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# Ocean acidification exacerbates the impacts of global warming on embryonic little skate, *Leucoraja erinacea* (Mitchill)

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ABSTRACT

Ocean acidification and warming have the potential to profoundly impact marine fishes by reducing embryo fitness and survival. Local adaptation to thermal gradients may reduce the impact of global warming, but whether fish from different populations may respond differently to climatic stressors remains unknown. The hypothesis that acidification and warming may have an effect on development, aerobic scope, and survival was tested in little skate (*Leucoraja erinacea*) embryos from two latitudinally separated populations. Temperature had the strongest effect on development, survival and metabolic rates, but acidification further exacerbated stress on embryos from the Gulf of Maine population by increasing the costs of activity, development time, and reducing body condition of newly hatched skates. Active metabolic rates of both populations exhibited countergradient variation with peak of performance at 18 °C, but were affected differently by acidification. These findings demonstrate that even adjacent fish populations may respond differently to increasing temperature and acidification and emphasize the need for multi-stressor studies on different populations of fishes with wide geographic range to understand complex responses to climate change and other environmental challenges.



## 1. Introduction

Since the industrial revolution, atmospheric concentrations of carbon dioxide ($pCO_2$) have risen by 41% to approximately 400 ppm, thus exceeding levels experienced over the past 65 Ma (IPCC, 2013). In addition to accelerating atmospheric and oceanic warming, about 30% of $CO_2$ introduced in the atmosphere enters the oceans causing a decrease in pH, a phenomenon known as ocean acidification (Baumann et al., 2011; Raven et al., 2005; Rosa et al., 2014). Current climate models project that atmospheric $pCO_2$ will reach about 1100 ppm by year 2100, causing an increase in temperature of about 3–5 °C (IPCC, 2013; Meinshausen et al., 2011). As warming and acidification may act synergistically to decrease fitness of fishes, researchers are investigating their combined effect on physiological processes (Rosa et al., 2014; Todgham and Stillman, 2013). Fishes are particularly vulnerable to warming as nearly every metabolic function depends on temperature (Di Santo and Bennett, 2011a,b; Fry, 1971; Gillooly et al., 2001), and shifts in migration and reproductive timing as well as in geographic ranges have already been widely observed (Greenstein and Pandolfi, 2008; Gregory et al., 2009; Perry et al., 2005). Furthermore, acidification has the potential to exacerbate the effect of warming by increasing osmoregulatory costs to buffer body fluid acidosis (Claiborne et al., 2002). However, there is little direct evidence that the $CO_2$ levels projected by the end of the century will significantly affect adult fishes because of their efficient acid–base capacities (Ishimatsu et al., 2004; Rummer et al., 2013). Recent studies on the effect of increased acidification on fishes report a range of effects (or no effect), thus underscoring the necessity for studies on different groups (Kroeker et al., 2010). For instance, recent data suggest that when reared at low pH, teleosts exhibit a reduction in survival and tissue function (Baumann et al., 2011; Chambers et al., 2013), impaired olfactory abilities (Munday et al., 2008), and abnormal otolith growth (Bignami et al., 2013a,b; Checkley et al., 2009) suggesting that early life stages (i.e., embryos and juveniles) may be particularly vulnerable to increased $pCO_2$. On the other hand a few studies on decreased ocean pH show no or even a positive effect on fishes (Bignami et al., 2013a,b; Kim et al., 2013; Kroeker et al., 2013; Munday et al., 2011; Rummer et al., 2013). As warming and acidification are likely to be experienced simultaneously by organisms and may trigger complex responses in fishes, understanding their combined effect on metabolic functions has the potential to transform our prediction of future responses to climate change.

Local adaptation to thermal gradients could have important consequences in climate change scenarios as warm-adapted individuals may survive rapid increase in temperature and replace cold-adapted conspecifics (Angilletta et al., 2004). Previous studies have already documented the different responses of fishes to environmental change (Baumann and Conover, 2011; Fangue et al., 2006, 2009; Schulte et al., 2000; Schultz et al., 2002). Such data may provide some evidence that stress responses and physiological performance following climate change may depend on local adaptations in fish species that have a

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relatively wide geographic range, a phenomenon already documented in marine invertebrates (Dong and Somero, 2009; Sorte et al., 2011). To answer this crucial question as to whether fish from latitudinally separated populations respond differently to environmental stressors, it is necessary to conduct "common garden" experiments where physiological responses to abiotic changes are measured in individuals from different populations that are reared at the same conditions (Angilletta, 2001; Baumann and Conover, 2011; Fangue et al., 2006; Munch and Conover, 2002).

Here, little skate *Leucoraja erinacea* (Mitchill, 1825) embryos from two latitudinally separated populations were reared at current and projected pH and temperatures according to the model RCP 8.5 (Meehl et al., 2007; Meinshausen et al., 2008) in a fully-crossed experimental design to quantify the combined and single effects of acidification and warming on key morphological and physiological traits. *L. erinacea* is an oviparous elasmobranch that is found along transitional regions of the northwestern Atlantic, such as the Gulf of Maine (GoM) and Georges Bank (GB), where impacts of sharp thermal discontinuities are evident (Frisk, 2002). *L. erinacea* only shows weak seasonal distribution patterns that consist of short distance movements from coastal and shallow waters to offshore and deeper waters during colder months (McEachran, 2002). Perhaps as a consequence of its strong site fidelity, this species exhibits a latitudinal gradient in growth and body size. Indeed, although laboratory-controlled studies have not yet confirmed observations made in wild specimens, a few studies have documented regional variations in life history patterns in the little skate including increasing body size with latitude (Bigelow et al., 1953; Frisk, 2002; Frisk and Miller, 2006, 2009; McEachran, 2002). This relationship between body size and latitudinal gradients suggests a potential metabolic adaptation to the local environment.

A critical stage in the life of oviparous elasmobranchs is the relatively long development time (between about five months and one year for this species) (Luer and Gilbert, 1985) because embryos are unable to utilize or avoid variations in the environment by undertaking thermotaxic behavior (Di Santo and Bennett, 2011a). Although increasing temperature is likely to reduce survival and aerobic performance in elasmobranch embryos (Luer and Gilbert, 1985; Palm et al., 2011), there is as yet no evidence that the low pH conditions expected by the end of the century will affect embryonic skate metabolism. By investigating separated populations from two geographic locations (GoM, GB), it is possible to test whether different populations may respond similarly or not to climate change related stressors. Comparisons between treatments will allow us to determine individual and combined effects of acidification and warming on different physiological processes that are linked to fitness and survival. Specifically, in this study the effect of simulated ocean warming and acidification was tested on: i) embryonic development and survival, ii) embryonic metabolic performance, and iii) hatchling body condition and initiation of feeding.

## 2. Material and methods

### 2.1. Egg incubation and experimental system

Newly laid (about 1 week old) little skate eggs were obtained from wild caught females at two distinct locations, the Gulf of Maine (43°N, 68°W) and Georges Bank (41.21°N, 67.38°W), USA (northern and southern populations, respectively), and transported to Boston University in temperature-controlled containers. Once in the environmental chamber, embryos were randomly assigned to a treatment group (3–5 per replicate tank; 5 replicate tanks per treatment) and reared in common garden conditions. A fully-crossed experimental design was employed to match current (15 °C) and projected temperature increases (+3, +5 °C) as well as current and decreased pH (8.1, 7.7) as suggested by the *Guide to best practices for ocean acidification research and data reporting* (Riebesell et al., 2010) according to high emission scenarios by year 2100 model RCP 8.5 (n = 5 replicate tanks per treatment; Table 1) (IPCC, 2013; Meinshausen et al., 2011). Each tank (150 L) had independent temperature and $CO_2$ control. Embryos were held in a temperature-controlled environmental chamber (Harris Environmental Systems, Inc., Andover, MA, USA) set at 12 °C and each experimental tank was maintained at constant temperature (either 15, 18 or 20 °C) by a submersible titanium heater unit (Finnex 300 W) controlled by a digital thermostat (Aqua Logic Inc., San Diego, CA, USA). In addition, each tank was provided with a mix of air:$CO_2$ (water pH = 7.7; $pCO_2$ ~1100 ppm) or present-day ambient air (water pH = 8.1; $pCO_2$ ~400 ppm) controlled by an Aqua Medic pH computer (Aqua Medic of North America, Loveland, CO). Temperature and $pH_{NBS}$ (National Bureau of Standards) were maintained to simulate the ocean temperature and $CO_2$ levels projected for 2100 under RCP 8.5 (Meinshausen et al., 2011) and controlled twice a day. Total alkalinity was estimated using titration and certified reference materials (Dickson, Scripps Institute of Oceanography). Water parameters were calculated in CO2SYS (Pierrot et al., 2006) using suggested constants (Dickson and Millero, 1987) (Table 1). Embryos were reared at constant salinity of 33 ppt and photoperiod (14L:10D) and after hatching were fed frozen mysis shrimp daily ad libitum.

### 2.2. Development, survival and body condition

Yolk area was initially measured in a subsample of 1 week old embryos from both populations (n = 10 each). Each embryo was monitored daily under a light source to detect mortality. Survival was measured again 30 days after hatching. Within 24 h of hatching, skates were weighed and measured to determine body condition as mass (g) × disc area$^{-1}$ (cm$^2$). Skates were offered thawed mysis shrimp every day after hatching, and food was removed if uneaten.

### 2.3. Metabolic performance curves

Skate embryos possess a long whip-like appendage on the tail which is inserted into a horn of the egg case where it is rapidly oscillated (Leonard et al., 1999). This activity can increase oxygen consumption by 81% at 15 °C from resting state (Leonard et al., 1999). Therefore, as classic swimming performance tests to determine aerobic costs are not feasible in embryos, the approach in this study was to quantify oxygen consumption of embryos moving in the egg case, or active metabolic rate (AMR) and compare it to standard metabolic rate (SMR). To achieve this goal, individual embryos were placed in a custom-made 1 cm-thick acrylic intermittent-closed respirometer (0.465 L) fitted with a YSI ProODO oxygen meter. In both experiment series, embryonic metabolic rates were measured every 30 min for 2 h after a 1 hour adjustment to experimental conditions (Leonard et al., 1999); oxygen saturation never fell under 80% (Di Santo and Bennett, 2011b; Steffensen, 1989). To measure SMR, embryos were anesthetized using tricaine methanesulfonate (MS-222) buffered with $NaHCO_3$ and NaOH to stop voluntary tail beating while retaining gill movement (Benetti et al., 1995; Leonard et al., 1999). Benetti et al. (1995) showed that MS-222 had no significant effect on fish RMR. Only near-hatch embryos (with yolk diameter ~1 mm) were used to determine metabolic rates (Leonard et al., 1999). Metabolic rates ($MO_2$) were calculated following the formula: $MO_2 = (O_{2\ start} = O_{2\ end}) \times \text{volume} \times \text{time}^{-1} \times \text{mass}^{-0.67}$; where $O_{2\ start}$ and $O_{2\ end}$ are oxygen concentrations at the start and the end (mg L$^{-1}$), volume represents the total volume of the respirometer (L), time is expressed in hours and mass is expressed in g. The mass exponent of 0.67 was used to correct for the allometric relationships between metabolic rates and mass in elasmobranchs (Di Santo and Bennett, 2011a,b; Meloni et al., 2002). Performance curves were constructed by fitting a binomial curve to metabolic data (Baumann and Conover, 2011).

**Table 1**
Mean temperature, pH, carbonate chemistry, total alkalinity (TA), and salinity (±SD) of experimental tanks during common garden experiments.

| Parameter | Treatment 1 n = 5 | Treatment 2 n = 5 | Treatment 3 n = 5 | Treatment 4 n = 5 | Treatment 5 n = 5 | Treatment 6 n = 5 |
|---|---|---|---|---|---|---|
| Temperature (°C) | 15 ± 0.5 | 15 ± 0.5 | 18 ± 0.5 | 18 ± 0.5 | 20 ± 0.5 | 20 ± 0.5 |
| $pH_{NBS}$ | 8.1 ± 0.05 | 7.7 ± 0.05 | 8.1 ± 0.05 | 7.7 ± 0.05 | 8.1 ± 0.05 | 7.7 ± 0.05 |
| $pCO_2$ (ppm) | 422.07 ± 16.57 | 1115.62 ± 59.33 | 414.68 ± 5.22 | 1075 ± 67.10 | 423.94 ± 16.58 | 1078.21 ± 53.06 |
| $\Omega_{Ca}$ | 2.91 ± 0.32 | 1.37 ± 0.17 | 3.38 ± 0.61 | 1.22 ± 0.23 | 2.64 ± 0.31 | 1.28 ± 0.11 |
| $\Omega_{Ar}$ | 1.86 ± 0.20 | 0.88 ± 0.11 | 2.17 ± 0.39 | 0.79 ± 0.15 | 1.71 ± 0.20 | 0.82 ± 0.07 |
| $CO_3$ (μmol/kg) | 114.47 ± 12.97 | 52.0 ± 6.82 | 132.05 ± 24.54 | 45.23 ± 9.22 | 101.71 ± 12.45 | 46.69 ± 4.53 |
| TA (μmol/kg) | 2037.37 ± 156.01 | 2077.78 ± 172.57 | 2069.52 ± 216.74 | 1795.49 ± 190.53 | 1755.08 ± 119.81 | 1767.39 ± 102.56 |
| Salinity (ppt) | 33 | 33 | 33 | 33 | 33 | 33 |

### 2.4. Statistical analysis

The effects of temperature and pH on morphological and physiological responses were explored by analysis of variance (ANOVA) using fish population, temperature, and pH as factors, followed by Tukey–Kramer HSD to test differences between group means. Percentage data (% survival) were subjected to arcsine square root transformation prior to analysis. Statistical significance was determined based on $\alpha = 0.05$. Data are shown as mean ± standard error. Statistical analyses were run in JMP Pro (version 11).

## 3. Results

### 3.1. Development, survival and body condition

The 3-WAY ANOVA revealed that acidification, temperature and origin of population had a significant, but complex effect on embryonic development ($F_{7,39} = 10.09$, $p < 0.0001$) and hatchling body condition ($F_{7,106} = 7.87$, $p < 0.0001$). Significant interactions were detected between population and acidification ($p < 0.0001$), and temperature and acidification in the GoM population ($p = 0.04$) for embryonic development. At current oceanic pH (8.1), GoM embryos developed faster than GB embryos at all temperatures, showing countergradient variation between northern and southern populations, with thermal optima at 18 °C ($F_{7,39} = 10.09$, $p = 0.001$; Fig. 1). Additionally, low pH had a significant effect only in the GoM embryos by increasing development time, and thus reducing performance, across temperatures ($p = 0.03$; Fig. 1). Low pH did not significantly decrease hatching success in either population (2-WAY ANOVA, $p = 0.6$; Fig. 2A).

Even accounting for the ~20% mortality that occurred in the control treatment (15 °C, pH 8.1), embryonic survival declined at the highest temperature (20 °C) in both populations (3-WAY ANOVA, $F_{7,49} = 1.12$, temperature: $p = 0.01$), suggesting that this temperature may approximate the thermal pejus for performance and survival (Fig. 2A). Likewise, post-hatch survival decreased at 20 °C regardless of pH in the GB population (2-WAY ANOVA, $F_{3,26} = 5.76$, $p = 0.0004$) while survival was not significantly affected by either stressors in the GoM population (2-WAY ANOVA, $F_{3,21} = 0.59$, $p = 0.6$; Fig. 2B). Although initial yolk area of newly-laid embryos did not differ significantly between populations (GoM: 26.08 ± 0.41 $cm^2$, GB: 25.96 ± 0.43 $cm^2$; 1-WAY ANOVA, $F_{1,18} = 0.04$, $p = 0.8$), hatchlings from the GoM population had higher weight and larger disc size ($F_{7,106} = 45.05$, $p < 0.0001$), than GB population, regardless of treatment ($p > 0.05$; Table 2). However, body condition of skates was reduced by 5 °C warming ($F_{1,106} = 17.18$, $p < 0.0001$) and acidification ($F_{1,106} = 14.6$, $p = 0.0002$) in both populations. Body condition indices correlated with the latency of hatchlings initiating feeding in both populations ($F_{1,106} = 8.46$, $p < 0.0001$, Fig. 3).

### 3.2. Metabolic performance curves

The 3-WAY ANOVA revealed that acidification, temperature and origin of population had a significant effect on aerobic performance ($F_{7,51} = 3.01$, $p = 0.01$). However no significant interactions between temperature, acidification and population were detected for aerobic performance. Active metabolic rates peaked at 18 °C, again showing countergradient variation between GoM and GB populations (Fig. 4A). Overall, there was a significant effect of treatments on AMR (3-WAY ANOVA, $F_{7,58} = 7.63$, $p < 0.0001$) with temperature and population having the highest impact ($p < 0.0001$, $p = 0.005$, respectively). Active metabolic rates were significantly affected by temperature ($p < 0.0001$) and pH ($p = 0.01$) in GB embryos (2-WAY ANOVA, $F_{3,26} = 21.62$, $p < 0.0001$), but only significantly affected by temperature ($p = 0.0008$) in GoM embryos (2-WAY ANOVA, $F_{3,25} = 5.30$, $p < 0.0001$; Fig. 4A). Low pH significantly increased AMR at 20 °C in the GM population when compared to high pH (2-WAY ANOVA, $F_{1,8} = 31.93$, $p = 0.0005$). Low pH significantly increased SMR at 15 °C in GB

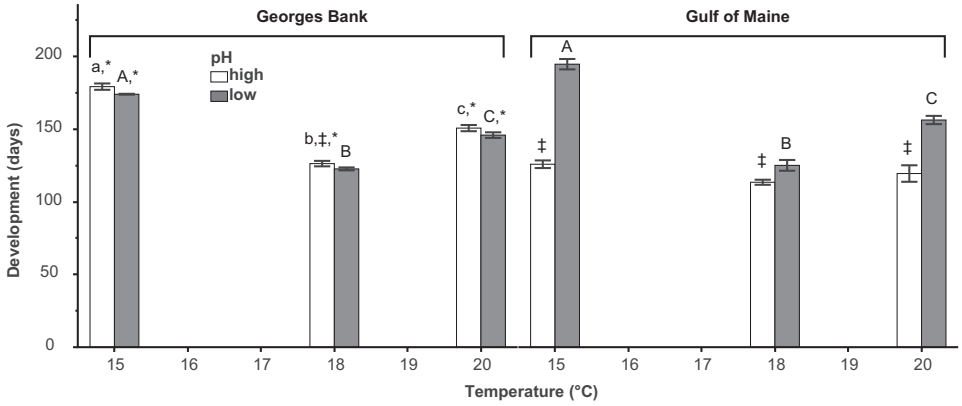


**Fig. 1.** Developmental time (mean ± s.e.m.) of *Leucoraja erinacea* embryos from two populations (Georges Bank n = 24, Gulf of Maine n = 23), at three temperatures and two pH conditions. Different lower and upper case letters represent significant differences within high (8.1) and low (7.7) pH conditions, respectively; double daggers represent significant differences between pH treatments at each temperature; asterisks represent significant differences between populations ($p < 0.05$).

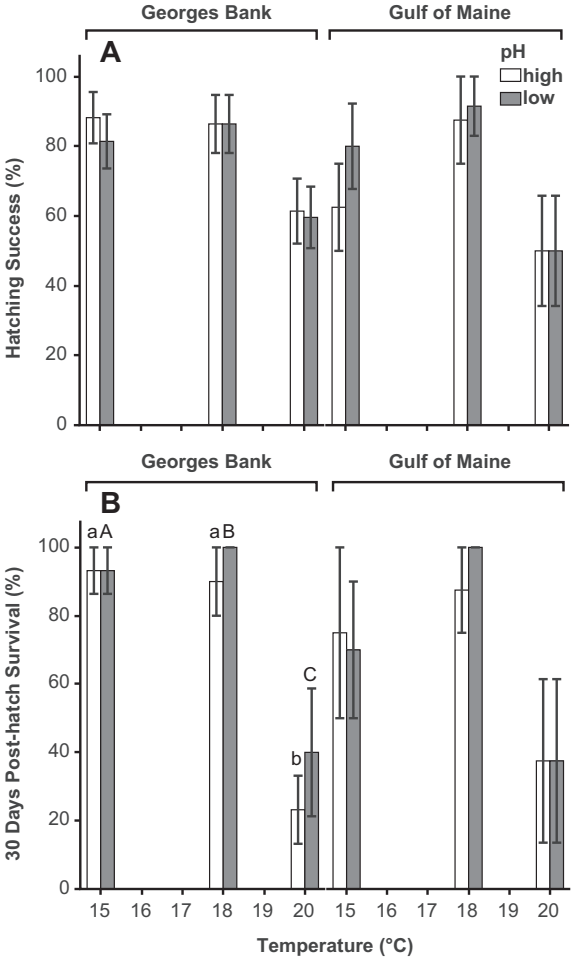


**Fig. 2.** (A) Hatching success and (B) 30 days post-hatching survival of *Leucoraja erinacea* from two populations (Georges Bank n = 77, Gulf of Maine n = 37), at three temperatures and two pH conditions. Different lower and upper case letters represent significant differences within high (8.1) and low (7.7) pH conditions, respectively; double daggers represent significant differences between pH treatments at each temperature; asterisks represent significant differences between populations ($p < 0.05$).

embryos ($F_{1,8} = 12.23$, $p = 0.008$, Fig. 4B) but had no significant effect on SMR at the optimal temperature for performance, 18 °C (2-WAY ANOVA, $F_{1,8} = 0.57$, $p = 0.4$, Fig. 4B). Conversely, in GoM embryos, low pH only significantly increased SMR at the peak of their performance (2-WAY ANOVA, $F_{1,8} = 469.33$, $p < 0.0001$, 18 °C). Overall, the metabolic scope (AMR-SMR) increased up to the optimal temperature (18 °C) and declined at the highest temperature (20 °C) in both populations (3-WAY ANOVA, $F_{7,51} = 3.01$, $p = 0.01$), while low pH increased the costs of activity of GoM embryos at higher temperatures (2-WAY ANOVA, $F_{1,8} = 8.87$, $p = 0.01$, 18 °C; $F_{1,8} = 23.25$, $p = 0.001$, 20 °C; Fig. 4C).

**Table 2**
Effect of temperature and pH on key morphological traits in two skate populations.

| Treatment T (°C); pH | Population | Mass (g) | Disc width (cm) | Body condition (g/cm$^2$) |
|---|---|---|---|---|
| 15; 8.1 control | GoM | $6.442 \pm 0.089^{a,}$ ‡ | $6.19 \pm 0.112^{a}$ | $0.168 \pm 0.005^{a}$ |
| | GB | $4.203 \pm 0.112$‡ * | $4.24 \pm 0.069^{a,}$ ‡ * | $0.234 \pm 0.006^{a,}$ ‡ * |
| 18; 8.1 | GoM | $6.218 \pm 0.201^{b}$ | $6.01 \pm 0.106^{b,}$ ‡ | $0.172 \pm 0.005^{b,}$ ‡ |
| | GB | $3.859 \pm 0.109$‡ * | $6.16 \pm 0.230^{a,}$ ‡ | $0.106 \pm 0.006^{b,}$ ‡ * |
| 20; 8.1 | GoM | $5.564 \pm 0.131^{c,}$ ‡ | $7.00 \pm 0.25^{c}$ | $0.115 \pm 0.011^{c}$ |
| | GB | $4.716 \pm 0.170$‡ * | $5.06 \pm 0.156^{c,}$ ‡ * | $0.187 \pm 0.011^{c,}$ ‡ * |
| 15; 7.7 | GoM | $6.077 \pm 0.089^{A}$ | $6.54 \pm 0.26^{A}$ | $0.114 \pm 0.012^{A}$ |
| | GB | $3.855 \pm 0.047^{A,}$ * | $5.26 \pm 0.041^{A,}$ * | $0.138 \pm 0.001$ |
| 18; 7.7 | GoM | $6.16 \pm 0.080^{B}$ | $6.35 \pm 0.078^{A}$ | $0.152 \pm 0.004^{B}$ |
| | GB | $3.538 \pm 0.097^{B,}$ * | $5.33 \pm 0.046^{B,}$ * | $0.124 \pm 0.002$* |
| 20; 7.7 | GoM | $5.038 \pm 0.044^{C}$ | $7.12 \pm 0.083^{B}$ | $0.099 \pm 0.003^{C}$ |
| | GB | $5.234 \pm 0.141^{C}$ | $6.15 \pm 0.181^{C,}$ * | $0.141 \pm 0.007$* |

Mean (±s.e.m.) for little skate (*Leucoraja erinacea*) exposed to different temperature levels (T) and pHs. GoM = Gulf of Maine population (n = 37); GB = Georges Bank population (n = 77). Different lower and upper case letters represent significant differences within high and low pH conditions, respectively; double daggers represent significant differences between pH treatments at each temperature; asterisks represent significant differences between populations ($p < 0.05$).

## 4. Discussion

This study shows a significant effect of stressors associated with climate change on elasmobranch embryos by providing empirical evidence that, when exposed to increased warming and acidification, little skate embryos exhibit: 1) increased developmental time outside optimal conditions, 2) higher metabolic costs with decreasing pH, 3) decline in body condition, and 4) decreased survival. Furthermore, although initial yolk area did not differ between populations when raised in common garden conditions, hatchlings from the southern population (GB) showed smaller body size than the ones from the northern population (GoM). This suggests local adaptation in metabolic processes by countergradient variation (Baumann and Conover, 2011), a pattern also observed in the wild by Frisk and Miller (Frisk, 2002; Frisk and Miller, 2006, 2009). As embryos were collected from sets of mothers held in different laboratories, feeding and size could not be determined for the parental generation. However, when comparing populations, maternal effect is generally measured by looking at the reserve (yolk) of embryos (Angilletta et al., 2004; Bengtson et al., 1987). In this study, yolk size from the two populations was not statistically different, which implies that the mothers' condition did not significantly affect energy reserves in embryos, which are key for growth and metabolic activities (Storm and Angilletta, 2007). In addition, both labs maintained skates at 15–16 °C thus reducing the potential effect of different thermal acclimation across generations (Donelson et al., 2011).

Larger body size and increased performance (active and developmental) in the GoM skates have potential tradeoffs. For instance, body condition was overall lower in the GoM population and embryos were more susceptible to acidification. In fact, even though weight and disc size were greater at high acidification and temperature, embryos grew in disc area much more than in weight, which resulted in reduced body condition. These results corroborate previous findings on *L. erinacea* that showed smaller but healthier hatchlings at lower temperatures when compared to higher temperatures (Palm et al., 2011). Given that embryos were reared at the same conditions, the responses should be attributed to genetic differences rather than physiological plasticity (Baumann and Conover, 2011). It is possible that, given the higher metabolic costs associated with activity in the GoM embryos when compared to GB ones, the additional physiological challenge induced by acidification may have exacerbated chronic stress in the northern population. Alternatively, frequent upwelling in the Georges Bank may cause wider fluctuations in pH when compared to the Gulf of Maine (Pershing et al., 2001). However more data are needed to increase resolution of spatial changes in pH in the GoM and GB. If a significant difference in pH were to be measured between the sites, this could at least partially explain why the GB embryos seem to be 'pre-adapted' and relatively insensitive to increased acidification. Poor body conditions may have far-reaching consequences for skate populations. In this study, hatchling body condition had a direct relationship with the time elapsed from hatching to first feeding event, likely as a way to compensate for low stored energy. In nature, the necessity to quickly initiate exploration of the environment in order to procure prey may dramatically increase predation risks and mortality in juveniles (Munch and Conover, 2003).

In both populations, metabolic scope increased up to 18 °C (thermal optimum), but decreased at 20 °C. However, the GB (southern population) was less sensitive to the highest temperature suggesting

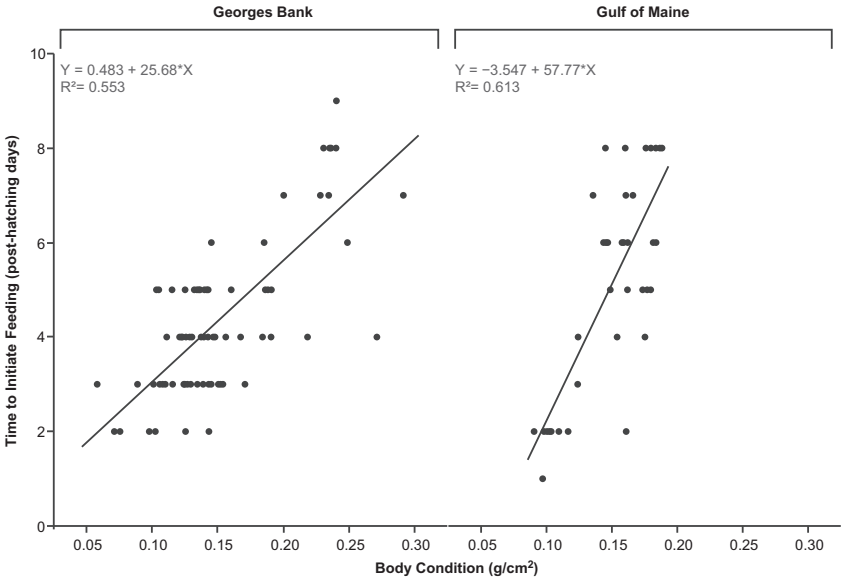


**Fig. 3.** The time elapsed from hatching to first feeding in hatchling *Leucoraja erinacea* from the Gulf of Maine (n = 37) and the Georges Bank (n = 77).

a narrower thermal window for the GoM (northern population). Increasing hypercapnia is also known to further exacerbate metabolic costs and therefore reduces the amount of energy allocated to growth (Baumann et al., 2011; Rosa et al., 2014). In this study, low pH increased metabolic costs of activity at the highest temperature in the GoM population. Therefore, acidification may exacerbate stress in embryonic skates from the GoM population thus making them more vulnerable to projected changes in the ocean. Although the maximum metabolic rate could not be measured in embryos as activity cannot be controlled, a measure of metabolic scope as the amount of energy that the embryo uses to be active in the egg case is useful to understand the costs of environmental change on performance (Vleck and Vleck, 1986). In fact, Vleck and Vleck (1986) observed a linear response of growth and metabolism in embryos. Embryonic bamboo sharks, *Chiloscyllium plagiosum*, also modulate their metabolic rates during development to closely match growth rates and body size (Tullis and Peterson, 2000). Likewise, *L. erinacea* from GoM exhibit higher metabolic rates to perhaps accommodate faster growth and development.

Finally, mortality in embryos occurred only in the first five weeks of development when the egg case jelly and plugs were not absorbed yet, suggesting that perhaps temperature rather than $pCO_2$ determined survival. These findings are different from previously observed results in which acidification decreased embryonic teleost survival (Baumann et al., 2011; Chambers et al., 2013). A possible interpretation is that skate embryos are initially protected by egg jelly and a plug (Hoff, 2009). The role of the egg jelly has not been fully understood, however it has been suggested that it provides protection to the embryo during the first stage of development (Hoff, 2009; Koob and Straus, 1998; Leonard et al., 1999). A few studies on embryos of another oviparous elasmobranch, the big skate *Raja binoculata*, have shown that during the early stage of development the jelly may protect the embryos from the surrounding water until it develops the ability to osmoregulate (Evans, 1981; Hoff, 2009; Read, 1968). It is possible that at the earliest stage, *L. erinacea* embryos were most sensitive to temperature and that acidification did not have a significantly lethal effect once the gills were developed. This differs from teleost embryos which are directly exposed to the surrounding environment, making them more vulnerable to changes in acidification (Baumann et al., 2011; Bignami et al., 2013a,b; Chambers et al., 2013).

## 5. Conclusions

The conservation of elasmobranchs presents a challenge because it is relatively difficult to determine population declines and how many individuals are remaining in a population. Because the vast majority of skates are not managed, vulnerability to local extinctions tends to be ignored (Chin et al., 2010; Dulvy and Reynolds, 2002; Dulvy et al., 2003; Stevens et al., 2000). Although *L. erinacea* has a relatively wide geographic range, this species may be vulnerable to local extirpation because of the limited capacity for shifting its range (Dulvy and Reynolds, 2002; Dulvy et al., 2005). In fact, skates tend to be philopatric, with only short distance (less than 50–100 miles) movements (Dulvy and Reynolds, 2002; Frisk and Miller, 2006), and there is little evidence for recolonization after local extirpation despite the presence of nearby populations (Dulvy and Reynolds, 2002). Until now, a lack of empirical evidence on the effect of climatic stressors on elasmobranchs has constrained the development of conservation and adaptation strategies related to global warming. Results from this experimental study show that embryonic development and energetics are affected differently by increasing warming and acidification in two little skate populations, and low pH exacerbates the effect of increasing temperature. Decreased body condition, as a result of the combined effect of acidification and warming, triggers newly-hatched skates to start exploring the environment to feed sooner, potentially making them more vulnerable to predation. Furthermore, performance curves in the two populations suggest local adaptation by countergradient variation. Lastly, in light of this study it is apparent that an increase in temperature beyond 18 °C will likely reduce fitness and survival of little skates and that the Gulf of Maine population may be more vulnerable at acidification levels expected by the end of the century. Based on these potential impacts, it would be advisable that the Gulf of Maine and the Georges Bank populations of *L. erinacea* were to be considered as different stocks.

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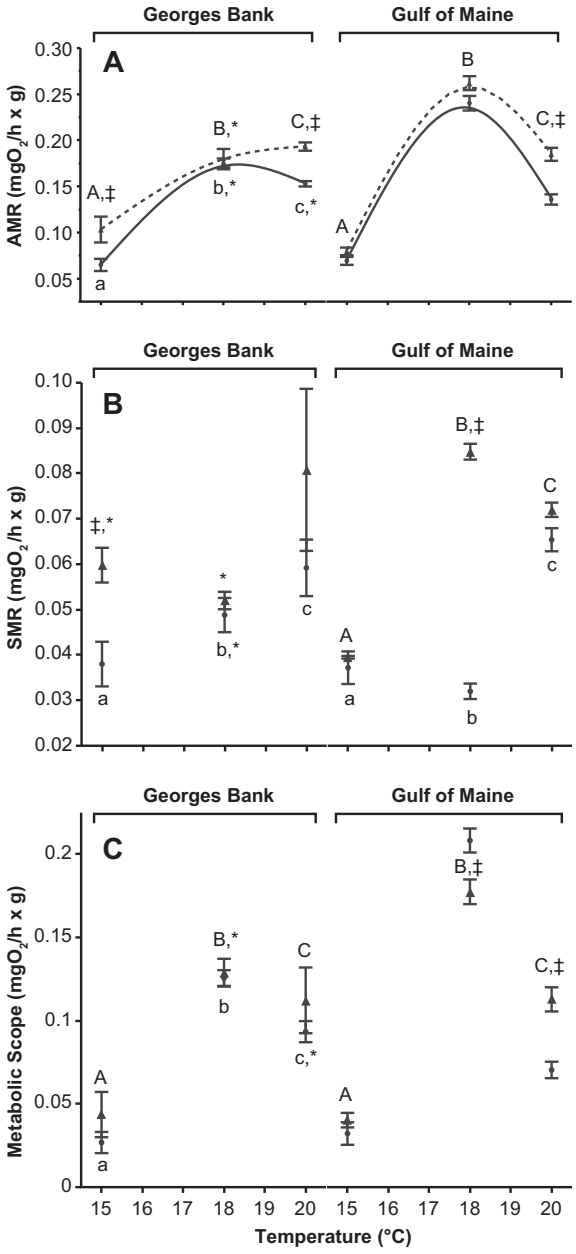


**Fig. 4.** Mass-adjusted (A) active metabolic rates, (B) standard metabolic rates, and (C) metabolic scopes (mean ± s.e.m.) of *Leucoraja erinacea* from the Gulf of Maine (n = 29) and the Georges Bank (n = 30) at three temperatures and two pH conditions (high, 8.1: circle, low, 7.7: triangle). Different lower and upper case letters represent significant differences within high (8.1) and low (7.7) pH conditions, respectively; double daggers represent significant differences between pH treatments at each temperature; asterisks represent significant differences between populations ($p < 0.05$).

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## Glossary

*Common garden experiment:* Experiment in which two or more species or populations of organisms living in different environments are reared in a common environment.
*Countergradient variation:* A geographic pattern in which phenotypic variation among populations is reduced along an environmental gradient, and results in increased performance at the thermal optimum of the northern population when compared to the southern population.
*Metabolic scope:* The metabolic scope is the difference between active metabolic rate and standard metabolic rate and gives an estimate of the cost of activity.
*Performance curve:* It represents the response of performance (i.e. growth, development, and metabolism) to changes in the environment (typically temperature).
*Thermal optimum:* Temperature at which performance reaches the peak.
*Thermal pejus:* Temperatures (low and high) at which performance declines.