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ARTICLE

Low tolerance of salt water in a marine fish: new and historical evidence for surprising local adaption in the well-studied commercially exploited capelin

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Abstract: Intraspecific biodiversity among populations is ecologically and evolutionarily notable. Substantial adaptations in well-studied commercial species should be obvious and ought to have been uncovered. The tenet of marine stability posits relatively little local adaptation in the sea, except for temperature responses to latitude for widespread species. However, aquatic reproduction is tightly constrained by water chemistry, which may lead to abstruse adaptations. Capelin (*Mallotus villosus*) reproduction has been studied for over a century, occurring within pebble substrate on beaches or the seafloor of continental shelves. Offshore spawners (Iceland/Barents Sea) have embryos with tolerance of high salinity. I provide new and historical data on beach spawners from several areas indicating that their embryos perform well from ~2 to 28 psu, but at higher salinities indicative of coastal seawater, there is poor hatch success, larvae take longer to hatch, hatch at a smaller size, and starve more quickly. The body of evidence supports the hypothesis that beach spawning evolved from anadromy, with offshore spawning a derived state, enabled by increased salinity tolerance. The results have recovery implications for depleted Newfoundland stocks, which have been spawning seasonally late under relatively high salinity for over 25 years.

Résumé : La biodiversité intraspécifique entre populations est un phénomène notable sur le plan tant de l'écologie que de l'évolution. Les adaptations importantes chez des espèces d'intérêt commercial bien étudiées devraient être évidentes et avoir déjà été découvertes. L'hypothèse de la stabilité marine prévoit relativement peu d'adaptation locale dans la mer, outre des réactions à la température associées à la latitude pour les espèces à grande aire de répartition. La chimie de l'eau exerce toutefois un contrôle serré sur la reproduction aquatique, ce qui peut mener à des adaptations obscures. La reproduction du capelan (*Mallotus villosus*), étudiée depuis plus d'un siècle, a lieu dans des substrats caillouteux sur les plages ou sur le fond marin de plateformes continentales. Les embryons d'individus qui frayent au large (Islande/mer de Barents) sont tolérants à de fortes salinités. Je présente des données nouvelles et historiques sur des individus frayant sur des plages de plusieurs endroits, qui indiquent que leurs embryons vont bien dans la fourchette de ~2 à 28 psu, mais à de plus fortes salinités caractéristiques d'eaux marines littorales, le succès d'éclosion est faible, les larves prennent plus de temps à éclore, sont plus petites au moment de l'éclosion et meurent de faim plus rapidement. Les preuves disponibles supportent l'hypothèse voulant que le frai sur la plage soit une évolution découlant de l'anadromie, le frai au large étant un état dérivé rendu possible par une augmentation de la tolérance à la salinité. Ces résultats sont importants pour la compréhension du rétablissement de stocks terre-neuviens décimés qui, depuis plus de 25 ans, frayent tard dans la saison dans des conditions de salinité relativement élevée. [Traduit par la Rédaction]

Introduction

Within species, local adaptation among populations often occurs when sufficient reproductive isolation, selective pressures, and heritability are present. This intraspecific biodiversity is important both evolutionary and ecologically. Local adaptation may encompass many aspects of a life history, including reproductive characteristics, such as reduced secondary sexual characteristics of sockeye salmon (*Oncorhynchus nerka*) in natal streams of relatively high bear predation (Quinn et al. 2001) and abiotic adaptations such as increased developmental rate of Atlantic silversides (*Menidia menidia*) in compensation for shorter growing seasons at northern latitudes (Conover and Present 1990). Although thermal responses are well studied in fishes, the importance of local adaptation in reproductive traits to variable water chemistries is poorly understood. Fragmented freshwater systems are predicted to promote adaptation, for example in a cichlid, Morita et al. (2010) found sperm motility to be tuned to the local environment. However, while strong thermal gradients exist, the paradigm of marine stability posits that water chemistry generally does not vary enough to cause intraspecific adaptation in the oceans. Notable exceptions include along unique salinity gradients in the Baltic Sea (DeFaveri and Merila 2014; Berg et al. 2015).

External developing species are evolutionarily constrained to reproduce in specific habitats. Amphibian embryos for example cannot develop in the sea. The great majority of teleost fishes reproduce in one of fresh or salt water. Very few species can reproduce in both and spawning is relatively uncommon at intermediate (estuarine) salinities. Diadromous species migrate to and from fresh and salt water but must return to natal habitats to spawn. Diadromy at temperate latitudes is most commonly in the form of anadromy (Gross et al. 1988), where fish grow at sea and reproduce in fresh water. Anadromous salmons often show local adaptation to the chemistry of their home rivers. Several fish taxa have species that spawn in the intertidal zone of marine beaches (Martin 2015). Hypotheses for beach spawning include it being

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intermediate between marine and anadromous life cycles or a mechanism to place marine young out of the reach of predators or in more favorable temperature and oxygen conditions (reviewed by Martin 2015). However, the reason is unlikely to be consistent across taxa (Bloom and Lovejoy 2014). Species that spawn both on beaches and in other habitats may provide novel insights into this problem. Within species, although temperature responses among different locations have been examined (e.g., Penton and Davoren 2013), I have found no work that has investigated local adaptation to water chemistries in beach spawners.

The capelin (*Mallotus villosus*) is a keystone species in the food webs on the continental shelves of the North Atlantic (see Buren et al. 2014). It converts small zooplankton protein into forage for larger fish, seabirds, and marine mammals. Capelin spawn on the same size substrate and at similar temperatures throughout their distribution (Carscadden et al. 1989; Davoren et al. 2006). The general hypothesis for spawning site selection is simply where these two factors meet (Carscadden et al. 1989): at depth on the seafloor where surface waters are colder, and bottoms relatively warm in comparison (e.g., Iceland, Barents Sea), and on beaches where the surface is relatively warm compared to the bottom (e.g., Norwegian and Alaskan fjords). On the island of Newfoundland, large runs of capelin spawn both on nearshore seafloors <40 m and more famously in intertidal areas of pebble beaches around much of the coast. Few if any fish species reproduce in a manner that is more easily observed and research on this natural wonder has been ongoing for decades (Lanman 1874; Jeffers 1931; Sleggs 1933; Templeman 1948). Although this key fish is well studied, many known unknowns of capelin biology remain, including mate choice and sperm competition during spawning, what drives high recruitment variability, food web interactions, and the effects of directed fishing.

Being strictly a marine species, one would predict capelin reproduction to be optimal at salinities indicative of sea water (30–35 psu). It is known that stocks spawning on the continental shelves of Iceland (Davenport 1989) and in the Barents Sea (Præbel et al. 2013) have embryos that perform well at high salinity, and this is presumed to be typical. The eggs are adhesive once fertilized and thus, beach-spawning capelin embryos remain in the intertidal zone for several weeks, which is a problem at times due to desiccation (Sleggs 1933). The initial objective of this study was to examine how much fresh water would be required to kill developing embryos of this marine fish (rain directly falls on eggs and increases stream flows on spawning beaches) and whether salinity sensitivity interacts with temperature. I report on a great unknown.

Methods

General framework

A split-brood experimental design was used to examine family-level reaction norms in phenotypic plasticity of capelin development to salinity and temperature. Siblings exposed to one environment were compared (with procedural replicates) to siblings in another. Experiment-wide replication was achieved through the use of multiple families, using fish collected from two spawning beaches, and different experiments conducted over three years.

2011 experiment: broad brush approach

Fish were collected on 19 July with a cast net as they were spawning on Middle Cove Beach (47°39′2.75″N, 52°41′45.24″W), on the Avalon Peninsula of Newfoundland, Canada. They were brought to a laboratory and held in a flow-through tank at 5–8 °C. Ten fish were taken from the tank and used to create five full-sibling families on 20 July. Another 10 fish created families 6–10 on 27 July. Lengths of parents and mean dry egg masses from each female are given in Table A1 (Appendix A).

As it was assumed that low salinity would be problematic for a marine animal, all fertilizations were conducted using 30 psu water at 10 °C (subsequent experiments indicate that fertilization is possible over a wide salinity range). Fish were killed and then dried with a paper towel prior to gamete collection. Approximately 1.5 mL of eggs from a given female was placed in a flexible teflon weigh boat. Capelin sperm leave the body already activated (Beirão et al. 2018). Very high sperm to egg ratios were created by adding two drops of semen from a single male to each group of eggs and then mixed dry with a toothpick and allowed to sit on ice for 30 s (most fertilizations are expected to occur within a few seconds).

Capelin eggs are sticky, making detailed examination of embryo development difficult. To remove egg adhesiveness, a mixture of 10 °C 600 mg/L tannic acid (mixed in 30 psu water) was added and swirled for 30 s (Walker et al. 2010). This was then poured off and the eggs were rinsed three times with 30 psu water. Preliminary experiments indicated that fertilization success and hatch success were unaffected by the tannic acid, that lower concentrations did not adequately prevent eggs from sticking to glass surfaces, and that eggs could not be unstuck if they came into contact with water (for fertilization) before tannic acid. The container of fertilized eggs was kept on ice while they were transferred to incubation beakers.

The design tested hatch characteristics under 18 conditions. A goal was to determine if temperature (5, 10, and 15 °C) had an interaction effect with salinity. Experimental incubation water was created by adding Instant Ocean sea salt to declorinated city water that had been oxygenated with an air stone for 24 h. Salinity treatments were 0, 5, 10, 20, 30, and 35 psu as measured by a conductivity meter. For each group of five families, new test water was stored in bulk at 10 °C until 200 mL was transferred to 180 prelabeled 250 mL glass beakers on a given setup day. Small groups of fertilized eggs were removed from the family stock container with a disposable pipette and placed in a 50 mL beaker containing test salinity water. These eggs were counted and then transferred to the incubation beaker, where final counts of 50 were confirmed three times. This process was repeated until each family was transferred to 36 beakers (6 salinities × 3 temperatures × 2 replicates). Eggs were incubated in the dark (following Jeffers 1931) using plant growth chambers to control temperature, with the location of each family and replicate beakers A or B randomized. All salinities for a given family and replicate (six beakers) were kept together. Every second day, 50% of the water in each beaker was decanted off and replaced with new water of the same salinity and temperature. After initial gamete mixing, 100–200 surplus eggs were placed in a petri dish of test salinity and incubated at 5 °C overnight. These were examined the next day for fertilization success. All eggs at 0 psu were dead at this time, as were all eggs from family 10; thus, 270 beakers of embryos were monitored for hatch characteristics.

Beakers were checked daily for hatched larvae, which were immediately removed and recorded by beaker. The first 10 larvae to hatch from each beaker (if 10 hatched) were preserved in 2.2% buffered formalin for later determination of hatch size (standard length measured from digital pictures taken under a dissecting microscope). Subsequently, if at least five more larvae hatched together on a given day, including the day the preserved larvae hatched, they were removed for starvation experiments. Two groups of five were taken from each beaker if available, either on the same day or on subsequent days. These five larvae were gently collected with a wide-mouth disposable pipette and placed in 50 mL glass vials that had been filled with water of test salinity and temperature. The number of days required for all five larvae to die was recorded as starvation time. For a given incubation beaker, once hatching started, if there were no new hatched larvae for 3 consecutive days, it was assumed no more would hatch (confirmed in preliminary experiments) and the batch was discarded.

Table 1. Summary of 2011 hatch characteristics (mean hatch success, mean hatch time, mean larval hatch size, and mean starvation time) across nine capelin (*Mallotus villosus*) families.

	Hatch success (%)				Hatch time (days)				Hatch size (mm)				Starvation time (days)			
Salinity (psu)	5 °C	10 °C	15 °C	Mean	5 °C	10 °C	15 °C	Mean	5 °C	10 °C	15 °C	Mean	5 °C	10 °C	15 °C	Mean
0	0	0	0	0												
5	49.3 (11.1)	77.3 (8.7)	63.6 (7.2)	63.4	31.8 (0.3)	16.6 (0.3)	11.6 (0.2)	20.0	5.4 (0.1)	5.4 (0.1)	5.7 (0.1)	5.5	20.6 (0.6)	13.3 (0.9)	8.6 (0.6)	13.6
10	54.2 (10.8)	83.0 (8.7)	65.4 (7.9)	67.6	32.0 (0.4)	16.6 (0.3)	11.4 (0.1)	20.0	5.5 (0.1)	5.3 (0.1)	5.5 (0.1)	5.4	20.7 (0.4)	14.5 (0.4)	8.5 (0.5)	14.3
20	54.2 (11.5)	78.7 (8.8)	62.9 (8.1)	65.3	31.7 (0.4)	17.2 (0.6)	11.8 (0.2)	20.2	5.3 (0.1)	5.3 (0.1)	5.4 (0.1)	5.3	19.5 (0.7)	11.8 (0.3)	7.5 (0.6)	12.7
30	24.1 (6.1)	17.2 (4.8)	32.4 (7.0)	24.6	30.9 (1.3)	18.7 (0.5)	12.2 (0.2)	20.5	5.0 (0.1)	5.2 (0.1)	5.0 (0.1)	5.0	18.0	9.3 (0.4)	6.2 (0.7)	9.2
35	3.6 (1.3)	11.2 (6.4)	4.6 (2.0)	6.4	35.8 (0.5)	20.1 (0.5)	14.0 (0.7)	22.3	5.1 (0.1)	4.9 (0.1)	5.4 (0.1)	5.0	7.0	6.0		6.5
Mean	37.1	53.5	45.8	45.5	32.2	17.8	12.2	20.5	5.3	5.2	5.4	5.3	19.5	12.6	8.0	13.0

Note: Standard errors among families are given in parentheses (unless data not available from enough families (Fig. 1)). Results of 0 psu are not included in the temperature means for hatch success. Data are not available for some treatment–family combinations and thus the grand means are not equal to the means of the subcomponents. Predicted time to hatch and observed degree-days to hatch follow each temperature. Days to hatch as predicted by Frank and Leggett (1981) are at 5 °C = 23.6, 10 °C = 16.1, and 15 °C = 12.7. Hatch time in degree-days (thermal summed units) from 5 to 35 psu (and for only 5–30 psu): 5 °C = 161 (158), 10 °C = 178 (172), and 15 °C = 183 (176).

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2012 experiment: fine-tuned upper salinity

Spawning capelin were collected on 11 July with a cast net from Bellevue Beach (47°38′12.27″N, 53°46′42.50″W) on the Avalon Peninsula of Newfoundland. These were held overnight at 8 °C and used to make five full-sib families on 12 July. Another group of fish were collected from Middle Cove Beach on 18 July and used to create families 6–10 on 19 July. Fish from these two beaches are thought to be from the same population. Body and egg sizes of experimental parents are given in Table A1.

All handling, fertilization, incubation, and data collection was done in the same way as in 2011. In 2012, all incubations were conducted at 10 °C. There were eight salinity treatments (20, 22, 24, 26, 28, 30, 20/30, and 30/20 psu). The variable salinity treatments started at 20 or 30 psu (day 0) and then were switched to the other on day 9 (in 2011, larvae took 17–18 days to hatch at 10 °C). One hundred and sixty groups of embryos were followed for hatch characteristics (10 families × 8 salinities × 2 replicates).

2013 experiment: fine-tuned lower salinity and upper temperature

Spawning fish were collected from Middle Cove Beach on the morning of 8 July. Fertilization and experimental treatment allocation was conducted on the same day. Five full-sib families were used. Fish sizes are presented in Table A1. Fertilization, incubation, and data monitoring were identical to 2011 and 2012; fertilization success, larval size, or starvation was not determined. Two mini-experiments were conducted. The first tested hatch characteristics in 20 psu water at three temperatures (15, 18, and 21 °C), thus giving 5 families × 3 temperatures × 2 replicates = 30 groups. The second tested five salinities (1, 2, 3, 4, and 5 psu) at 10 °C and thus 50 groups.

Statistical analyses

Hatch success data from each year were analyzed separately, as treatments varied across years. Odds ratios of events (number hatched) and trials (50 eggs per beaker) were analyzed in a generalized linear model with binomial error distribution using Minitab 16. Temperature and salinity were fixed effects categorical variables. In 2011, the model included temperature, salinity, and their interaction along with the random effect of family. Subsequent years had simplification of this design. In 2012, all salinities were tested at the same temperature. In 2013, the two experiments were treated separately (a low-salinity analysis and a high-temperature analysis). In both cases, family remained a random effect.

Model parameters were the same for hatch and starvation timing and larval size, which were analyzed using normally distributed errors in a general linear model. A weighted average was created for hatch timing for each beaker by multiplying the quantity hatched on any given day by the number of days they took to hatch and taking the sum across hatching days and dividing by the total number of eggs that hatched. One value was generated per family at each treatment by averaging the replicate beakers. Starvation time was only determined in 2011; number of days to starvation of all five larvae per vial was averaged between vials (if available) and then between replicate beakers. Both hatch and starvation timing have a resolution of a whole day, which limits detection of small differences among treatments if for example most hatch in less than a 24 h period, starting shortly after they were checked on given day. Degrees of freedom vary among tests, as varying numbers of hatched larvae were produced per family/temperature/salinity combination.

Results

2011 experiment

After 24 h of incubation, cell cleavage indicated good fertilization followed by development at all salinities (data not shown) except deionized water, where eggs had turned opaque. There was thus no hatching at 0 psu (Table 1). The logistic regression for hatch success indicated a significant interaction between salinity and temperature (chi square = 291.00, df = 10, $p < 0.001$), and the model was rerun at each temperature. Hatch success salinity patterns and significance were similar at all temperatures but the interaction was driven by varying magnitudes (Fig. 1A). Across all temperatures, using mean hatch at 35 psu as reference, the odds of hatching were 10 times (95% CI = 7–14) higher at 30 psu, 50 times (35–71) higher at 20 psu, 59 times (41–84) higher at 10 psu, and 52 times (36–74) higher at 5 psu. The experimental technique creates the potential for good hatching performance; for example, excluding families 7 (which had relatively poor hatch success under all conditions (Fig. 1A)) and 10 (which had no hatch), high hatch success occurred at intermediate salinities and temperatures (for eight families, the mean = 92% at 10 °C and 10 psu).

Time to hatch was faster at warmer temperatures but longer at very high salinity (Table 1; Fig. 1B). The interaction between temperature and salinity (T × S) was barely nonsignificant ($F_{[8,104]} = 2.00$, $p = 0.054$). In the simplified model, higher temperatures significantly reduced hatch time ($F_{[2,112]} = 1773.02$, $p < 0.001$), each temperature being different from the other. A significant effect of salinity ($F_{[4,112]} = 16.72$, $p < 0.001$) was caused by 35 psu having longer hatch times than those at 5–30 psu, which were similar. Hatch time (Table 1) was near that predicted by Frank and Leggett (1981) at 10 °C (predicts 16.1 days) and 15 °C (predicts 12.7 days) but was substantially slower at 5 °C (predicts 23.6 days). Embryos took less degree-days (thermal summed units) to hatch at 5 °C than at 10 or 15 °C (Table 1).

Hatch size also varied with salinity (Table 1; Fig. 1C). The T × S interaction was significant ($F_{[8,92]} = 7.69$, $p = 0.001$), and the model

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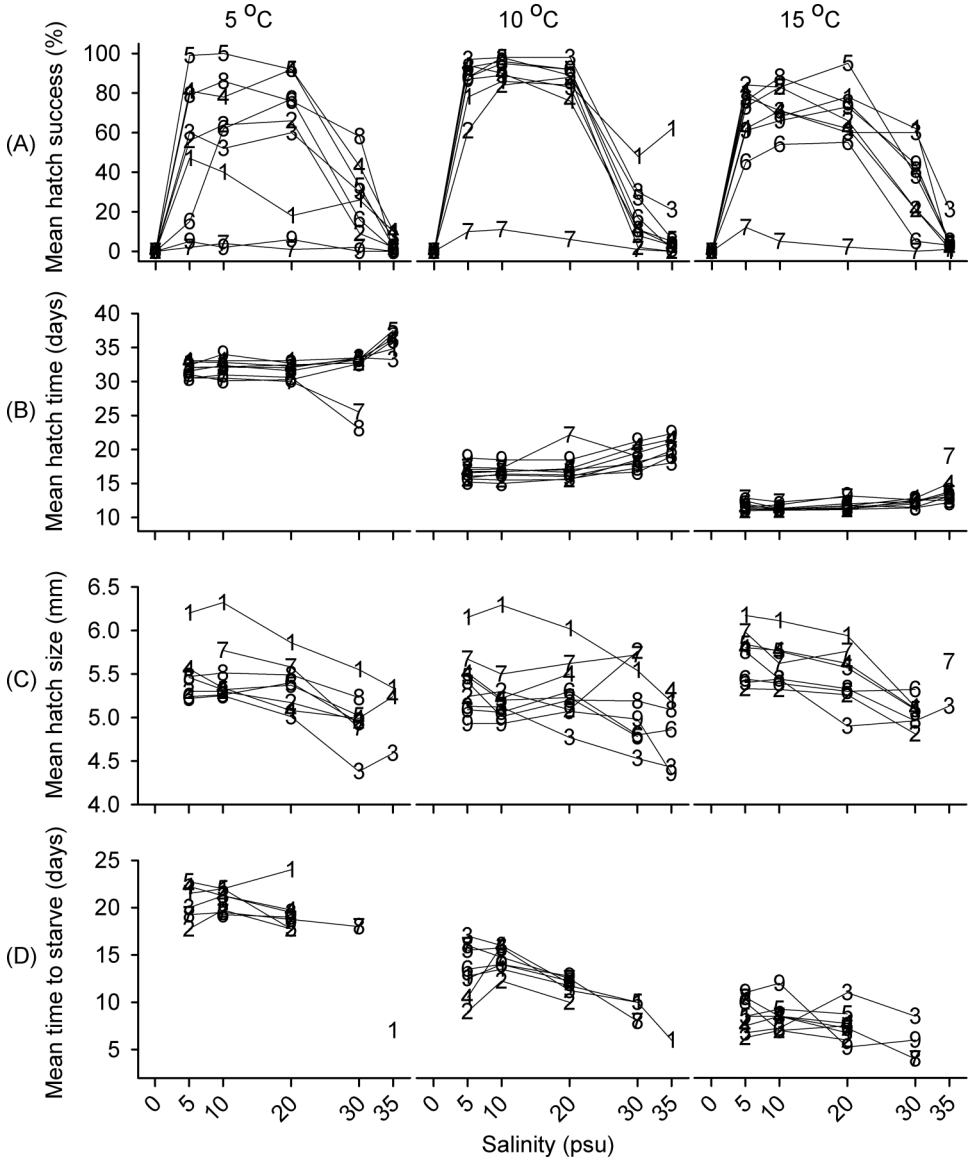
Fig. 1. The 2011 reaction norms of hatch characteristics of nine capelin (*Mallotus villosus*) families by temperature and salinity. Each datum for (A) percent hatch, (B) hatch time, and (C) hatch size is the mean of two incubation replicates. (D) Time to starvation is the mean of two incubation replicates of the means of two groups of larvae. Reaction norm lines do not connect if adjacent salinity data are lacking between data points (i.e., not enough hatched). Summary data are presented in Table 1.



was rerun at each temperature. Salinity was significant in all cases ($p < 0.001$ for 5 and 15 °C and 0.009 at 10 °C). Samples sizes of hatched larvae are small in some salinity/temperature/family combinations, making fine-tuned interpretation difficult. In general, larvae were similar in size at 5, 10, and 20 psu and smaller at 30 and 35 psu (Table 1).

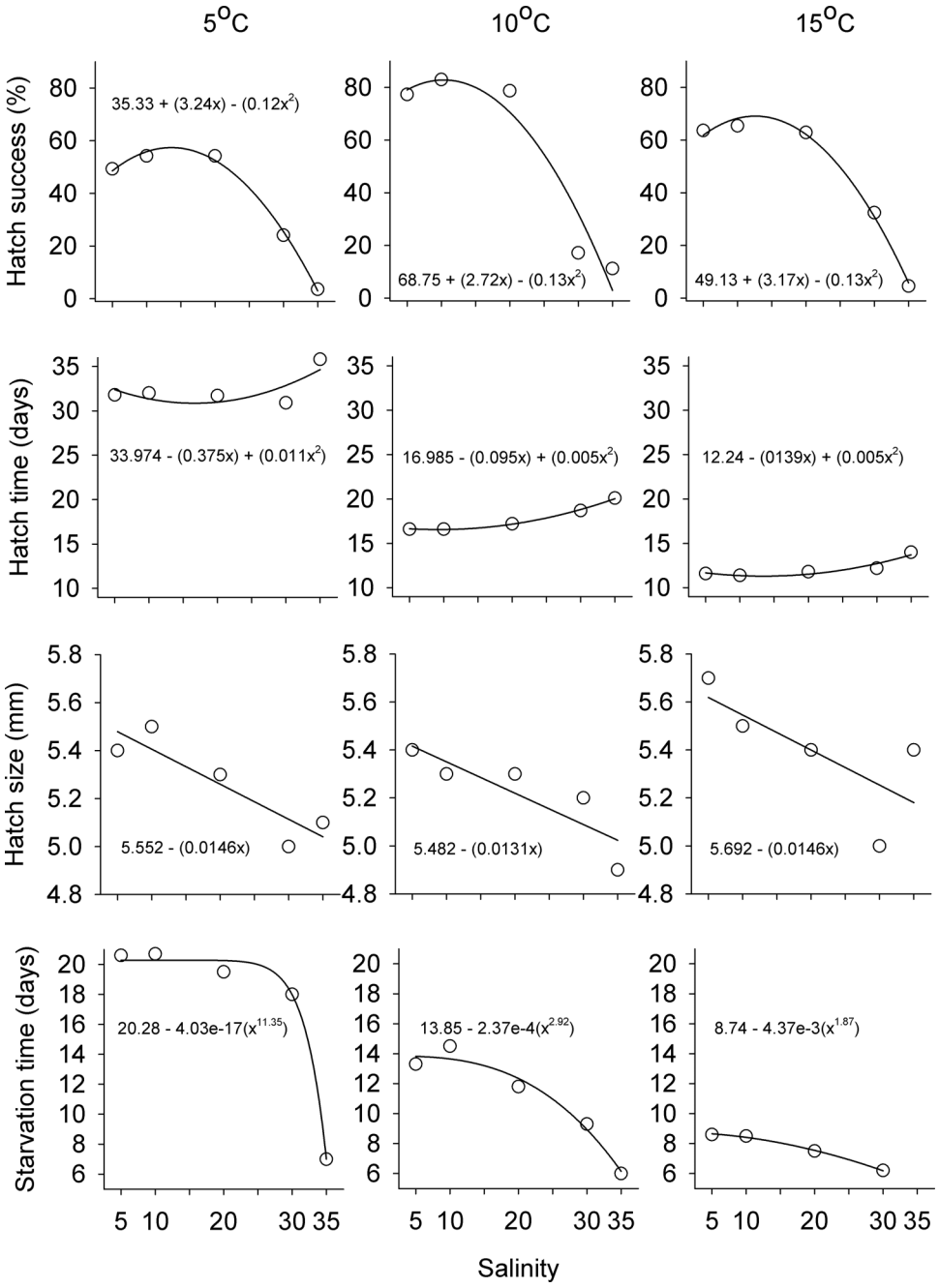
Low temperature or salinity increased starvation time of hatched larvae. Poor hatch success in some treatments meant starvation time could not be determined in all cases. No data are available for family 7, and 35 psu data only exist for a single family (family 1) at only two temperatures (removed from statistical analyses). There was no significant T × S interaction ($F_{[6,56]} = 0.84$, $p = 0.548$), which was removed and the model simplified. As expected, hatched larvae starved significantly faster ($F_{[2,62]} = 328.44$, $p < 0.001$) in warmer water (Table 1; Fig. 1D). Tukey comparisons indicated all three temperatures to be different from each other. Salinity also had a significant effect ($F_{[3,62]} = 12.46$, $p < 0.001$). Starvation time at 30 psu was significantly shorter than at 20 psu, which was shorter than at 10 psu, which was not significantly different from that at 5 psu (Table 1).

Predictive equations for hatch characteristics at 5, 10, and 15 °C as a function of salinity are provided in Fig. 2; these data are the means among nine full-sibling families.

2012 experiment

Salinity had a significant effect on the odds of hatching (chi square = 788.814, df = 7, $p < 0.001$) (Fig. 3). As in 2011, the lowest hatch success occurred at the highest tested salinity (30 psu, reference salinity). The odds of hatching were 6 times (95% CI = 4–7) higher at 28 psu, 8 times (6–10) higher at 26 psu, 10 times (8–12) higher at 24 psu, 9 times (7–12) at 22 psu, and 13 times (10–16) higher at 20 psu. Problems hatching at high salinity are associated with the second half of incubation, as hatch after 1 week at 30 psu followed by 1 week at 20 psu was nearly identical (91.3%) to that at 20 psu for the entire time (91.5%), while 20/30 psu experienced lower (74.8%) hatch success (Fig. 3).

Fig. 2. Predictive equations for beach-spawning capelin (*Mallotus villosus*) hatch characteristics as a function of salinity. Data taken from Table 1 and are the means among families.



There was a minor, but significant effect of salinity on hatch time ($F_{[7,63]}$ = 5.07, p < 0.001), being about half a day longer (15.8 days at 10 °C) at the highest salinity (data not plotted).

2013 experiment

Binary regression indicated that the odds of hatching varied with salinity (chi square = 208.762, df = 4, p < 0.001) (Fig. 4A). The odds were similar between 5 psu (reference salinity) and 2 psu but marginally significantly higher at 3 psu (1.3 times, 95% CI = 1.01–1.79) and significantly lower at 4 psu (0.65 times, 0.49–0.86) and 1 psu (0.18 times, 0.14–0.24). The biologically relevant drop seems to occur between 2 and 1 psu. In the second experiment, the odds varied significantly among temperatures (chi square = 78.596, df = 2, p < 0.001) (Fig. 4B). Nothing hatched at 21 °C and the odds of hatching were 6 times (4–8) higher at 15 than at 18 °C (reference temperature).

There was no difference in hatch time from 1 to 5 psu (mean 17.4 days at 10 °C) in the low-salinity experiment ($F_{[4,16]}$ = 1.49, p = 0.250) or between 15 and 18 °C (mean 10.9 days at 20 psu) in the high-temperature experiment ($F_{[1,3]}$ = 0.11, p = 0.764); none hatched at 21 °C (data not plotted).

Discussion

The rate of early development of Newfoundland beach-spawning capelin increases with temperature as anticipated, and there are no major interactions between temperature and salinity. The first half of embryo development seems to occur normally over a very

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Fig. 3. The 2012 reaction norms of hatch success to salinity for ten capelin (*Mallotus villosus*) families. Each datum is the mean of two incubation replicates. Numbers inserted above the data are the mean (and SE) among families at each treatment. All fertilizations were performed at 30 psu. Embryos were transferred to experimental salinity within 2 h. Treatment 30/20 had embryos remain at 30 psu for 8 days and then were switched to 20 psu for another ~7.5 days until hatch (the reciprocal happened for 20/30).

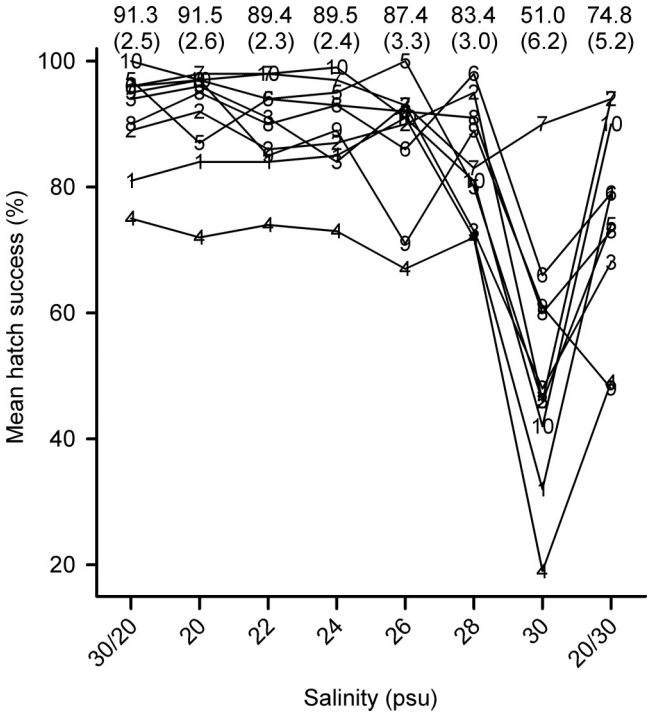
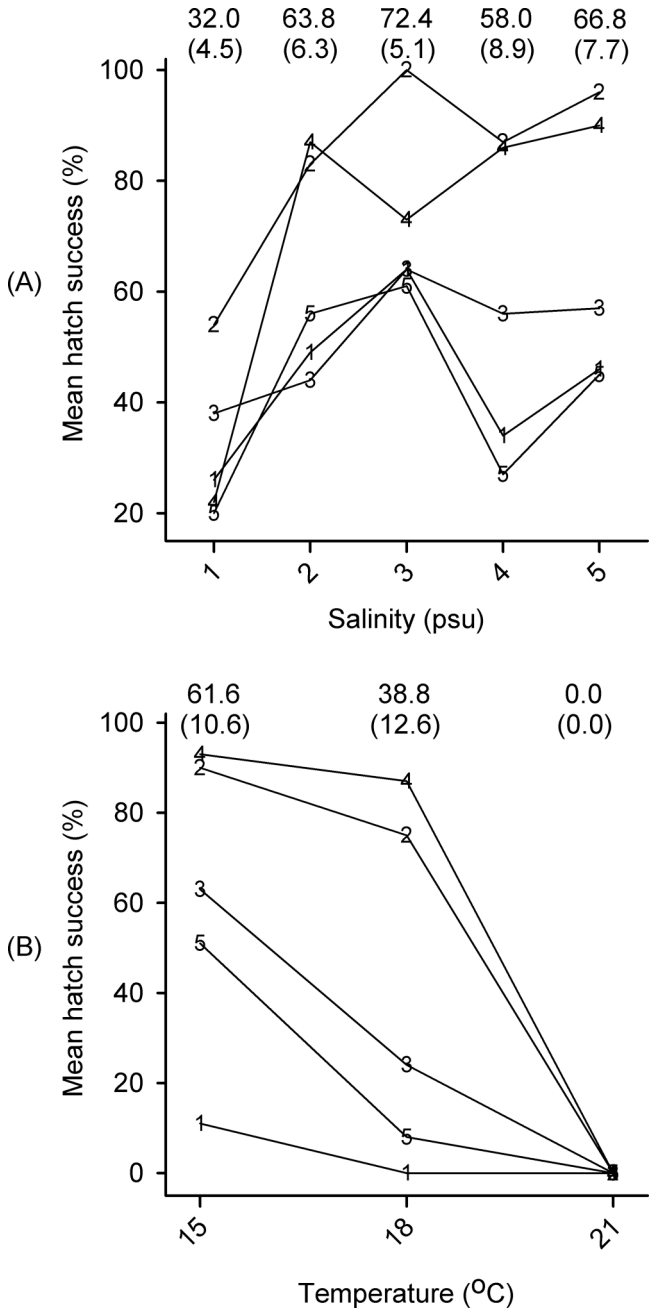


Fig. 4. The 2013 reaction norms of hatch success for five capelin (*Mallotus villosus*) families. Each datum is the mean of two incubation replicates. Numbers inserted above the data are the mean (and SE) among families at each treatment. All fertilizations were performed at 30 psu. (A) five salinities at 10 °C; (B) three temperatures at 20 psu.



wide range of salinities. However, salinity does have substantial and unexpected influences on reproduction. Although no larvae hatched in deionized water, many did at 1 psu, and good hatch success occurred from 2 to 28 psu. Surprisingly, at higher salinities indicative of coastal seawater, there was poor hatch success, larvae took longer to hatch, hatched at a smaller size, and starved more quickly than those exposed to lower salinity. These results are not expected for a marine spawning fish and have both evolutionary and ecological implications.

Combined with previous work (Table 2), the results from my experiments suggest substantial local adaptation to salinity between beach and offshore (deepwater seafloor) spawners from different regions, despite neutral genetic markers not showing much differentiation among capelin stocks (e.g., Dodson et al. 2007; Præbel et al. 2008). However, conclusions drawn from population genetics are dependent on the exact samples collected and what can be gleaned from specific markers and may fail to detect valuable diversity (Hutchings et al. 2007). Importantly, previous capelin salinity studies focused on low salinities, which may underlie why upper limit differences among stocks have not been previously acknowledged. For example, Præbel et al. (2013) rejected the hypothesis that offshore spawners should have lost tolerance of low salinity but did not address whether they evolved tolerance of high salinity.

Demersal offshore spawners from Europe have a wide salinity tolerance and notably no negative effects of high salinities on development. Davenport (1989) showed that offshore spawning Icelandic capelin have good hatch from 1.7 to 34 psu (the highest measured), while Barents Sea capelin experienced hatch success of 85%–95% (depending on temperature) at salinities of 51 psu (Præbel et al. 2013). Comments in two older studies (see footnote *b* in Table 2) on Barents Sea capelin also show tolerance of high salinities (Friðgeirsson 1976; Gjøsaeter and Gjøsaeter 1986). In contrast, Balsfjord (near Tromsø, Norway) capelin beach spawn during the spring melt at 5.2–28 psu, and salinity within the gravel can be 1.8–6.3 psu (Præbel et al. 2009). Although not discussed in detail, Davenport and Stene (1986) showed that these capelin have good hatch success up to 13.6 psu but reductions at intermediate salinities (20.4, 27.2, and 34.0 psu) and no hatch at high salinities (40.8 and 47.6 psu). At 47.6 psu, embryo development was okay for the first half of incubation, matching observations from my experiments. The Balsfjord population also has similar patterns to my work in hatch timing (delayed at higher salinity) and starva-

Table 2. Summary of studies examining effects of salinity on capelin (*Mallotus villosus*) embryo development.

	Barents Sea (offshore)[a]	Iceland (offshore)[b]	Norway (fjord)[c]	Newfoundland (coastal north), 1927 and 1929[d]	Newfoundland (coastal north), 1930[d]	Newfoundland (coastal south), 2011–2013[e]
Location	Seafloor	Seafloor	Beach	Beach	Beach	Beach
Salinities (psu)	3.4, 17.0, 34.0, 51.0	0.0, 1.7, 3.4, 6.8, 13.8, 20.4. 27.2, 34.0	0.0, 3.4, 6.8, 13.6, 20.4, 27.2, 34.0, 40.8, 47.6	0.0, 7.5, 15.0, 22.5, 30.0	0.0, 3.0, 6.0, 9.0, 30.0 or 0.0, 7.5, 15.0, 22.5, 30.0	0.0–35
Notes at 0 psu		All dead by day 4 (5 °C)	Most dead by day 14, all dead by day 34 (5 °C)	Some hatched in coldest conditions (5 °C)	40% hatched at 10 °C	All dead by day 1
Hatch success	Good (>85%): all salinities	Good (79%–95%): 1.7–34.0 psu with no trend	Good (82%–96%): 3.4–13.6 psu Poor (28%–32%): 20.4–34.0 psu No hatch: 40.8–47.6 psu*	Good (>70%): 7.5–30.0 psu at intermediate temperatures	Good (>70%): 3.0–30.0 psu	Good (58%–92% at 10 °C): 2–28 psu Poor (17%–51%): 1, 30 psu Very poor (4%–11%): 35 psu**
Hatch timing	Delayed at higher salinities (1.4 °C), no difference (5.5 °C)	Same at 1.7 and 34 psu, later at intermediate salinities	No difference in artificial crosses but hatch delayed at higher salinities in "wild" eggs	Delayed at 30.0 psu at intermediate temperatures	No clear trend	Delayed at higher salinities
Larvae			Starvation faster above 27.2 psu	Hatched smaller at 30.0 psu	Hatched smaller at 30.0 psu	Hatched smaller at higher salinities Starvation faster at higher salinities

[a]Præbel et al. (2013) (results taken from the text; 1.4 and 5.5 °C). Additionally, Friðgeirsson (1976) commented that some eggs in 1974 from the same stock were kept at 29.5 and 35 psu and showed similar development (no data are given). Gjøsæter and Gjøsæter (1986) mixed gametes and then placed them in 0, 10, 15, 20, or 34 psu water. After 10 days, all were dead in 0 psu (some fertilized) and 10 psu (most fertilized); mortality was higher at reduced salinity and development delayed compared to 34 psu (no data are given).

[b]Davenport (1989) (results taken from Table 1; 5 °C). Experiment used early season spawners, which reproduce south of Iceland and always in deep water. Later spawners are to the north and sometimes intertidal.

[c]Davenport and Stene (1986) (results taken from Tables 8–10; 5 °C). Fish remain in the fjord their entire life and spawn during spring melt with significant fresh water influence. Single asterisk indicates no hatch >34 psu but embryos survived half way through development.

[d]Jeffers (1931) (results taken from the text and Tables II and III). Newly fertilized eggs were collected from a beach in Raleigh in northern Newfoundland and subjected to a series of temperatures and salinities. No "high" salinities were used.

[e]This study (several experiments with varying salinity/temperature combinations). Double asterisk indicates problems in the second half of the incubation period.

tion timing (faster at higher salinities). The beach-spawning Balsfjord capelin and those from southern Newfoundland examined in this study thus have remarkably similar responses to salinity. A third group, from northern Newfoundland, was examined in the 1920s (Table 2) and also displayed delayed hatching and smaller size at hatch at "high" salinities (Jeffers 1931). Hence, there are three studies on beach spawners (two with experiments over multiple years) and four studies on offshore spawners showing consistently different sensitivity to salinity.

The data endorse the hypothesis that anadromy led to marine beach spawning in capelin and two other osmerids (Martin and Swiderski 2001), which fits with a freshwater (McDowall 1997) but not marine (Dodson et al. 2009) ancestor for the Osmeridae. Tolerance for higher salinity by embryos of only offshore-spawning capelin supports the notion that beach spawning is the ancestral capelin spawning condition (Stergiou 1989; Vilhjálmsson 1994; Martin 2015). I thus hypothesize that the large offshore seafloor-spawning capelin stocks are at least partially enabled due to an adaptation for higher salinity tolerance by embryos. Moreover, reproduction of beach-spawning capelin is also constrained by sperm performance. Newfoundland beach-spawning capelin have unique sperm that may be an adaptation for a freshwater taxon to spawn in the sea (Beirão et al. 2018). Unlike other marine spawners, beach-spawning capelin sperm are quickly immobilized by seawater. As a consequence, as in embryos (Table 2), Beirão et al. (2018) predicted that sperm of offshore spawners should be more tolerant of high salinity than those that spawn on beaches. That prediction has not been tested.

There are substantial ecological implications from these findings for the Newfoundland beach-spawning capelin stock. Historically, Newfoundland capelin spawned in mid-June, but since 1991, spawning has been delayed about a month (DFO 2015). Weather patterns in coastal Newfoundland are dramatically different in the average June versus July. June is typically colder and much wetter with frequent drizzle and fog generated by northeasterly onshore winds that also push relatively low-salinity surface waters onto beaches. July is much warmer and drier, being dominated by southwest winds that push surface waters offshore, causing upwelling of relatively high-salinity water at beaches. Reduced rainfall also creates less runoff from streams that occur in nearly all capelin spawning beaches. Delayed spawning thus places developing embryos into higher salinity environments in most years, which based on these experiments would lead to reduced hatch success and size and result in shorter times to starvation, a problem that would be amplified by higher late-summer temperatures. Furthermore, later larval emergence would shorten the amount of time fish would have to attain any critical sizes needed for overwinter survival. In addition, delayed spawning resulting in higher temperatures may lead to increased use of inshore "off-beach" spawning sites (Davoren 2013). Such areas are likely to have higher salinity and thus would be predicted to have lower reproductive success than beaches.

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To more thoroughly understand the effect of salinity on capelin reproduction, further directions should include an examination of tidal influence on the development of beach-spawning capelin embryos at high salinity, effects of salinity on growth rates of larvae, and whether inshore/coastal seafloor-spawning capelin in Newfoundland (Penton and Davoren 2012) are more similar to European offshore spawners or European/Newfoundland beach spawners in their sensitivity to coastal seawater.

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Appendix A

Appendix Table A1 appears on the following page.

Table A1. Total length of each parent used in experimental families and mean dry mass of eggs (not measured in 2013).

Year	Family	Male size (mm)	Female size (mm)	Mean dry mass of 30 eggs (g)
2011	1	161	149	0.0023
	2	150	140	0.0018
	3	165	137	0.0017
	4	162	142	0.0013
	5	155	140	0.0019
	6	152	158	0.0022
	7	170	133	0.0020
	8	172	144	0.0022
	9	160	142	0.0015
	10	162	150	0.0015
2012	1	164	130	0.0026
	2	163	158	0.0024
	3	160	130	0.0022
	4	185	160	0.0030
	5	175	140	0.0025
	6	184	142	0.0020
	7	184	144	0.0017
	8	158	126	0.0017
	9	191	133	0.0014
	10	159	150	0.0021
2013	1	164	157	
	2	184	135	
	3	180	163	
	4	185	145	
	5	182	169	

Note: Masses are for 30 eggs and are the mean of three replicates (90 eggs) from each female.

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