and constant light as a control group. Each group was checked on a 5-7 day basis counting the live and dead eggs. The water salinity was checked and the water was changed in all groups at least every fourth day.

Simulated tidal cycle and sampling of egg fluids

To study the effect of simulated tidal cycles on the osmolality approximately 5000 live eggs where transferred from the 34‰ beakers to distilled water at 5°C. Eggs were collected in the interval 10 seconds to 6 hours after which the remaining eggs where transferred back into the 34‰ water at 5°C and a similar sampling procedure was conducted. At each collection approximately 100 eggs were placed on a Kimvipe to remove any excess and capillary water. The eggs were then transferred to a 0.5 ml Eppendorff tube and overlaid with paraffin oil to prevent evaporation. After homogenisation with a glass piston the suspension was centrifuged at 10000 ***g*** for 5

minutes. The supernatant was then transferred to a new 0.5 ml tube, overlaid with paraffin oil and frozen at -80°C for later analyses.

Before the experiment started the outer diameter (OD) and the diameter of the yolk (YD) of 100 eggs were measured using a binocular microscope equipped with a measuring ocular and a cold light source. Furthermore samples of the yolk fluid were collected by a micro glass pipette mounted on a micromanipulator to obtain an initial yolk osmolality value.

Osmolality measurements

The osmolality of the different egg fluids was measured using a Clifton Nanolitre Osmometer (Clifton Technical Physics, Hartford, NY, USA), mounted on a microscope. The samples were loaded with a capillary micropipette into the centre of oil-filled wells and the sample size was approximately one-third of the well size. The samples were then quickly cooled to -40°C and the temperature was raised at a rate of 0.19ºC /min until the last ice crystal disappeared which was taken as the melting point. The melting point was determined six times for each sample to account for the melting points dependency of sample size. The melting points were given in mosmole/kg (mOsm) and the colligative freezing point was obtained by multiplying the osmolality values with -0.001858°C $mosmol^{-1}kg^{-1}$ (Levine, 1995).

The osmolality of the seawater and seawater blends were measured as duplicates with a Wescor 5100C vapour pressure osmometer (Wescor Inc., Logan, UT, USA).

Calculations

To determine the osmolality of the yolk fluid (C_Y), the following equation was developed:

$$C_Y = \frac{(V_T \times C_T) - (V_P \times C_P)}{V_Y}$$

Where V_T is the total volume of the egg (in cm^3) calculated from the OD of the egg, C_T is the total osmolality of the egg (in mOsm), V_p is the volume of the perivitelline space (in cm^3) calculated by subtracting the volume of the yolk – calculated from the YD – from the total volume of the egg, C_p is the osmolality of the perivitelline fluid (in mOsm) and V_Y is the volume of the yolk (in cm^3). It was assumed that the eggs and the yolk were spherical and the perivitelline fluid was isoosmotic to the external medium. Hence calculations of yolk osmolality in the range 10 seconds to 5 minutes after transfer to a new medium were excluded.

RESULTS

Abiotic factors

The temperature measurements revealed that eggs laid at the spawning ground may experience daily temperature fluctuations of 3.9° to 20.8° during the period of incubation (Fig. 1). Lowest recorded temperature during the measuring period was -2.70°C. Comparing the surface and sub-surface temperature of the gravel it is seen that the temperature fluctuations are much less pronounced sub-surface (0.8°- 8.9°). The temperature fluctuations at the reference station (0.2°- 4.6°), was smaller than both that of the surface and sub surface loggers.

The salinity recordings at the reference station revealed that capelin eggs spawned demersally may experience a stable salinity regime except in periods of snow and ice

melting (Fig. 1). At the surface of the spawning ground the salinity were lower than at the reference station (Fig. 2). The capillary water surrounding the Barents Sea eggs at low tide had an osmolality of 522 mOsm corresponding to a salinity of 18.3‰. Surprisingly the salinity at the sub-surface station (4.7-9.2‰), was only half of that on the surface of the spawning ground (Fig. 1).

Survival

The influence of temperature and salinity on survival and hatching success of capelin eggs during development was studied. Eggs incubated at ambient water temperature and salinity showed a survival rate of 45% until the hatching started 31 days post-fertilization (Fig. 3). Eggs incubated at 5°C showed a higher survival rate (15-50%), than those at 0°C (0-14%), until hatching. The incubation period was affected by temperature, which is seen by one month difference in hatching time.

The salinity only seems to affect the survival rate when the incubation takes place in hyperosmotic conditions (51‰). Less than 10% of the eggs hatched in the 5°C group and all eggs died within 20 days when incubated at 0°C and 51‰.

Simulated tidal cycle

As demonstrated by the *in situ* measurements of the abiotic factors capelin eggs may experience extreme variation in both temperature and salinity during development. Exposure of artificially fertilized eggs to distilled water revealed that the chorion is very permeable to ions and water. In fact, the osmolality of homogenized eggs dropped to 158 mOsm within 3 minutes, with the most dramatic drop in osmolality within the first minute (770 to 275 mOsm) (Fig. 4). However only small changes in the osmolality were seen in the interval from 3 minutes to 6 hours (116 mOsm) of

exposure. An opposite trend was seen when the eggs were transferred back to 34‰ seawater (Fig. 4).

To estimate the osmolality changes in the yolk fluid during exposure to distilled water an equation was developed that gives the osmolality from measured diameters, total osmolality, and the osmolality of the perivitelline fluid. The measurements show that the osmolality of the yolk remains nearly constant during exposure to distilled water which was seen by comparing the initial measured yolk osmolality value (406 mOsm) with the calculated value after 6 hours exposure to distilled water (419 mOsm) (Fig. 4). Only a slight increase in osmolality was observed during the transfer to salt water (Fig. 4).

DISCUSSION

It has been shown previously (Davenport et al., 1979; Davenport and Stene, 1986; Davenport, 1989), that capelin eggs tolerate extreme variations in abiotic factors such as salinity and temperature. However, none of these studies have reported the magnitude of these factors. Continuous high-resolution temperature and salinity data of this study revealed that capelin eggs spawned intertidally may be exposed to large fluctuations in both abiotic factors. This contrasts the demersally spawned eggs of the Barents Sea capelin (Gjøsæter, 1998). The question that arises from this observation is whether eggs from capelin with two different spawning modes, i.e. the Barents Sea and Balsfjord capelin, have adapted different physiological mechanisms to deal with these abiotic factors.

The survival experiment with eggs from the Barents Sea capelin showed that the temperature during the incubation has an effect on the survival and hatching success

of the eggs. This observation is consistent with the findings by (Gjøsæter, 1998). Whether the temperature has the same effect on eggs from the Balsfjord remains to be answered. However, as pointed out in several studies, the reproductive mode is not genetically predetermined but rather a response to temperature and substrate (Carscadden et al., 1989; Dodson et al., 1991; Nakashima and Taggart, 2002; Nakashima and Wheeler, 2002). Therefore physiological differentiation of the adaptation to temperature and salinity fluctuations between the two stocks of capelin is doubtful. This hypothesis is further supported by the eggs from the Barents Sea capelin response to short and long term exposure to different salinities shown in the present study. Short-term exposure (6 hours) to distilled water did not cause any major changes in yolk sac osmolality and only small differences were seen in survival and hatching success when eggs were incubated at different salinities.

In conclusion the preliminary results obtained in the present study shows that eggs from the Barents Sea capelin is less tolerant to temperature than salinity changes. Furthermore the results indicate that eggs from the Barents Sea capelin tolerate similar abiotic factors as found on the beaches in Balsfjord.

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FIGURES

Figure 1.

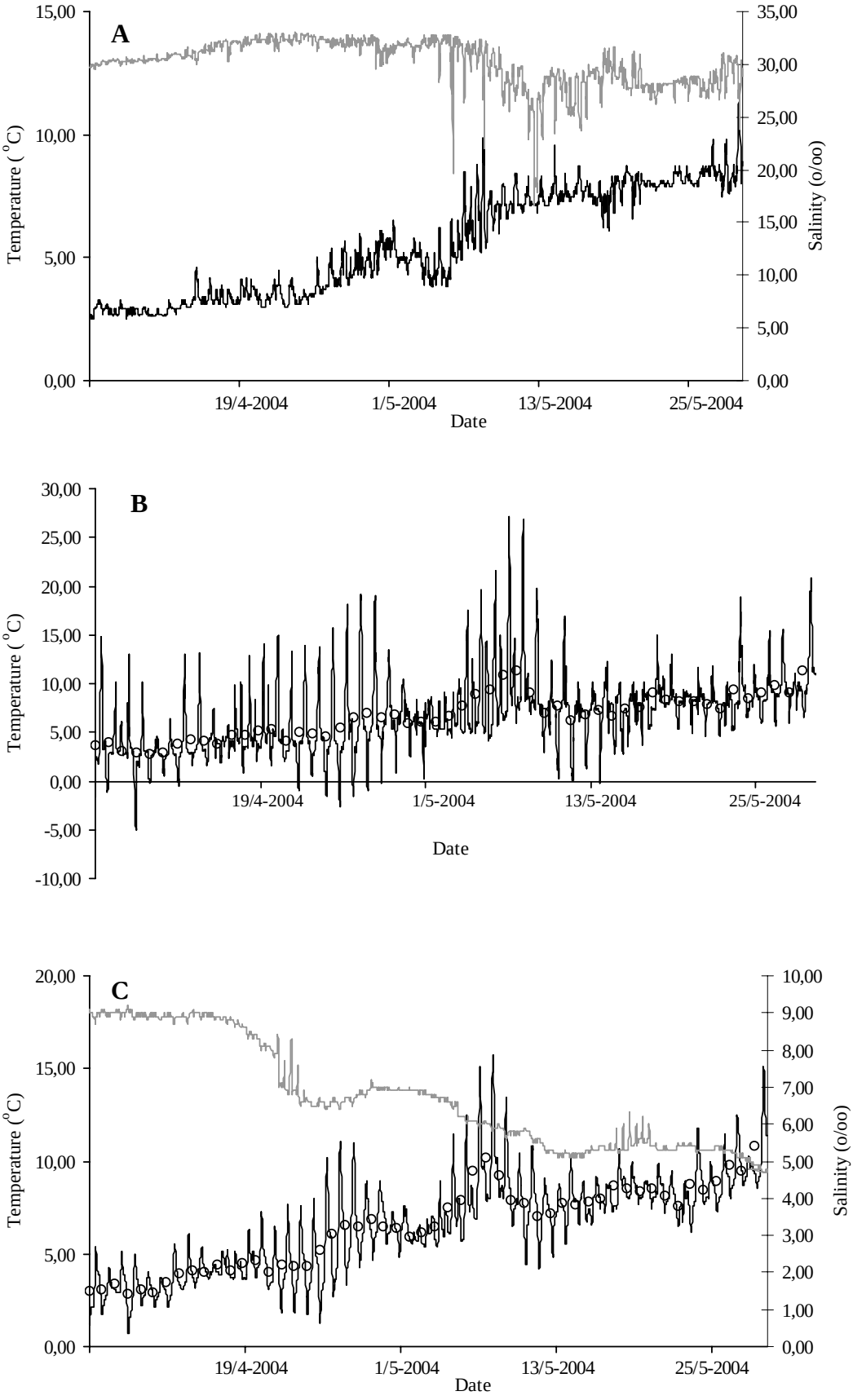

Figure 2.

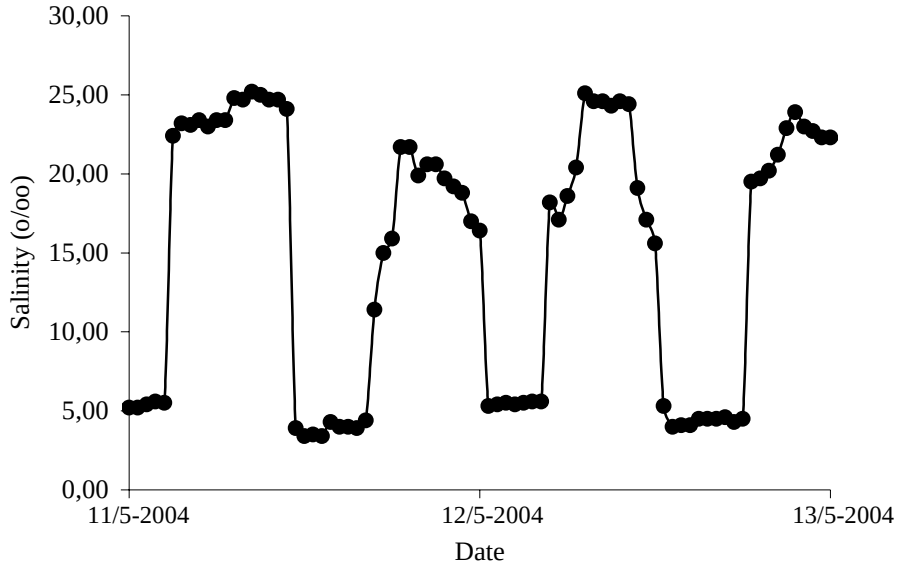

Figure 3.

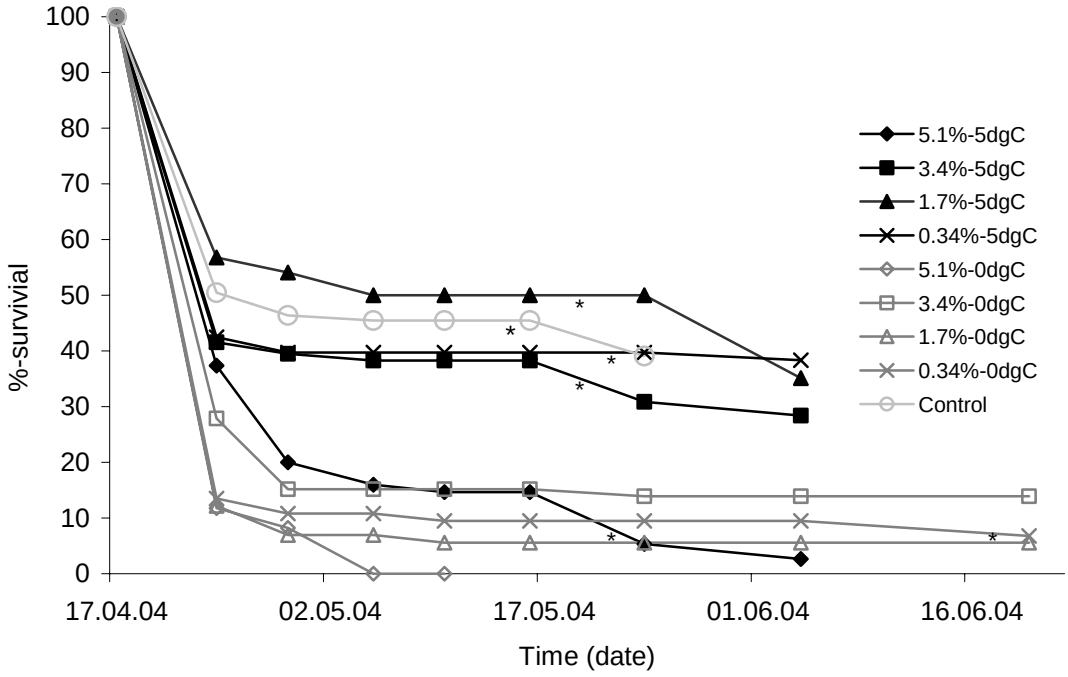

Figure 4.

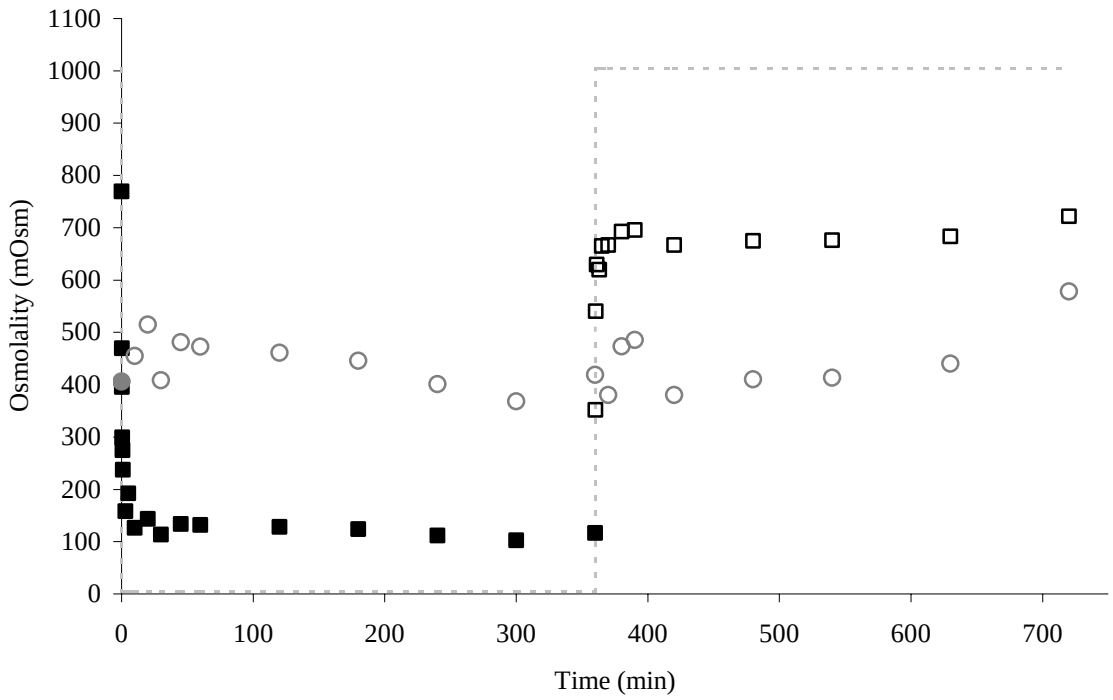

FIGURE LEGENDS

Figure 1. Water temperature and salinity at the reference station (A), on the spawning ground (B) and sub-surface in the gravel on the spawning ground (C). The black line represents the raw date and open circles show the mean water temperature for 24 hours periods. The light grey line illustrates the salinity changes at the reference station (A) and sub-surface at the spawning ground (C).

Figure 2. The salinity at the surface of the spawning ground (see Fig 1B). An extract of 48 hours is shown.

Figure 3. Survival of capelin eggs during incubation at different salinities and temperatures. Asterisks mark the day of first hatching.

Figure 4. Changes in the total osmolality and yolk osmolality of capelin eggs during exposure to distilled water and 34‰ seawater at 5 °C. The filled squares mark the osmolality changes in homogenised eggs when transferred from 34 ‰ salt water to distilled water and the open squares mark the osmolality changes for the opposite transfer. The filled grey circle mark the measured initial osmolality value of the yolk and the open circles mark the calculated osmolality changes of the yolk during exposure to distilled water and sea water. The dotted line shows the osmolality of the medium.