BIOLOGY LETTERS

Review

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Biological responses of sharks to ocean acidification

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Sharks play a key role in the structure of marine food webs, but are facing major threats due to overfishing and habitat degradation. Although sharks are also assumed to be at relatively high risk from climate change due to a low intrinsic rate of population growth and slow rates of evolution, ocean acidification (OA) has not, until recently, been considered a direct threat. New studies have been evaluating the potential effects of end-of-century elevated CO_2 levels on sharks and their relatives' early development, physiology and behaviour. Here, we review those findings and use a meta-analysis approach to quantify the overall direction and magnitude of biological responses to OA in the species of sharks that have been investigated to date. While embryo survival and development time are mostly unaffected by elevated CO_2, there are clear effects on body condition, growth, aerobic potential and behaviour (e.g. lateralization, hunting and prey detection). Furthermore, studies to date suggest that the effects of OA could be as substantial as those due to warming in some species. A major limitation is that all past studies have involved relatively sedentary, benthic sharks that are capable of buccal ventilation—no studies have investigated pelagic sharks that depend on ram ventilation. Future research should focus on species with different life strategies (e.g. pelagic, ram ventilators), climate zones (e.g. polar regions), habitats (e.g. open ocean), and distinct phases of ontogeny in order to fully predict how OA and climate change will impact higher-order predators and therefore marine ecosystem dynamics.

1. Introduction

Chondrichthyan fishes (sharks, skates, rays and chimaeras) are one of the most successful marine groups, having been able to survive all five mass extinctions over the last 400 million years [1]. The first reports of their most reliable diagnostic feature—the tesserate mode of cartilage mineralization—are from late Devonian deposits (approx. 380 Mya) [2], though the first scales and spines of Chondrichthyans appeared already in the Lower Silurian [3,4]. Presently, the cartilaginous fishes (comprising approximately 1200 species) are found throughout all of the world's oceans, and many occupy high trophic levels in marine habitats [5,6] where they can exert a fundamental influence (top-down control) on the structure and function of communities [7,8]. Although chondrichthyans have evolved to fill many aquatic habitats and niches, their ability to adapt quickly to human-induced environmental changes is assumed to be limited [9–11]. In contrast to most marine fishes, they generally have a K-selected life-history strategy: slow growth, late age at maturity, low fecundity, and few offspring, long gestation periods and long lifespans [6]. These life-history traits have important implications for fisheries' sustainability, management and conservation [12–14]. For sharks in particular, it is widely accepted that the major threats to their populations are overfishing and habitat degradation, but this group is also at relatively high risk from climate change [15,16].

2. Ocean acidification

The anthropogenic emissions of greenhouse gases to the atmosphere are driving rapid changes in the Earth's climate system, which are expected to accelerate in the current century. Atmospheric carbon dioxide (CO_2) levels have risen to 400 μatm [17] for the first time in at least 800 000 years and are expected to exceed 900 μatm by 2100 if the current emissions trajectory is maintained [18]. The pCO_2 of the ocean is rising as the same rate as the atmosphere [19], and the uptake of additional CO_2 from the atmosphere is causing ocean pH to decline. Ocean surface pH is projected to decrease by 0.13–0.42 units by the end of the 21st century, depending on CO_2 emission scenarios [18]. These changes in seawater chemistry and the subsequent shift in the relative proportion of species of dissolved inorganic carbon (DIC), together known as ocean acidification (OA), will have cascading effects on marine ecosystems [20,21].

Higher ambient CO_2 levels act to acidify the blood and tissues of water-breathing marine organisms [22]. Teleost fishes, however, are assumed to be quite resilient to elevated CO_2 because of their ability to regulate acid-base balance by bicarbonate accumulation and ion exchange across (primarily) the gills [23–25]. Sharks and their relatives use a similar mechanism to that of teleost fishes [26]. Yet, it is worth noting that they are osmoconformers, whereas teleost fishes are osmoregulators. Being an osmoconformer in seawater means that the blood plasma has roughly the same osmolality as the seawater, which largely comes from high concentrations of urea and trimethylamine N-oxide (TMAO) [27–29].

Until recently, there were no experimental data with which to assess the possible effects of OA on sharks and their relatives. Only through 'Ecological Risk Assessments' (ERAs) for climate change was it argued that OA would not directly affect sharks, although it may indirectly affect them via changes in habitat, marine community structure and prey availability [11]. The underlying reasoning behind these conclusions was the fact that the modern sharks evolved in the Devonian, when atmospheric CO_2 levels were many times greater than the current day [29], and therefore it was expected that they should be highly tolerant of high CO_2 due to their evolutionary history. However, teleost fishes also evolved in a period of high CO_2 and this has not conferred an overarching tolerance to high CO_2 in all extant species [30]. Furthermore, new studies have revealed empirical evidence that sharks and their relatives may be more sensitive to OA than previously predicted, especially during their early life stages (embryos, newborns and juveniles). Here, we present the first review on the subject and apply a meta-analysis approach to assess the direction and magnitude of biological responses of sharks to OA (i.e. simulated end-of-century elevated CO_2 conditions). More specifically, we use the meta-analysis to test whether OA will have a negative effect on survival, growth, physiology and behaviour in the species investigated to date and how these effects will interact with environmental warming (methodological details in electronic supplementary material).

3. Early development

Although all shark species display internal fertilization, there is variation in the mode of fertilization, ovulation cycle, gestation period and mating systems (reviewed in [31]).

Also, depending on how long the embryos are retained by the mothers, shark species can be divided by oviparous (egg-laying) or viviparous (live-bearing) reproduction. Oviparous species retain the fertilized eggs for short periods, after which the eggs are attached to benthic structures until hatching. To our knowledge, no studies have yet examined the possible effects of OA on embryonic development in viviparous species, and only three studies to date have evaluated the possible effects of OA on the embryonic development of oviparous species—the tropical bamboo (*Chiloscyllium punctatum*) and epaulette (*Hemiscyllium ocellatum*) sharks and the temperate Port Jackson shark (*Heterodontus portusjacksoni*) (table 1). Overall, these studies found no significant effects of OA on embryo survival and development time (figure 1). Moreover, specific growth rates, yolk consumption, tail oscillations and gill movements were not significantly different in embryos exposed to control conditions when compared with those reared under elevated CO_2 conditions [32,37,39]. As expected, elevated temperature significantly increased the rate of embryonic development, but there was no interaction with elevated CO_2 [32,39] (figure 2). It is possible that oviparous shark species may display adaptive mechanisms that confer tolerance to elevated CO_2 conditions inside the egg capsules. Nonetheless, it is worth noting that in another chondrichthyan group (skates), Di Santo [42] found that CO_2-induced acidification exacerbated the effects of high temperature stress on the embryogenesis in the little skate (*Leucoraja erinacea*). Although early development was not significantly affected by elevated CO_2 in the shark-related studies conducted to date, more studies are necessary to increase replication, to focus on critical developmental periods (e.g. the pre-gill formation period is normally linked to higher mortalities [37]) and to consider oviparous species that thrive at higher latitudes.

4. Physiology

The physiological effects of simulated end-of-century elevated CO_2 conditions have only been evaluated in four relatively sedentary, benthic species: the temperate lesser-spotted (*Scyliorhinus canicula*) catshark [38] and Port Jackson (*H. portusjacksoni*) sharks [39,40] and the tropical bamboo (*C. punctatum*) [32–34] and epaulette (*H. ocellatum*) sharks [35,36] (table 1). Previous studies investigating physiological processes under elevated CO_2 in sharks have been conducted at very high CO_2 levels (>8–10 mm Hg, approximately 10 000–13 000 μatm) (e.g. [29]) that are not ecologically relevant to the impacts of near-future ocean acidification. Consequently, they are not included in this review and meta-analysis. Although there was no overall effect of OA-relevant CO_2 levels on the survival of recently hatched and juvenile sharks, some physiological impairments have been detected (figure 1). For instance, while there were no modifications to growth in *S. canicula*, significant changes in resting metabolic rate, aerobic scope and blood chemistry (increased HCO_3^- and Na^+ levels) were detected. Similar acid-base compensation, blood haematology variables (e.g. haematocrit, haemoglobin concentration, and mean cell haemoglobin concentration) and respiratory (resting oxygen consumption rates, citrate synthase activity and hypoxia tolerance *via* Pcrit) responses to elevated CO_2 were observed in *H. ocellatum* [35]. The latter authors suggested that these physiological responses

Table 1. Summary of the available experimentally based studies on the impacts of ocean acidification (OA) in sharks. Abbreviations: C, control; T, treatment; dph, days post-hatching; Hct, haematocrit; Hb, haemoglobin; MCHC, mean cell haemoglobin concentration; R-h, recently hatched; NA, not applicable.

shark species	region and life strategy	life stage	pCO_2 (µatm) C	T	pH C	T	temperature (°C) C	T	acclimation period (days) C	T	OA effects	references
Chiloscyllium punctatum	tropical and benthic	embryos and r-h juveniles (30 dph)	371–382	1383–1481	8.0	7.5	25.9–26.2	29.9–30.1	approximately 230[a]	approximately 210[a]	= embryo survival; ⇓ juvenile survival; ⇓ metabolic rates; ⇓ ventilation rates; ⇓ Fulton condition index	[32]
Chiloscyllium punctatum	tropical and benthic	R-h juveniles (30 dph)	371–382	1383–1481	8.0	7.5	25.9–26.2	29.9–30.1	approximately 230[a]	approximately 210[a]	⇓ brain and muscle aerobic (CS) potential; ⇑ brain and muscle anaerobic (LDH) potential; ⇑ peroxidative damage and brain AchE levels. OA effects enhanced with warming.	[33]
Chiloscyllium punctatum	tropical and benthic	R-h juveniles (30 dph)	371–382	1383–1481	8.0	7.5	25.9–26.2	29.9–30.1	approximately 230[a]	approximately 210[a]	⇓ pancreatic trypsin levels and alkaline phosphatase activity in shark's intestine. ⇑ both enzyme activities with warming.	[34]
Hemiscyllium ocellatum	tropical and benthic	juveniles	397–384	608–876	8.2	8.0–7.9	28.5	NA	60	NA	⇑ plasma [HCO_3^-], [Hb] and MCHC; no differences in metabolic rates, muscle CS and brain, Pcrit, [Hct], plasma [Na^+], [K^+], [Cl^-] and [urea]	[35]
Hemiscyllium ocellatum	tropical and benthic	juveniles	400	615–910	8.0	7.9–7.8	28.8	NA	30	NA	= foraging and shelter-seeking behaviour.	[36]
Hemiscyllium ocellatum	tropical and benthic	embryos	420	945	8.1	7.9	28.3	NA	82	NA	= growth, yolk usage, tail oscillations, gill movements and survival.	[37]
Scyliorhinus canicula	temperate and benthic	juveniles	401	993	8.1	7.7	12.7	NA	30	NA	⇑ plasma [HCO_3^-] and [Na^+]; ⇑ absolute lateralization; ≠ swimming patterns; no changes in [K^+], [Ca^{2+}], [Cl^-], [Hct], MCHC, metabolic rates, dentide ultrastructure and growth.	[38]

Table 1. (Continued.)

| shark species	region and life strategy	life stage	pCO$_2$ (μatm) C	pCO$_2$ (μatm) T	pH C	pH T	temperature (°C) C	temperature (°C) T	acclimation period (days) C	acclimation period (days) T	OA effects	references
Heterodontus portusjacksoni	temperate and benthic	embryos and juveniles	400	1000	8.0	7.8	16	19	approximately 225ᵃ	approximately 200ᵃ	⇑ energetic demands, ⇓ metabolic efficiency and ⇓ ability to locate food through olfaction. ⇓ growth rates alone or in combination with temperature.	[39]
Heterodontus portusjacksoni	temperate and benthic	embryos and juveniles	400	1000	8.0	7.8	16	19	147	108	⇓ chemical and behavioural responses to effective hunting. ⇑ energetic demands.	[40]
Mustelus canis	temperate and benthopelagic	adults	405–412	734–1071	8.1	7.7–7.8	19.6	NA	5		Food odour avoidance and ⇓ attack behaviour	[41]

ᵃIncludes the entire egg rearing period.
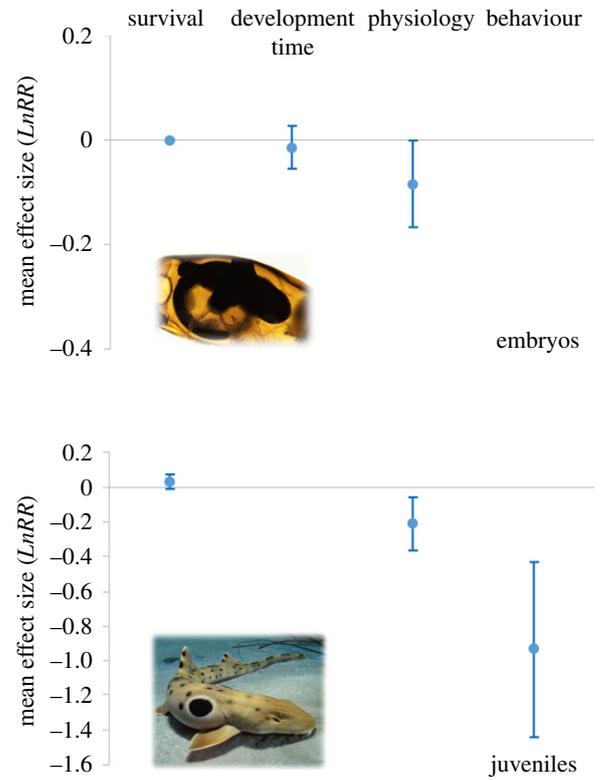
Figure 1. Mean effect of near-future CO$_2$ levels on major biological response variables, namely survival ($n = 4$ studies for embryos and $n = 5$ for juveniles), development time (embryogenesis, $n = 4$), physiology (e.g. oxygen consumption rates, respiratory enzyme activities, ventilation rates, or Fulton's condition index; $n = 4$ studies for embryos and $n = 5$ for juveniles), and behaviour (e.g. food detection or absolute lateralization; $n = 5$ for juveniles). Means are from weighted, random effects categorical meta-analyses for each separate response variable. Significance (*) is determined when the 95% bootstrapped confidence interval does not cross zero. For additional details, see electronic supplementary materials. Images are of said species used in some of the studies.

were associated with *H. ocellatum* living in shallow reef and lagoon habitats that naturally experience variable CO$_2$ levels, which could confer them a certain degree of tolerance to projected future CO$_2$ concentrations. Contrary to these two studies that encompass acclimation periods varying between 30 and 60 days (table 1), other studies performed in recently hatched juveniles exposed to elevated CO$_2$ during the entire embryogenesis (more than 200 days of acclimation; table 1) observed significant changes in Fulton's condition index [32], aerobic potential (citrate synthase activity), peroxidative damage in the brain, cholinergic neurotransmission [33] and digestive enzyme activities [34], among other physiological variables. Most of these effects also exhibited significant interactions with elevated temperatures (figure 1). Thus, in addition to the limited number of studies, the different duration of the experiments also challenges our ability to make strong inferences on effect size, since studies with shorter acclimation periods (less than three months; see table 1) have contrasting effects to studies with much longer acclimation times.

Another key aspect of shark physiology that could alter blood pCO$_2$ and thus make some species more or less sensitive to elevated water CO$_2$ is the ability to pump water over their gills. While some sharks use buccal pumping (usually less active and benthos-associated species), others oxygenate
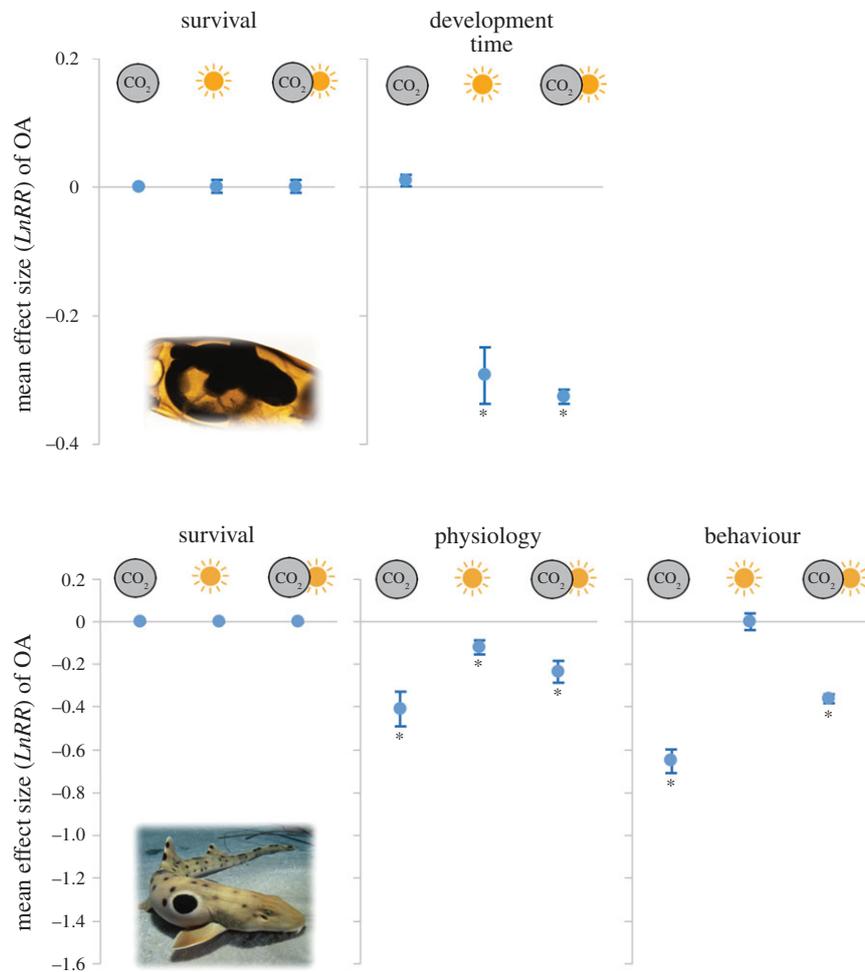
Figure 2. Differences in the mean effect of near-future CO_2 at control and elevated temperature conditions for the different biological response variables of embryos ($n = 3$) and juveniles ($n = 3$). An asterisk (*) denotes a significant difference from zero. For additional details, see electronic supplementary materials. Symbols: CO_2 circle—ocean acidification effect, sun—warming effect; circle and sun—combined effects of both variables. Images are of said species used in some of the studies.

the gills by opening their mouth while swimming – ram ventilators (more active and pelagic species). All species studied to date fall within the first category and, therefore, there is a complete lack of knowledge necessary to predict the effects of OA on the behavioural and physiological ecology of the larger and more active sharks, such as those belonging to the Carcharhinidae (requiem) and Sphyrnidae (hammerhead) families. In other fast-swimming (non-obligate) ram ventilators (e.g. mackerels) it has been shown that as swimming speed increases blood pCO_2 declines in a linear way [43]. Thus, ram-ventilating sharks may be more susceptible to OA because they have lower internal pCO_2 values than their benthic counterparts. The smaller differential between ambient and internal $pCO2$ in ram ventilators could make them more sensitive to OA because of the larger relative increase in internal $pCO2$ when exposed to increasing ambient CO_2 levels [44].

5. Behaviour

Sharks are generally considered to possess superior olfactory sensitivities when compared to teleost fishes due to sharks' particularly large olfactory structures, which play a key role in their capabilities for predator avoidance, prey detection and navigation [45]. Sharks and their relatives also possess unique and elaborate (ampullary) electroreceptor systems that facilitate geomagnetic navigation and detection of preys' bioelectric fields [46]. Contrary to the other phenotypic responses, elevated CO_2 has been found to significantly affect shark behaviour (figure 1). For instance, Pistevos and colleagues showed that Port Jackson sharks reared under simulated end-of-century elevated CO_2 conditions (approx. 67 days in mesocosms) took nearly four times longer to detect their prey than those reared in control conditions [39]. However, in combination with elevated temperatures, the amount of time to detect prey was reduced by one third. This temperature effect was recurrent in other studies and is highlighted in figure 2. In a subsequent study [40], the same team revealed that although warming increased prey location rate in *H. portusjacksoni*, future CO_2 conditions inhibited the chemical and visual behavioural responses that allow effective hunting in the test arenas. Similarly, Dixson *et al.* [41] found that high CO_2-treated smooth dogfish (*Mustelus canis*) were less attracted to a water stream containing a food stimulus and reduced attack behaviour when compared to their control counterparts. Lesser-spotted (*Scyliorhinus canicula*) catsharks exposed to elevated CO_2 levels exhibit increased absolute lateralization and significantly fewer swimming events [38]. Together, these studies

demonstrate that exposure to elevated CO_2 can significantly impair critical feeding behaviours and activities in sharks. On the contrary, however, the behaviour of the benthic reef-dwelling *H. ocellatum* was unaffected by the projections for ocean CO_2 by 2100 [36]. Again, the absence of phenotypic effects in this species was explained by adaptations to the diel fluctuations in oxygen and CO_2 that are found in their shallow, reef habitat, illustrating that sensitivity to higher CO_2 levels may be habitat specific.

The broadly similar behavioural changes to OA observed in marine teleosts have been attributed to interference between acid-base regulation in a high CO_2 environment and the function of the GABA-A receptor, the primary inhibitory neurotransmitter receptor in the vertebrate brain [47]. The GABA-A receptor is an ion-channel with conductance for Cl^- and HCO_3^- and under normal (control) conditions, ion inflow leads to membrane hyperpolarization and inhibited neural activity. Under elevated CO_2, marine teleosts make regulatory adjustments in blood and tissues that affect such transmembrane gradients in some neurons [22]. Consequently, GABA-A receptors can become depolarizing and excitatory, resulting in behavioural impairments [22,47–49]. The same explanatory mechanism could apply to sharks, since they possess the same GABA-A neurotransmitter receptor [50] and accumulate HCO_3^- from the environment in exchange for Cl^- from the body to buffer eventual pH disturbance. A pharmacological approach (e.g. the use a GABA-A receptor antagonist such as gabazine) to test this hypothesis in sharks has not yet been undertaken but warrants investigation.

6. Conclusion and future directions

The structure of marine food webs has already been altered due to dramatic declines in shark populations from overfishing and habitat deterioration [7,9,10,14]. Until recently, ocean acidification was not considered a direct threat to sharks. Recently available empirical evidence suggests that they may be more susceptible than previously assumed. Physiological impairments due to elevated CO_2 exposure were less evident than behavioural impairments, and revealed greater interspecific variability. Future studies should choose species with different lifestyles, from different climate zones and habitats, and/or species with different niche preferences. Aware that short-term studies do not consider the potential for adaptation over the time frame that CO_2 levels will rise by the end of the century [51], future research should also attempt to conduct transgenerational (multiple generations; namely in oviparous species) approaches. More empirical data are thus required to directly assess the risk and vulnerability of sharks to climate change and ocean acidification, which will assist managers and policy makers to make informed decisions targeting the most endangered species.

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