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RESEARCH ARTICLE

Cardiorespiratory interactions in the Pacific spiny dogfish, *Squalus suckleyi*

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ABSTRACT

Elasmobranchs are a group of cartilaginous fish with no direct sympathetic innervation of the heart or gills. Fast cardiorespiratory regulation is controlled solely by the parasympathetic branch of the autonomic nervous system. Cardiovascular changes associated with ventilation are commonly present in the form of respiratory sinus arrhythmia (RSA) and as cardiorespiratory synchrony (CRS, in which there is a 1:1 beat to breath ratio). The latter has been hypothesized to maximize oxygen uptake, coupling the pulsatile flows of blood and water in the gills. Given this, we hypothesized that CRS should be more prevalent in situations of low oxygen supply and RSA should be abolished by vagotomy. To test this, we investigated the role of the vagus nerve in mediating cardiorespiratory responses to changing environmental oxygen conditions in the elasmobranch *Squalus suckleyi*. Hypoxia and hyperoxia had little effect on heart rate but did alter breathing frequency and amplitude. Atropine yielded an overall tachycardia in all oxygen conditions and abolished all heart rate variability (HRV), suggesting that HRV solely reflects fluctuating vagal tonus on the heart. Regardless of the presence of atropine, hypoxia still induced an increase in ventilation rate and depth. CRS was only found during progressive hyperoxia post-atropine, when heart rate was uninhibited and ventilation was slowed owing to the increase in oxygen supply, suggesting that in *S. suckleyi*, CRS is an epiphenomenon and not actively regulated to maximize gas exchange efficiency.

KEY WORDS: Cardiorespiratory synchrony, Dogfish, Hypoxia, Hyperoxia, Heart rate variability, Vagus nerve

INTRODUCTION

Elasmobranchs are the earliest phylogenetic group of vertebrates with a well-developed autonomic nervous system that has clearly differentiated parasympathetic and sympathetic components (Nicol, 1952). All fish hearts with the exception of cyclostomes (jawless fishes) are controlled by inhibitory parasympathetic innervation via the vagus nerve (Taylor et al., 1977; Barrett and Taylor, 1985; Taylor and Barrett, 1985). This input is mediated by muscarinic cholinoreceptors associated with the pacemaker and the atrial myocardium. The sympathetic system of elasmobranchs consists of a series of irregularly arranged paravertebral ganglia that do not extend into the cephalic or pharyngeal regions of the body (Taylor, 1992). Therefore, there is no direct sympathetic innervation of the heart or the branchial circulation. The hearts of elasmobranchs are under some sympathetic humoral influence, however, exerted by catecholamines released from the chromaffin cells of the kidney. It is thought that variations in the degree of cholinergic vagal tone are the main control mechanism of cardioregulation in elasmobranchs (Taylor et al., 1977; Barrett and Taylor, 1985).

The magnitude of inhibitory vagal tone on the heart varies depending on physiological state and environmental conditions. Butler and Taylor (1971) found a direct inverse relationship between vagal tone and normoxic heart rate in lightly restrained unanaesthetized dogfish. In hypoxia, oxygen-sensitive chemoreceptors increase vagal tonus on the heart causing a reflex bradycardia or a slowing of heart rate (Taylor et al., 1977; Taylor, 1992). This bradycardia was thought to increase cardiac filling time and hence stroke volume via the Frank Starling mechanism, resulting in improved filling of the gill lamellae and improved gas exchange. The latter, however, has now been shown not to be the case (McKenzie et al., 2009; Iversen et al., 2010). There are also relatively high levels of circulating catecholamines in elasmobranch blood under normoxic conditions, and these levels increase in hypoxia (Butler et al., 1978). It is possible that they exert excitatory, tonic control on the cardiovascular system (Short et al., 1977) and compensate for the lack of direct sympathetic innervation. However, the relative importance of this influence is unknown (Taylor et al., 1999).

The majority of studies examining the duality between excitatory and inhibitory influences on the heart have been conducted via pharmacological blockade (Axelsson et al., 1987). Atropine blocks muscarinic acetylcholine receptors, thereby blocking parasympathetic vagal inhibitory output. This results in a tachycardia, abolishes the reflexive hypoxic bradycardia and reduces heart rate variability (HRV) (Campbell et al., 2005; Butler and Taylor, 1971; Altimiras et al., 1997). HRV reflects the responsiveness of the cardiovascular system to changes in external stimuli over heart beat-to-beat time intervals and is used to investigate autonomic modulation of cardiac function (Mcdonald, 1980). The abolition of HRV after pharmacological cardiac vagotomy in intact fish supports this hypothesis (Campbell et al., 2005).

Life in an aquatic environment yields a different set of physiological limitations than that experienced by terrestrial organisms. The marine environment is often oxygen limited and, to cope, fish must have a respiratory system that maximizes oxygen uptake. This is accomplished by matching the oxygen capacities of blood and water at the gills and by countercurrent flow (Piiper and Scheid, 1977). Because the flows of blood and water are pulsatile at the gills, it has been suggested that coupling both pumping systems could optimize gas exchange (Taylor, 1992). This would be a form of cardiorespiratory synchrony (CRS) in which there was a 1:1 ratio of heart rate to ventilation frequency. The harmonization of the pressure pulses associated with ventilation and gill perfusion are hypothesized to increase the relative efficiency of oxygen uptake (Taylor, 1992). CRS has been demonstrated in several fish species,

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including the European dogfish (Satchell, 1961; Barrett and Taylor, 1985), where the heart beat and respiratory frequency were often synchronized in a 1:2 or 1:3 ratio with the P-wave of the ECG occurring most often during the mouth opening phase of the respiratory cycle. This has been suggested to arise within the central nervous system, possibly by irradiation from a central respiratory rhythm generator (Taylor et al., 2009a,b), and is generated by efferent parasympathetic inputs via the vagus nerve (Leite et al., 2009).

If CRS is occurring to maximize oxygen uptake in elasmobranchs, we would expect to see it in situations of high oxygen demand or low oxygen availability. However, Taylor and Barrett (1985) demonstrated CRS for long periods in dogfish (*Scyliorhinus canicula*) settled in normoxic seawater at 23°C, whereas Taylor and Butler (1971), working with the same species, found no evidence of any maintained synchrony between heart beat and respiratory movements in normoxic or hypoxic waters. In the pacu (*Piaractus mesopotamicus*), there was also a loose 1:1 relationship between heart rate and breathing frequency in normoxia. During progressive hypoxia, this coupling was initially strengthened but disappeared when aquatic O_2 partial pressure ($P_{W_{O_2}}$) fell below 75 torr as a bradycardia developed and ventilation increased further (Leite et al., 2009); i.e. it disappeared as oxygen supply decreased. In the rainbow trout (*Oncorhynchus mykiss*), heart rate was higher than breathing frequency under normoxic conditions and CRS was only present when the fish were exposed to severe hypoxia (Randall and Smith, 1967). While these studies suggest that CRS occurs in many fish species and that the vagus nerve is instrumental in its production, they raise questions as to whether this is a regulated phenomenon or simply an indirect consequence of chemoreceptor-driven changes in the frequencies of breathing and heart beat.

In the present study, we examined the degree of inhibitory parasympathetic vagal influence on the cardiac and respiratory systems in the Pacific spiny dogfish, *Squalus suckleyi*, in normoxia, hypoxia and hyperoxia using time domain analysis, as well as power spectral analysis. In this species under resting normoxic conditions, breathing frequency is higher than cardiac frequency and CRS is not present. If CRS is occurring to maximize oxygen uptake, we hypothesized that CRS should develop in situations of high oxygen demand and that both CRS and HRV should be abolished by pharmacological vagotomy.

MATERIALS AND METHODS

Animals

Adult male Pacific spiny dogfish, *Squalus suckleyi* (Girard 1854) (1.5–3.0 kg, N=10), were caught by fishers in Barkley Sound, on the west coast of Vancouver Island, and were held at the Bamfield Marine Sciences Center for 30 days. The fish were held in a large 75,000 liter cable tank at 9–10°C and were fed once a week with herring. They were not fed for at least 48 h prior to experimentation. Experimental procedures were performed according to University of British Columbia Animal Care Committee protocols A15-0100 under the guidelines of the Canadian Council on Animal Care. Wild animals were collected in accordance with Fisheries and Oceans Canada (DFO) permit XR128 2015.

Surgical preparation

Fish were first anaesthetized by immersion in a solution of MS222 (1.0 g l^{-1}) until righting responses were abolished (~5 min). Each fish was then transferred to a surgical table, where its gills were kept irrigated with an aerated supply of the same anaesthetic solution. A polyethylene cannula (PE50) was inserted percutaneously into the caudal artery from the ventral midline approximately 10 cm caudal to the anus. The cannula was sutured in place three times on the lower midline, and on the back of the dogfish. This allowed us to record blood pressure and to perform intra-arterial injections. Another cannula (PE60) was inserted into the buccal cavity through the skin above the palatoquadrate cartilage of the mandible to measure the changes in pressure associated with breathing, and sutured to the roof of the mouth and near the dorsal fin to hold it in place. After surgery, each fish was placed into aerated seawater and monitored until it recovered spontaneous ventilation. All cardiovascular cannulae were filled with heparinized (50 i.u. ml^{-1} sodium heparin) saline (500 mmol l^{-1} NaCl). After surgery, fish were revived, and each fish was then placed on a water table in a chamber that partially restricted turning movements of the fish. The fish were left in the chamber overnight in running water at 9–10°C.

Experimental protocol

Both cannulae were first connected to pressure transducers (Deltran, Utah Medical Products, Midvale, UT, USA) that were connected to a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO, USA). The O_2 concentration in the water (9–10°C) was measured (Hach, Loveland, CO, USA) and the dogfish was allowed 1 h to acclimatize to the normoxic (~9.5 mg l^{-1-1} O_2) experimental conditions. The oxygen levels were then reduced by N_2 bubbling, making the water progressively hypoxic over a period of approximately 1 h until the oxygen concentration reached ~2.5 mg l^{-1-1}. Subsequently, we increased the oxygen concentration by bubbling O_2 into the water for approximately 1.5 h until the oxygen concentration reached ~20.0 mg l^{-1-1}. These oxygen levels were both maintained for approximately 15 min to allow for adequate analysis time in each oxygen condition. We then stopped oxygen flow and increased water flow until oxygen levels returned to normal. One hour later, we injected 1 ml kg^{-1} of an atropine solution (1 mg ml^{-1}) into the animal via the arterial cannula. Atropine blocks muscarinic acetylcholine receptors, thereby stopping parasympathetic vagal inhibitory output. Although we did not run a dose–response curve, the dose chosen was based on earlier experiments on Pacific dogfish (see McKendry et al., 2001) and was sufficient to eliminate all signs of parasympathetic activity in the power spectra (see Results). The dogfish was then allowed to rest for another 30 min before we

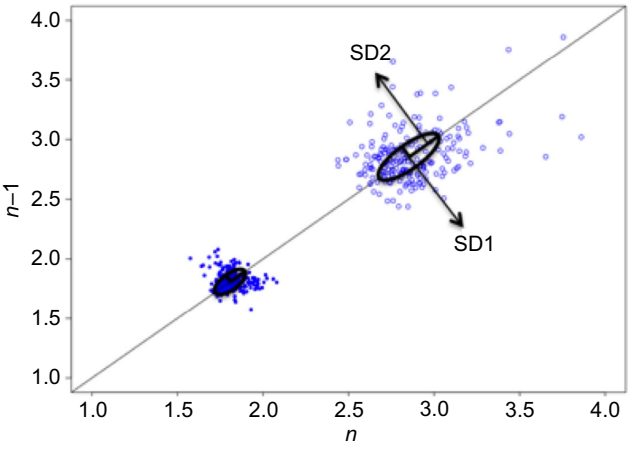

Fig. 1. Sample Poincaré plot of successive HH intervals (intervals between beats), each plotted against the following HH interval pre-atropine (open symbols) and post-atropine (closed symbols) for a representative animal (Dogfish 9) under normoxic conditions. SD1 and SD2 are the width and length of the ellipse, respectively. *n* is the inter-beat interval between two beats and *n*–1 is the preceding inter-beat interval.

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repeated the oxygen manipulations described above. Following the entire protocol, the dogfish was again anaesthetized using the same solution of MS222, and placed back on the surgical table with gill irrigation. We removed the buccal cannula, and cut and sealed the arterial cannula. The animal was then placed into aerated seawater until spontaneous ventilation resumed. At this point the fish was returned to the large cable tank.

Statistical analyses

All variables were analysed from 10-min periods of recording at each oxygen level before and after the drug treatment. Measurements were made of breathing frequency, buccal pressure amplitude, blood pressure and heart rate determined from the blood pressure trace. All data were checked for normality using the Shapiro–Wilk test. All statistics were performed in RStudio statistical software (https://www.rstudio.com/). Student's two-tailed *t*-test, two-way MANOVA and the *F*-test were used, and data that were not normally distributed were log transformed. Non-parametric tests were used for data that could not be transformed.

Peak detection software was used to determine the peak systolic pressure associated with each heart beat, and the intervals between beats (here designated as HH intervals) were substituted for RR intervals for the HRV analysis. Although the peaks detected from the pressure traces are not as distinct as those derived from an ECG, the results obtained this way are more than adequate to address the questions we pose in this study. A similar analysis was performed on breath-to-breath variability using the peak buccal pressure associated with each breath (BB intervals).

Poincaré plot analyses were performed using the HRV module in ADInstruments LabChart software. Fig. 1 is a sample Poincaré plot illustrating the HRV in one dogfish before and after atropine administration. The width (SD1) and length (SD2) of the ellipse is determined by the standard deviation of the distance of the points from each axis (Brennan et al., 2001) and are reported as milliseconds.

Power spectral analysis was also performed using the HRV module in ADInstruments LabChart software. The low-frequency (LF) band in our analysis was in the range of 0.04 to 0.15 Hz and the high-frequency (HF) band was in the range of 0.15 to 0.4 Hz. Power has the units of RR interval variation (ms^2).

It is commonly accepted in humans that the SD1 and HF components of the analyses represent the parasympathetic modulation or the short-term vagal control of the heart, and that the SD2 and LF components reflect a combination of sympathetic and parasympathetic influences. Though both methods of computation and analysis are different, it has been shown that they correlate well with each other in humans (Hsu et al., 2012).

All data are available on request.

RESULTS

Cardiorespiratory variables

Heart rate was not affected by hypoxia or hyperoxia, but increased significantly (~55%) with atropine administration across all three oxygen concentrations (two-way MANOVA, treatment, $P<<0.0001$) (Fig. 2A). There was a small degree of hysteresis in the relationship between heart rate and O_2 concentration during progressive hypoxia and recovery (heart rate higher during recovery) that was absent after atropine administration (Fig. 3A). There was also no hysteresis in the relationship between heart rate and oxygen concentration during progressive hyperoxia and recovery (Fig. 3A).

Breathing frequency increased significantly in hypoxia both before (27%) and after (20%) atropine administration (two-way MANOVA, oxygen, $P=0.0002171$; Fig. 2B) and decreased slightly but not significantly in hyperoxia both pre- and post-atropine. The amplitude of the pressure change associated with each breath also increased significantly in hypoxia, decreased modestly but not significantly in hyperoxia and was unaffected by the pharmacological blocker (two-way MANOVA, oxygen, $P=0.04025$; Fig. 2C). There was a small degree of hysteresis in the

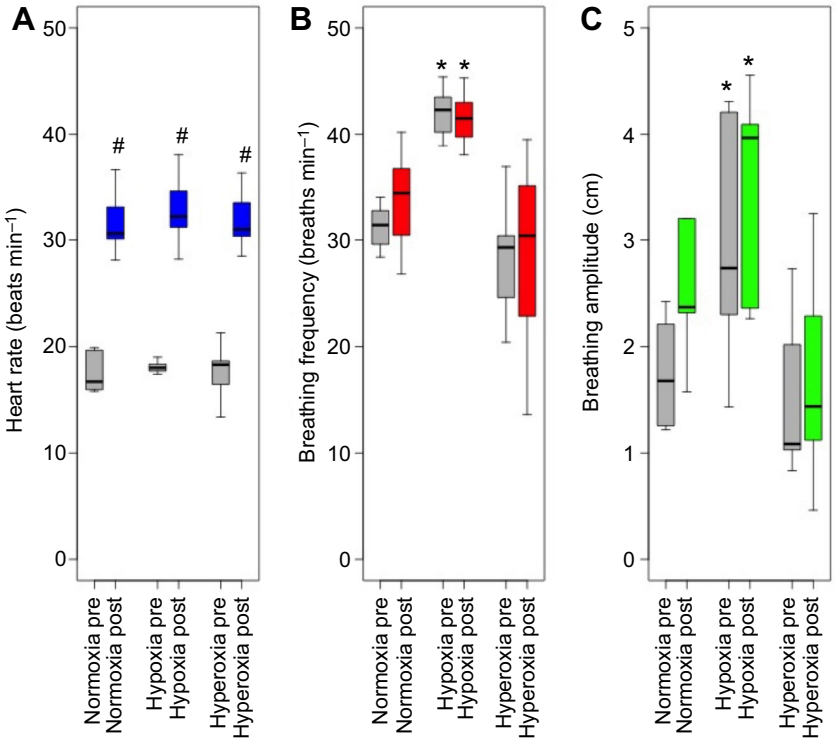

Fig. 2. Cardiorespiratory variables pre- and post-atropine administration in normoxic, hypoxic and hyperoxic conditions. (A) Heart rate, (B) breathing frequency and (C) breathing amplitude. * indicates hypoxia or hyperoxia values significantly different from normoxic values for the same treatment. # indicates post-atropine values significantly different from pre-atropine values for the same O_2 concentration. In all boxplots, the line within the box represents the median value, the ends of the box are the upper and lower quartiles and the whiskers represent the maximum and minimum values.

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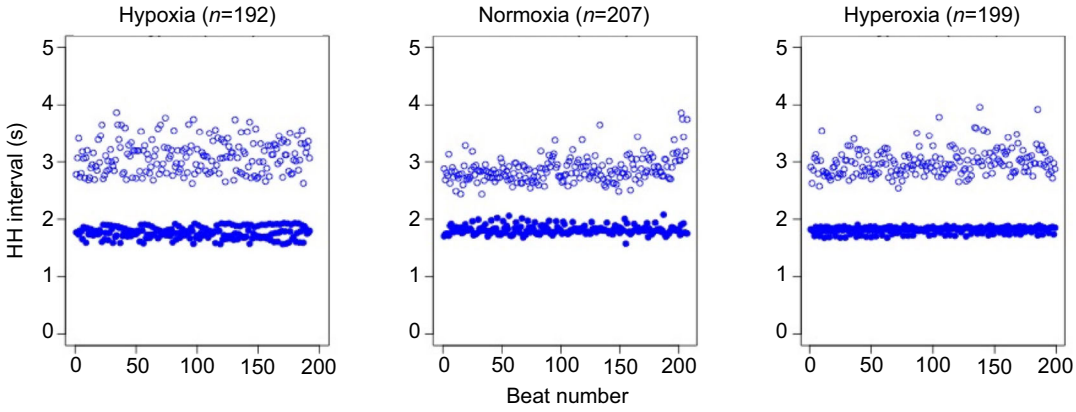

Fig. 3. Tachogram of successive HH intervals for a representative animal (Dogfish 9) in hypoxia, normoxia and hyperoxia pre- (open circles) and post-atropine (closed circles).

relationship between breathing frequency and O_2 concentration during progressive hypoxia and recovery (breathing frequency higher during recovery) that was not altered by atropine administration (Fig. 3B).

Heart rate variability

Heart beat-to-beat (HH) intervals significantly decreased after atropine administration in all oxygen conditions and the variability in these intervals was significantly reduced after pharmacological vagotomy (*t*-test, $P<<0.0001$, *F*-test, $P<<0.0001$; Fig. 4). Atropine administration resulted in a significant decrease (two-way MANOVA, $P<<0.0001$) in HH intervals, as well as in the standard deviation of the HH intervals (SDHH) under all oxygen conditions (Fig. 4B). Furthermore, both the SD1 and SD2 derived from the Poincaré plots and the ratio between the two decreased significantly after atropine injection (*t*-test, $P<0.05$; Fig. 5, Table 1).

Power within the low-frequency (0.04–0.15 Hz) and high-frequency band (0.15–0.4 Hz) and the ratio between them also significantly decreased post-atropine (*t*-test, $P<0.05$; Figs 6 and 7).

Mean BB intervals and SDBB decreased significantly in hypoxic conditions, but did not show any appreciable change after atropine administration (two-way MANOVA, oxygen, $P<<0.0001$; Fig. 8).

Cardiorespiratory synchrony

Cardiorespiratory synchrony is a 1:1 breath-to-heart beat ratio (Satchell, 1961). As demonstrated by Figs 9 and 10, the only situations where the breath-to-heart beat ratio approached 1 was in the normoxic to hyperoxic transition after atropine administration. The breath-to-heart beat ratio for the resting dogfish was closer to 1:2, as can be seen in Fig. 11. Even in instances where there was a 1:1 ratio between the two variables, the relationship drifted in and out of phase, and the peaks of each pulse did not remain aligned for any prolonged period of time.

DISCUSSION

One criticism of our methodology was the short recovery time between surgery and the experimental trials. It has been shown previously, for the shorthorn sculpin, that minor surgery or the

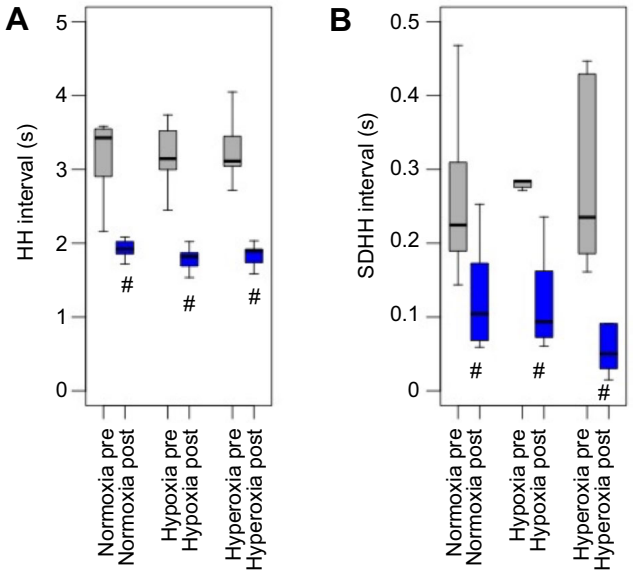

Fig. 4. Heart beat-to-beat (HH) intervals pre- and post-atropine administration in normoxic, hypoxic and hyperoxic conditions. (A) Mean HH intervals. (B) Mean standard deviation of the HH intervals (SDHH). # indicates post-atropine values significantly different from pre-atropine values for the same O_2 concentration. In all boxplots, the line within the box represents the median value, the ends of the box are the upper and lower quartiles and the whiskers represent the maximum and minimum values.

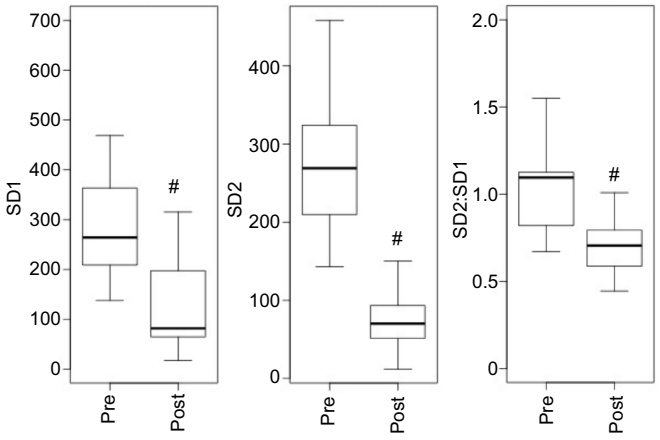

Fig. 5. SD1, SD2 and the ratio SD2:SD1 derived from the Poincaré plots for all dogfish in normoxia pre- and post-atropine. # indicates post-atropine values significantly different from pre-atropine values for the same O_2 concentration. In all boxplots, the line within the box represents the median value, the ends of the box are the upper and lower quartiles and the whiskers represent the maximum and minimum values.

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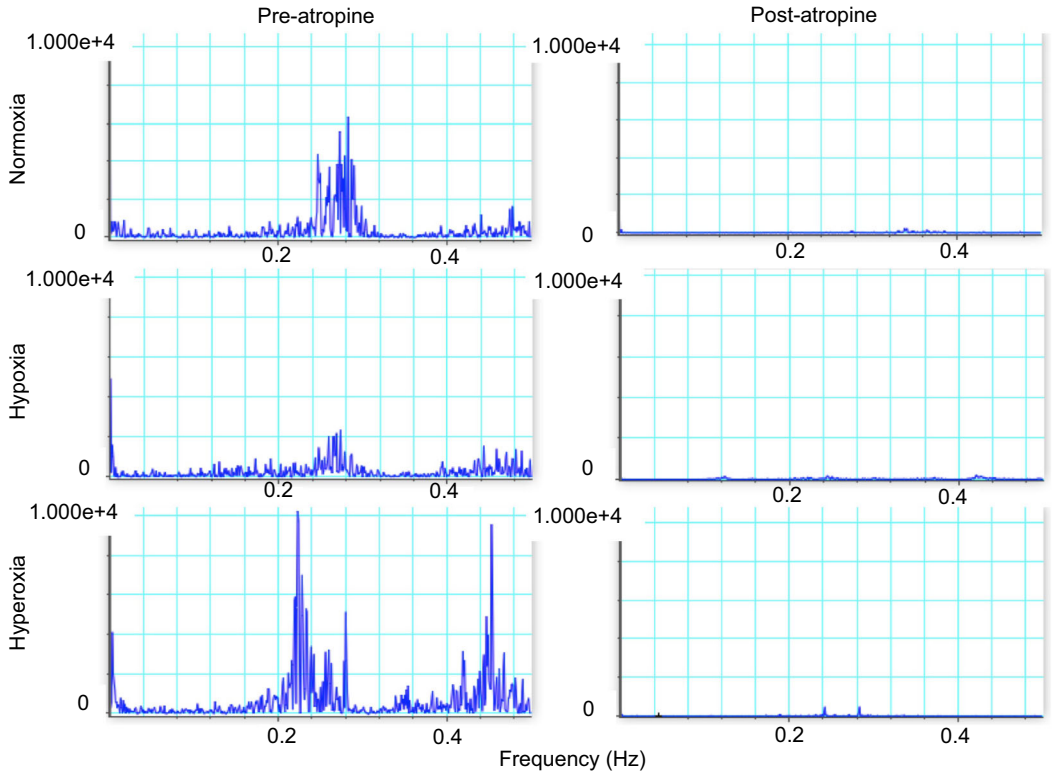

Fig. 6. Mean power spectra for all dogfish in normoxia, hypoxia and hyperoxia pre- and post-atropine.

placement of fish into respirometers produced a prolonged increase in heart rate and abolished the beat-to-beat variability (HRV) in instantaneous heart rate, which was only re-established slowly over 96–120 h (Campbell et al., 2004). Most of our fish were run less than 24 h post-surgery, and the protocol employed continuous progressive exposure to hypoxia and then hyperoxia, followed by injection of atropine (with efficacy judged by the data obtained rather than injection of the agonist) and a repeat of exposures. Our results, however, reveal low heart rates and the presence of established HRV, indicative that heart rate had returned to pre-stress levels. It is also possible, however, that this reflects a new steady-state with elevated levels of stress-related hormones with both positive and negative chronotropic effects, including circulating catecholamines with positive chronotropic effects as well as humoral substances with negative chronotropic effects (e.g. cardiac natriuiretic peptides and nitric-oxide-related compounds). If the latter were the case, however, the atropine administration should have revealed a much higher resting heart rate.

There was no effect of changing oxygen concentration on heart rate in the present study. We did not see a reflex bradycardia in animals held at 9–10°C. This is consistent with a previous study on *Scyliorhinus canicula* in which progressive hypoxia had no effect on heart rate or oxygen uptake at 7°C (Butler and Taylor, 1975), but did result in a bradycardia at 12°C and 17°C. This was not due to a lack of vagal tone. The response of the heart to vagal blockade was a significant increase in heart rate, similar to that seen previously (Butler and Taylor, 1971), reflecting the significant level of vagal tone present under all conditions. The temperatures used in the present study are those of the ambient seawater at this time of year (summer).

Hypoxia did produce a significant increase in breathing frequency and amplitude, whereas hyperoxia produced a modest, non-significant decrease in both variables. Although early studies suggested that dogfish, unlike teleosts, show little or no change in respiratory frequency during hypoxia (Hughes and Umezawa, 1968;

Table 1. SD1:SD2 ratios derived from Poincaré plots in different oxygen treatments, pre- and post-atropine administration in all dogfish

	Oxygen level		
Treatment	Normoxia	Hypoxia	Hyperoxia
Pre-atropine	0.920	1.132	0.855
Post-atropine	1.261	1.826	1.581

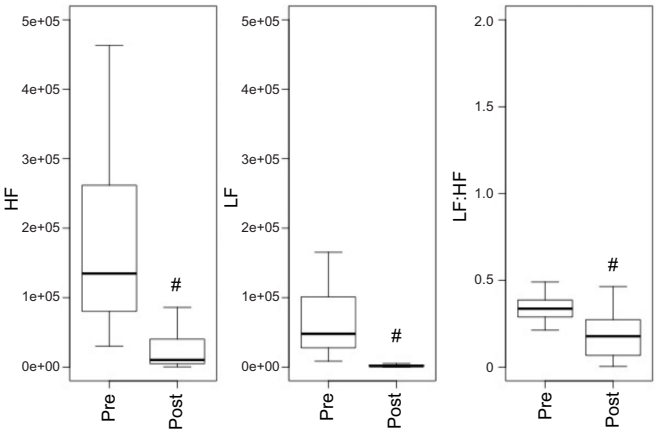

Fig. 7. Mean power in the high- (HF) and low-frequency (LF) bands and the ratio between them derived from all dogfish pre- and post-atropine. # indicates post-atropine values significantly different from pre-atropine values for the same O_2 concentration. In all boxplots, the line within the box represents the median value, the ends of the box are the upper and lower quartiles and the whiskers represent the maximum and minimum values.

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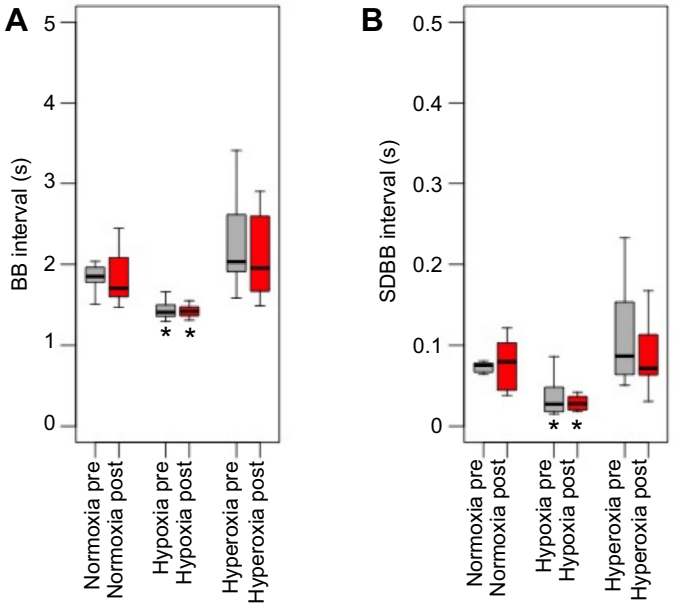

Fig. 8. Breath-to-breath (BB) intervals pre- and post-atropine administration in normoxic, hypoxic and hyperoxic conditions. (A) Mean BB intervals. (B) Mean standard deviation of the BB intervals (SDBB). * indicates hypoxia values significantly different from normoxic values for the same treatment. In all boxplots, the line within the box represents the median value, the ends of the box are the upper and lower quartiles and the whiskers represent the maximum and minimum values.

Piiper et al., 1970; Butler and Taylor, 1971), it was subsequently shown that this likely reflected the effects of stress and confinement and that a significant response was present in unstressed dogfish (Metcalfe and Butler, 1984). This further supports our belief that our fish were sufficiently recovered post-surgery to produce normal responses. These responses were unaffected by the administration of atropine, indicating that neither the afferent neurons responding to oxygen levels nor the efferent neurons responsible for respiratory motor output are adversely affected by atropine, again consistent with previous studies (e.g. Butler and Taylor, 1971). This would suggest that neither the hypoglossal and vagal afferent fibres carrying sensory information from the gills (Milsom, 2012) nor the branches of the VIIth, IXth and Xth cranial nerves innervating the

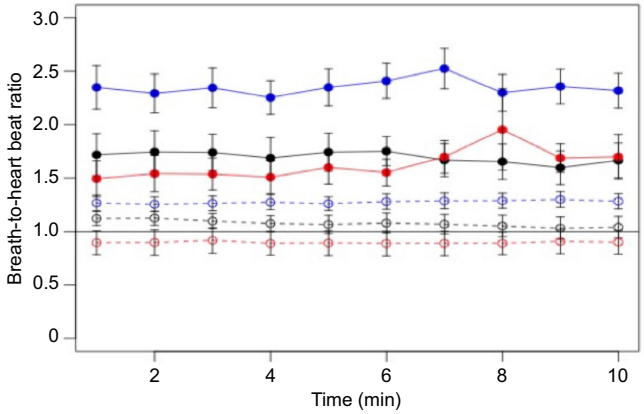

Fig. 9. Mean breath-to-heart beat ratio across all dogfish in normoxia (black), hypoxia (blue) and hyperoxia (red) pre-atropine (solid lines, filled circles) and post-atropine (dashed lines, open circles) for 10 consecutive minutes.

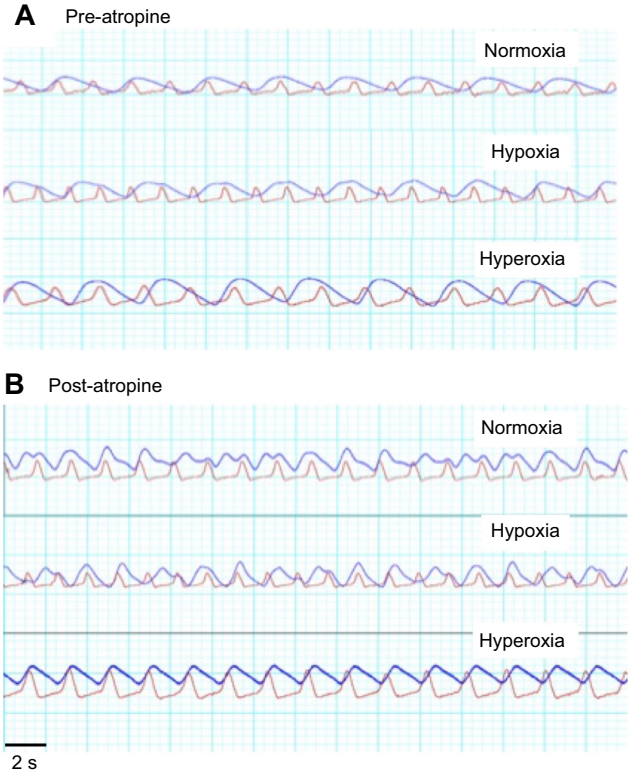

Fig. 10. ECG traces from Dogfish 5 showing the relationship between heart rate (blue) and breathing (red) in all oxygen conditions. (A) Pre-atropine and (B) post-atropine.

respiratory muscles (Butler et al., 1977; Barrett and Taylor, 1985) were affected by atropine.

HRV is the short-term variation that one sees in the heart beat-to-beat intervals. Using HRV to investigate cardiac autonomic modulation has become commonplace in the last decades (Malik, 1998). Fig. 4 visibly demonstrates the change in HRV after atropine administration in an example animal. Blocking the cardiac vagus resulted not only in a mean increase in heart rate owing to the loss of inhibitory innervation, but also a significant reduction in variability (Fig. 6). In most amniotes, either branch of the autonomic nervous system can produce HRV, as can a fluctuating balance between sympathetic and parasympathetic tone (Hsu et al., 2012). HRV analysis in higher vertebrates has been shown to be a useful way of determining this sympathovagal balance. There are many methods of analyzing HRV in both the frequency and time domains that provide non-invasive means of studying the autonomic system (Hsu et al., 2012). In the present study, we carried out both time domain analysis via Poincaré plots as well as frequency domain analysis via power spectral analysis. Both of these methods aim to quantify sympathovagal balance (Hsu et al., 2012). Fig. 1 shows a sample Poincaré plot that demonstrates SD1 and SD2, the indices used to determine sympathovagal balance in the time domain. Power spectral analysis, in contrast, determines sympathovagal balance by looking at the ratio between the high-frequency and low-frequency oscillatory components. It is commonly accepted in humans that the SD1 and HF components of the analyses represent the parasympathetic modulation or the short-term vagal control of the heart, and that the SD2 and LF components reflect a combination of sympathetic and parasympathetic influences. Though both methods of computation and analysis are different, it has been shown that they correlate well with each other in humans (Hsu et al., 2012).

Interestingly, in the present study, atropine greatly reduced both the SD1 and SD2 indices and all but eliminated the LF and HF components of the power spectra (Figs 6–8). These results suggest that parasympathetic tone dominates in both indices of the Poincaré plots and both components of the power spectra in *S. suckleyi*, and are consistent with suggestions that cardioregulation in fish is exclusively controlled through parasympathetic innervation via the cardiac vagus (Campbell et al., 2005).

Previous power spectral studies in fish have found a dual spectral peak in rainbow trout (DeVera and Priede, 1991), sea bream (Altimiras et al., 1995) and shorthorn sculpin (Campbell et al., 2004), but only a single peak in pike and brown trout (Armstrong et al., 1988), ballen wrasse (Altimiras et al., 1995) and Atlantic salmon (Altimiras et al., 1996). This species-specific difference in HRV may reflect differences in the degree of cardiac cholinergic inhibition and/or adrenergic excitation. It has been shown that the degree of cardiac vagal or adrenergic tone on the fish heart varies greatly between species (Taylor, 1992). However, results similar to ours were obtained in the shorthorn sculpin (*Myoxocephalus scorpius*), in which transection of the cardiac branch of the vagus nerve led to a dramatic increase in heart rate and eliminated the dual peaks in the power spectrum, also leading to the conclusion that both are the consequence of a reduction in an inhibitory cholinergic influence on the cardiac pacemaker (Campbell et al., 2004).

Piiper and Scheid (1977) hypothesized that because the flows of both the respiratory medium and the blood are pulsatile, matching of these relative rates would increase oxygen uptake efficiency. If CRS is occurring to maximize oxygen uptake, we would expect to see it in situations of high oxygen demand or reduced oxygen supply. In the dogfish (*Scyliorhinus canicula*), it was found in normoxia in one study under resting conditions (Taylor, 1992) but not in another (Taylor and Butler, 1971). In the pacu (*Piaractus mesopotamicus*), it was most prevalent in mild hypoxia but lost as hypoxia progressed (Leite et al., 2009). In the rainbow trout (*Oncorhynchus mykiss*), CRS was only present when the fish were exposed to severe hypoxia (Randall and Smith, 1967). Although these observations suggest that CRS occurs in many fish species and that the vagus nerve is instrumental in its production, they raise questions as to whether this is a regulated phenomenon or simply an indirect consequence of chemoreceptor-driven changes in the frequencies of these two variables. In *S. suckleyi* under resting normoxic conditions, breathing frequency is higher than cardiac frequency and CRS is not present. A 1:1 coupling ratio was only seen in situations where oxygen supply was high and where atropine had abolished all inhibitory influences on the heart, allowing the heart rate to increase and ventilation frequency to decrease. Even so, the peaks of the pulses did not remain matched for long periods of time, suggesting that the 1:1 coupling occurred by chance, and not in a regulated fashion designed to maximize oxygen uptake efficiency. With the resting heart rates and breathing rates seen in the dogfish used in our experiments, a 1:1 breath-to-heart beat ratio in the absence of atropine would require breathing to slow significantly. There is, of course, the possibility that the animals used for our study were not completely relaxed and fully recovered from surgery (Campbell et al., 2004), and thus maintained higher than resting heart and ventilation rates for the duration of the experiment. If this were the case, however, as animals recovered fully, not only would breathing frequency slow, so too would heart rate. Furthermore, any reflex bradycardia and reflexive increase in breathing frequency associated with hypoxia would further separate the two rates, supporting the conclusion that CRS does not occur in *S. suckleyi* with the purpose of maximizing oxygen uptake efficiency.

In summary, the administration of atropine abolished HRV. Power spectrum analysis, alongside the Poincaré analysis, confirm that all aspects of short-term cardiac regulation are controlled by the parasympathetic nervous system in dogfish. Thus, both the SD2 index of the Poincaré plot and the LF component of the power spectrum are dominated by parasympathetic and not sympathetic activity. Ventilatory responses, in contrast, were not affected by atropine administration. This disparity allowed for the establishment of a transient 1:1 ratio between ventilation and heart rate in situations of high oxygen supply (hyperoxia), and thus low ventilation rate, with the high heart rate post-atropine administration; i.e. cardiac vagal blockade did not abolish, but produced CRS under hyperoxic conditions.

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