



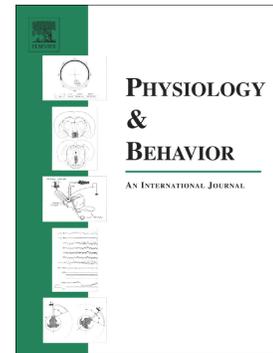
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Habituation of the cold shock response is inhibited by repeated anxiety: Implications for safety behaviour on accidental cold water immersions

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“Habituation of the cold shock response is inhibited by repeated anxiety: implications for safety behaviour on accidental cold water immersions”

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## Abstract

**Introduction.** Accidental cold-water immersion (CWI) triggers the life-threatening cold shock response (CSR) which is a precursor to sudden death on immersion. One practical means of reducing the CSR is to induce an habituation by undergoing repeated short CWIs. Habituation of the CSR is known to be partially reversed by the concomitant experience of acute anxiety, raising the possibility that repeated anxiety could prevent CSR habituation; we tested this hypothesis. **Method.** Sixteen participants (12 male, 4 female) completed seven, seven-minute immersions in to cold water (15 °C). Immersion one acted as a control (CON1). During immersions two to five, which would ordinarily induce an habituation, anxiety levels were repeatedly increased (CWI-ANX<sub>rep</sub>) by deception and a demanding mathematical task. Immersions six and seven were counter-balanced with another high anxiety condition (CWI-ANX<sub>rep</sub>) or a further control (CON2). Anxiety (20 cm visual analogue scale) and cardiorespiratory responses (cardiac frequency [ $f_c$ ], respiratory frequency [ $f_R$ ], tidal volume [ $V_T$ ], minute ventilation [ $\dot{V}_E$ ]) were measured. Comparisons were made between experimental immersions (CON1, final CWI-ANX<sub>rep</sub>, CON2), across habituation immersions and with data from a previous study. **Results.** Anxiety levels were sustained at a similar level throughout the experimental and habituation immersions (mean [SD] CON1: 7.0 [4.0 cm]; CON2: 5.8 [5.2] cm *cf* CWI-ANX<sub>rep</sub>: 7.3 [5.5] cm;  $p > 0.05$ ). This culminated in failure of the CSR to habituate even when anxiety levels were not manipulated (i.e. CON2). These data were different ( $p < 0.05$ ) to previous studies where anxiety levels were allowed to fall across habituation immersions and the CSR consequently habituated. **Discussion.** Repeated anxiety prevented CSR habituation. A protective strategy that includes inducing habituation for those at risk should include techniques to lower anxiety associated with the immersion event or habituation may not be beneficial in the emergency scenario.

**Key words:** Drowning, cold water, perception, cold shock.

## 1.0 Introduction

A conservative estimate suggests at least 372,000 people drown worldwide each year by accidentally entering water and failing to defend their airway against water ingress [1]. If the water is cold, the physiological responses evoked during the first few minutes of whole body cold water immersion (CWI) are life threatening [2] and are strongly implicated in this drowning statistic [3]. The initial responses to CWI include an “inspiratory gasp,” hyperventilation, tachycardia, peripheral vasoconstriction and hypertension, and are collectively known as the ‘Cold Shock’ response (CSR; [3]). The hyperventilatory component of the CSR significantly decreases maximum breath hold time in the majority of participants, thus increasing the chances of involuntarily aspirating water and drowning [4]; this represents a further hazard to that posed by the high cardiovascular strain [5]. The current behavioural recommendation to survive acute accidental CWI is to “float first and kick for your life” on the basis that the added buoyancy can enable greater freeboard (distance from water level to mouth of the victim) and the onset of leg-only exercise leads to a more rapid restoration of cerebral blood flow after its hyperventilation induced reduction on cold water entry [6]. The CSR subsides after the initial peak in the first two to three minutes following which swimming to safe refuge may become possible [7,8], with leg kicking being preferable to achieve this propulsion and to minimise heat loss [9].

For those at daily risk of accidental immersion (e.g. those undertaking leisure activities close to water, fishermen, aircrew or marine personnel) it is wise to take practical and safety precautions. One such precaution is to wear protective clothing to prevent rapid skin cooling on water entry, yet this is not always practical or logistically feasible [10,11]. An alternative is to reduce the extent of the CSR by inducing an habituation of the response; habituation is defined as reduced response to a stimulus of the same magnitude [12]. This can be achieved

by undergoing a series of cold-water immersions which has shown to induce an habituation after as few as four short (three or five-minute) exposures on consecutive days [13,14]. Indeed an habituation of the CSR reduces the respiratory portion of the CSR by approximately 44% and the extent of tachycardia by approximately 22% [15]. The benefit of this reduction is retained fully for seven months after consecutive exposures and is partially retained for up to 14 months [15]. Hence, retaining an habituation is not a labour intensive process and is practically feasible. Theoretically, reducing the CSR may confer some benefit to defending the airway in the emergency scenario as the hyperventilatory drive seen in unhabituated participants is significantly reduced [13].

The variation between individuals in the CSR on initial immersion and its habituation is large, and recent evidence suggests it may be strongly influenced by psychological state both prior to, and during a CWI [14,16-18]. Indeed, it has been shown that there are salient moderating influences on the extent of the CSR which are, at least in part, caused by high by contrast to low levels of anxiety [17]. The available evidence suggests that acute anxiety can significantly increase the magnitude of the CSR in unhabituated participants and partially reverse the habituation in those who have completed repeated CWIs [17]. Conversely, anxiety associated with the immersion scenario *per se* can be reduced by repeatedly experiencing the immersion sequence (i.e. repeated thermoneutral water immersion; 35°C) in the absence of a repeated cold-water stimulus. One consequence of this lowered anxiety was a partially reduced ventilatory (i.e. tidal volume;  $V_T$ ) response to CWI [18]. Accordingly, we concluded that repeated immersion in thermoneutral water induces a perceptual habituation of the threat posed by imminent immersion and this confers some benefit even when the water temperature is cold.

Collectively these data raise the possibility that it is the degree of the anxiety experienced prior to and during an immersion that determines if habituation occurs, with low levels of anxiety enabling habituation and high, continuous levels preventing it. The latter suggestion has yet to be examined experimentally probably because of the difficulty in sustaining high levels of anxiety throughout a series of experimental immersions. It is possible that the concomitant experience of anxiety disinhibits the transmission of thermal afferent information such that it magnifies the CSR response or prevents habituation [19]. Accordingly the present study examined the possibility that the repeated experience of anxiety during a series of cold-water habituation immersions prevents significant habituation of the CSR. These data will be compared to those from our previous study where habituation was achieved and subsequently reversed by the induction of acute anxiety on immersion after cold-water habituation had taken place [17].

We hypothesised that low levels of acute anxiety are permissive of CSR habituation, but heightened anxiety, maintained throughout the duration of the immersion and the series of habituating immersions, would prevent habituation occurring ( $H_1$ ). Similar to our previous studies, deception about the water temperature was used to elevate anxiety. In addition to this an anxiety-inducing maths task, with the punitive consequences of poor performance leading to an extended immersion duration, was also undertaken to elongate the anxiety that was induced.

## **2.0 Materials and Methods**

The Research Ethics Committee of Portsmouth University granted ethical approval for the study which was performed in accordance with the ethical standards of the 1964 Declaration

of Helsinki. The participants gave their written informed consent to participate in cold-water immersion experiments lasting up to seven minutes.

## 2.1 Experimental Design

The present study utilized a within participant repeated measures design with between groups comparisons also made to data from a previous study [17]. Two groups were tested:

Group 1 – Repeated Anxiety & CWI (CWI-ANX<sub>rep</sub>): Participants undertook seven, seven-minute CWIs (water temperature;  $T_w$  15°C). Immersion one was used to establish the extent of the CSR and acted as a control (CON1). During immersions two to five the participants' anxiety levels were raised using deception and a demanding maths task (see below). Immersions six and seven were counter-balanced to include one further anxiety inducing immersion (CWI-ANX<sub>rep</sub>) and one further control where no anxiety inducing manipulations were undertaken (CON2).

Group 2 – Acute Anxiety & CWI (CWI-ANX<sub>ac</sub>). Participants undertook seven, seven-minute CWIs (15°C). Immersion one was used to establish the extent of the CSR and acted as a control (CON1). Immersions two to five were conducted without intentionally increasing anxiety. Immersions six and seven were counter-balanced to include one acute anxiety inducing immersion (CWI-ANX<sub>ac</sub>) by way of deception about the water temperature only and one control where no anxiety inducing manipulations were undertaken (CON2); these data were drawn from our previous work ([17]; study 2). All experimental immersions (i.e. in both groups) were standardised; they took place at the same time of day (within-participant), with a minimum of 24 hours and a maximum of 48 hours between immersions, were to the same depth and each lasted 7-minutes. Figure 1 shows the order of the experimental conditions in each group.

\*\*\*INSERT FIGURE 1 NEAR HERE\*\*\*

## 2.2 Participants

### 2.2.1 Common Characteristics

The participants were non-smokers and were not cold water habituated. They abstained from alcohol and caffeine consumption for 24 hours before each test and from undertaking any exercise on the day of the test.

### 2.2.2 Group Specific Characteristics

Group 1 - CWI-ANX<sub>rep</sub>. Sixteen healthy participants (12 male, 4 female) volunteered for the experiment. Their physical characteristics were (mean [SD]): Age 21 [2] yrs; height 1.76 [0.1] m; mass 78.0 [18.0] kg; sum of skinfold 42 [18] mm).

Group 2 – CWI-ANX<sub>ac</sub>. Ten healthy participants (6 male, 4 female) volunteered for the experiment. Their physical characteristics were (mean [SD]): Age 19 [2] yrs; height 1.74 [0.1] m; mass 77.6 [16.8] kg). An independent samples t-test verified that the groups did not differ based on their physical characteristics of height ( $p = .549$ ) and mass ( $p = .949$ ).

### Procedure 2.3

Following arrival at the Extreme Environments Laboratory, each participant's height (m) and mass (kg) was recorded using a stadiometer (Bodycare Stadiometer, Leicester, U.K) and calibrated weighing scales (OHAUS digital weighing scales, New Jersey, USA). Each participant changed into their swimming costume. Males wore swimming trunks and females wore a swimsuit; the same swimming costume was worn by each participant on each occasion. Participants were then instrumented with a 3-lead ECG (HME Lifepulse, England) and entered an ambient temperature ( $T_a$ ) controlled laboratory. They sat on an immersion

chair attached to an electronic winch (CPM, F1-8; 2-8; 5-4, Yale, Shropshire, U.K) with a seat belt fastened around their waist to counteract buoyancy on immersion. The participant inserted a two-way mouthpiece (Harvard, USA) and attached a noseclip. The mouthpiece was connected to a spirometer (spirometric transducer module, KL Eng. Co, Northridge, USA) by respiratory tubing in order to measure the respiratory responses to immersion. The participant was winched above the immersion tank to rest for one-minute. Thirty seconds into the one-minute rest period participants provided a rating of their state anxiety on a visual analogue scale; they were familiarised with the scale in advance of the study. Towards the end of the one-minute period a ten-second verbal countdown preceded the participant being lowered at a reproducible rate ( $8 \text{ m}\cdot\text{min}^{-1}$ ) until immersed to the clavicle in stirred water. After one, three, five and seven-minutes of immersion they again reported their anxiety rating, following which they were winched from the immersion tank.

Prior to immersion number two, and before inserting their respiratory mouthpiece, the participants completed a demanding maths task and were told that the water would be  $1 \text{ }^{\circ}\text{C}$  colder than the previous immersion. This was done to induce additional anxiety about the impending immersion; however, the water temperature for *all* immersions was  $15 \text{ }^{\circ}\text{C}$  and was carefully controlled throughout but remained concealed from the participants. The demanding maths task was performed prior to immersions two to five and before either immersion six or seven which were counter-balanced to either maximise (i.e. maths task plus deception) or minimise (i.e. no maths and no deception) the extent of anxiety experienced during immersion.

### **2.3.1 Anxiety-Inducing Maths Task**

Participants completed a performance-based mental arithmetic task for three-minutes. Similar tasks have been shown to induce a cognitive and somatic anxious response [20]. Before the maths task was undertaken, the participants were informed that the immersion duration would be a minimum of three-minutes. Each incorrect answer led to an additional minute of immersion to complete; the maximum immersion time was limited to seven-minutes and a total of four mistakes were allowed. The task was to answer basic addition, subtraction, multiplication and division equations which contained a mixture of two and three digits in the question and answer. The task was performed to a metronome with an answer frequency of one answer every 3 seconds with a total of 60 answers to be given over the three-minute period. Pilot tests verified the logistical and experimental viability of this design. After the three-minute maths task was complete the participant self-inserted the equipment for measurement of respiratory parameters. As the end of the three-minute mandatory immersion period approached (i.e. in the last ten seconds of the third minute) the experimenter explained that the participant gave ‘at least one incorrect answer’ on the maths task signalling that one additional minute would be spent in the water. Subsequently, as the end of the fourth minute approached the experimenter again explained that ‘they gave at least two incorrect answers’ which would carry the same consequence. It was the intention to make the task performance deliberately difficult in order that the full seven-minute immersion was undertaken and to prolong the anxiety associated over the duration of the immersion.

## **2.4 Measurements**

### **2.4.1 Environmental Conditions**

$T_a$  and  $T_w$ , were measured and recorded using a calibrated thermistor (Grant Instruments (Cambridge) Ltd, Shepreth, U.K) secured to the wall of the immersion tank and a Wet Bulb

Globe Thermometer station respectively, both attached to a data logger (1000 series, Squirrel Data Logger, Grant Instruments (Cambridge) Ltd, Shepreth, U.K). Average  $T_w$  was closely matched within participant ( $\pm 0.2^\circ\text{C}$ ) between CON1, CON2 and the final CWI-ANX<sub>rep</sub> immersion and was;  $T_w$  CON1 15.2 [0.2] $^\circ\text{C}$ , CON2 14.8 [0.3] $^\circ\text{C}$ , CWI-ANX<sub>rep</sub> 14.8 [0.2] $^\circ\text{C}$ . The average  $T_a$  during the CWIs was: CON1 24.0 [2.5] $^\circ\text{C}$ , CON2 24.0 [2.4] $^\circ\text{C}$ , CWI-ANX<sub>rep</sub> 24.2 [2.3] $^\circ\text{C}$ .  $T_w$  and  $T_a$  during immersions two to five averaged 15.1 [0.1] $^\circ\text{C}$  and 24.0 [0.1] $^\circ\text{C}$  respectively across the four CWI-ANX<sub>rep</sub> immersions.

#### 2.4.2 Cardiorespiratory Responses

The ECG and spirometer were interfaced with a digital data acquisition system (16SP PowerLab, Castle Hill, Australia) which captured data continuously throughout the rest and immersion periods. Chart analysis software (Chart version 6, AD Instruments Ltd, Oxford, U.K) was used to automatically identify R-waves from the ECG and calculate cardiac frequency ( $f_c$ ); movement artefacts were visually identified and excluded from analysis. The spirometer was calibrated using a syringe of known volume (3 L syringe, Harvard Instruments, Harvard, USA). Respiratory frequency ( $f_R$ ) was recorded by Chart analysis software using auto-recognition of the peak after inspiration. The peak value after the onset of inspiration was recorded as tidal volume ( $V_T$ ) and multiplied by the calculated  $f_R$  to generate minute ventilation ( $\dot{V}_E$ ).

#### 2.4.3 Anxiety Perceptual Responses

The state anxiety response to immersion was quantified using a 20 cm visual analogue scale (VAS) with descriptive phrases ranging from 0cm (*not at all anxious*) to 20cm (*extremely anxious*). This previously validated scale [22] has been used to quantify state anxiety [20,21] with the instrument's reliability and validity receiving support. Participants reported their

anxiety by drawing a horizontal line on the vertical scale (see example in figure 2 y axis) that corresponded to their feeling of anxiety. The scale is anchored by worded descriptors demarking the extremes of anxiety (0 cm – *not at all anxious* and 20cm – *extremely anxious*) and the numerical value for anxiety is generated by measuring from the zero point of the scale to where the horizontal line begins. In order to classify the participants' levels of trait anxiety each participant completed a state-trait anxiety inventory (STAI; [23]). This was undertaken for exploratory purposes in group 1 only.

#### **2.4.5 Semi-structured interview and debrief**

At the end of the experiment participants in group 1 took part in a semi-structured interview and were asked a) whether they perceived the water to be colder during either of the final two immersions b) whether they felt more anxious prior to it c) if they thought at any stage the water was not colder and d) if so, at what stage. The participants were then debriefed about the aims of the experiment.

#### **2.4.6 Procedures: Group 2 – CWI-ANX<sub>ac</sub>**

A full description of the experimental procedures undertaken in this group is reported in Barwood et al [17]. Briefly, the cardiorespiratory and perceptual measurements were the same as described here. Nevertheless, the participant's were only deceived about the water temperature on one occasion after a series of habituating immersions, thereby inducing acute anxiety about that specific immersion (i.e. CWI-ANX<sub>ac</sub>); see Figure 1 for experimental design for each group.

## 2.5 Data Analyses – Common Features

Normality of data were checked using the Kolmogorov-Smirnov test. Univariate analyses were checked for sphericity using Mauchley's test and the Greenhouse-Geisser adjustment applied where non-spherical data sets were evident. The direction of statistically significant effects were determined using a *post-hoc* pair-wise comparisons procedure with *Bonferroni* correction for multiple comparisons where necessary. For all statistical tests  $\alpha$  level was set at 0.05. Data are presented as mean [SD]. All statistical tests were conducted using SPSS version 21 (Chicago, IL, USA).

### 2.5.1 Data Analyses – Group 1 Only

Mean [SD] data were calculated for each minute of immersion for the cardiorespiratory and perceptual variables which included  $f_c$ ,  $f_R$ ,  $V_T$ ,  $\dot{V}_E$  and the anxiety ratings respectively. The peak cardiorespiratory responses were manually checked and recorded for  $f_c$  and  $f_R$ . Comparisons were made for the peak and mean responses seen in CON1, CON2 and CWI-ANX<sub>rep</sub> within participant, across condition (3) and time (7; mean data only), using a repeated measures analysis of variance (ANOVA).

### 2.5.2 Between Group Comparisons

Comparisons were made between the two groups (2) across immersions (7) in the perceptual and cardiorespiratory variables  $f_c$ ,  $f_R$ ,  $V_T$ ,  $\dot{V}_E$ . These variables were averaged across the immersions to reduce the complexity of the analysis. The mean [SD] data analysed here were not reported in our previous study [17]. *Post-hoc* comparisons between groups were made using a *Scheffe* test as participants in each group were different in number.

## 3.0 Results – Group 1 Only

Errors in data capture occurred for some variables culminating in the removal of some data from analysis: of anxiety ratings ( $n = 15$ ),  $f_c$  mean and peak ( $n = 15$  &  $16$ ),  $f_R$ ,  $\dot{V}_E$  (experimental immersions  $n = 16$ , habituation immersions  $n = 14$ ), and  $V_t$  (experimental immersions  $n = 16$ , habituation immersions  $n = 13$ ).

### 3.1.1 Anxiety Ratings – Habituation Immersions

Statistical analysis verified that the CWI-ANX<sub>rep</sub> intervention was successful at sustaining the anxiety ratings at a similar level throughout the habituation immersions; no main effect for condition ( $F_{(3, 42)} = .694$ ;  $p = .510$ ) or interaction effect ( $F_{(9, 126)} = 1.304$ ;  $p = .280$ ). The levels of anxiety during the habituation immersions initially peaked in the first minute of immersion and declined significantly on each subsequent data point until the fifth minute of immersion following which no differences were seen; main effect for time ( $F_{(3, 42)} = 12.116$ ;  $p = .001$ ).

### 3.1.2 Anxiety Ratings – Experimental Immersions

Similar to the habituation immersions, the anxiety ratings seen across the experimental immersions peaked on immersion and declined significantly with each subsequent time point but continued to do so until the end of the immersion; main effect for time ( $F_{(3, 42)} = 11.105$ ;  $p = .001$ ). On average, the extent of the anxiety reported throughout the immersion did not differ between each condition despite the different experimental manipulations of anxiety levels; no main effect for condition ( $F_{(2, 28)} = 11.105$ ;  $p = .147$ ). The extent of the anxiety reported on immersion demonstrated a different pattern across time in each condition (interaction effect:  $F_{(6, 84)} = 2.318$ ,  $p = .040$ ). *Post hoc* analysis indicated that the anxiety levels tended to be higher in CWI-ANX<sub>rep</sub> condition than CON2 after 1,3 and 5 minutes of immersion ( $p = .005$ ,  $.019$  &  $.013$  respectively). CON1 and CON2 were only different at 3

minutes of immersion ( $p = .042$ ). Mean [SD] anxiety responses on immersion are shown in Figure 2.

\*\*\*INSERT FIGURE 2 NEAR HERE\*\*\*

### 3.1.3 Peak Cardiorespiratory Responses Prior and on Immersion

In anticipation of immersion (i.e. prior to water entry)  $f_c$  was significantly lower (main effect for condition:  $F_{(2, 30)} = 5.118$ ;  $p = .024$ ) in the CON2 condition (96 [13] b.p.m<sup>-1</sup>) compared to CWI-ANX<sub>rep</sub> (109 [20] b.p.m<sup>-1</sup>) but not CON1 (102 [15] b.p.m<sup>-1</sup>;  $p = .018$  &  $.093$ ). CON1 and CWI-ANX<sub>rep</sub> did not approach being different ( $p = .648$ ). On immersion these differences were reflected numerically (CON1 120 [12] b.p.m<sup>-1</sup>; CON2 112 [15] b.p.m<sup>-1</sup> & CWI-ANX<sub>rep</sub> 117 [17] b.p.m<sup>-1</sup>) but not statistically (no main effect for condition:  $F_{(2, 30)} = 2.361$ ;  $p = .112$ ).

### 3.1.4 Mean Cardiac Frequency $f_c$ Responses

Throughout the habituation immersions  $f_c$  showed consistent time effects peaking in the first three minutes of immersion and declining thereafter (main effect for time:  $F_{(6, 90)} = 23.486$ ;  $p = .001$ ). Contrary to the anxiety ratings data, there were differences between conditions (main effect for condition:  $F_{(3, 45)} = 4.248$ ;  $p = .012$ ) with immersion 3 showing a lower average  $f_c$  (by 5 [3] b.min<sup>-1</sup>) than immersion 2 ( $p = .026$ ) but not 4 ( $p = .851$ ); there were no further differences. There was no difference in the pattern of the response in each condition across time (no interaction effect;  $F_{(18, 270)} = 1.488$ ;  $p = .193$ ).

In the experimental immersions a similar response over time was seen (main effect for time:  $F_{(6, 90)} = 25.055$ ;  $p = .001$ ) but there were no differences in the  $f_c$  response, on average, between any of the individual conditions (no main effect for condition:  $F_{(2, 30)} = 2.645$ ;  $p =$

.092) and no difference in the pattern of the response over time (no interaction effect:  $F_{(12, 180)} = 2.109$ ;  $p = .084$ ); this was despite there being a different pattern in the anxiety response with higher anxiety ratings (interaction effect) in CWI-ANX<sub>rep</sub> than CON2. Mean [SD]  $f_c$  data are shown in figure 3A for the experimental immersions and Table 1 for the habituation immersions.

\*\*\*INSERT FIGURE 3 NEAR HERE\*\*\*

### 3.1.5 Respiratory Frequency ( $f_R$ ), Tidal Volume ( $V_T$ ) & Minute Ventilation ( $\dot{V}_E$ )

Analysis of the respiratory data indicated that an habituation of the response did not occur for any of the respiratory variables (no main effect for condition:  $f_R F_{(3,39)} = .781$ ,  $p = .084$ ;  $V_T F_{(3,36)} = .738$ ,  $p = .536$ ;  $\dot{V}_E F_{(3,36)} = .366$ ,  $p = .778$ ). Similarly, during the experimental immersions there were no differences between any of the conditions (no main effect for condition:  $f_R F_{(2,30)} = .188$ ,  $p = .829$ ;  $V_T F_{(3,36)} = .950$ ,  $p = .398$ ;  $\dot{V}_E F_{(2,30)} = 1.442$ ,  $p = .252$ ). The pattern of the response remained consistent for all variables during both the habituation immersions (main effect for time:  $f_R F_{(6,90)} = 25.946$ ,  $p = .001$ ;  $V_T F_{(6,72)} = 7.108$ ,  $p = .001$ ;  $\dot{V}_E F_{(6,72)} = 25.480$ ,  $p = .001$ ) and the experimental immersions (main effect for time:  $f_R F_{(6,90)} = 25.946$ ,  $p = .001$ ;  $V_T F_{(6,90)} = 10.943$ ,  $p = .001$ ;  $\dot{V}_E F_{(6,90)} = 33.955$ ,  $p = .001$ ) peaking during the initial minutes with a decline in the response seen to the 4<sup>th</sup> or 5<sup>th</sup> minute following which no differences were seen. This did not culminate in an interaction effect in any of the variables during habituation immersions ( $f_R F_{(18,234)} = .977$ ,  $p = .487$ ;  $V_T F_{(18,216)} = .835$ ,  $p = .657$ ;  $\dot{V}_E F_{(18,216)} = .733$ ,  $p = .775$ ) or the experimental immersions ( $f_R F_{(12,180)} = .568$ ,  $p = .866$ ;  $V_T F_{(12,180)} = .693$ ,  $p = .757$ ;  $\dot{V}_E F_{(12,180)} = .597$ ,  $p = .843$ ). This was despite there being a different pattern in the anxiety response with higher anxiety ratings (interaction effect) in

CWI-ANX<sub>rep</sub> than CON2. Mean [SD] respiratory data are shown in figure 3B-D for the experimental immersions and table 1 for the habituation immersions.

\*\*\*INSERT TABLE 1 NEAR HERE\*\*\*

### 3.1.6 Semi-structured Interview Responses

Of the 16 participants tested, 13 participants stated they felt colder during one of the experimental immersions with 10 of those 13 participants suggesting that the coldest condition was the final immersion with the maths test (i.e. the experimental immersion CWI-ANX<sub>rep</sub>); despite the  $T_w$  being closely matched between experimental immersions. Participants stated that the sensation of being colder occurred before (2 participants), during (6 participants) and after (2 participants) the maths test was undertaken. One participant remarked that the maths test helped distract them from the CWI and one remarked they felt “quite relaxed about the maths test” indicating some variability in the subjective response to the maths task.

### 3.1.7 Between Group Analysis Anxiety Response

The differing manipulations of anxiety in each study revealed that the absolute levels of anxiety were higher in the CWI-ANX<sub>rep</sub> group than the CWI-ANX<sub>ac</sub> group (main effect for group:  $F_{(1,23)} = 7.342$ ,  $p = .013$ ) and a different pattern was evident in the response between the immersions (interaction effect:  $F_{(6,138)} = 4.358$ ,  $p = .007$ ). *Post-hoc* analysis revealed a mean difference in anxiety ratings, irrespective of the immersions, of 4.3 [1.6] cm (i.e. 22% of the VAS scale) between the groups. While there were no differences observed in the extent of anxiety reported between immersion 1 and 2, differences were observed between immersion 3 ( $p = .009$ ), immersion 4 ( $p = .005$ ) and the intervention immersion (i.e. CWI-

$ANX_{rep}$  cf  $CWI-ANX_{ac}$ ;  $p = .003$ ). No differences across immersion were observed for CON2 ( $p = .114$ ). On each occasion the response of the  $CWI-ANX_{rep}$  group was higher; see Figure 4.

\*\*\*INSERT FIGURE 4 NEAR HERE\*\*\*

### 3.1.8 Mean Cardiorespiratory Responses

Mean  $f_c$  data indicated that the differing manipulations of anxiety rating did not produce differences in the  $f_c$  response between the two groups ( $F_{(1,24)} = .255$ ,  $p = .618$ ). As the series of immersions progressed there was no difference in the pattern of the  $f_c$  response with both groups showing a similar pattern from their first immersion through to their last, irrespective of their experimental manipulation (no interaction effect:  $F_{(6,144)} = 1.894$ ,  $p = .086$ ). The respiratory frequency data showed a different response with significant differences between groups on average (main effect for group:  $F_{(1,22)} = 5.600$ ,  $p = .027$ ) and an interaction as the immersion series progressed ( $F_{(1,22)} = 267.965$ ,  $p = .001$ ). The  $CWI-ANX_{rep}$  group experienced higher  $f_R$  overall but the groups were not different at the start of the immersion series with the  $f_R$  response significantly elevated in the  $CWI-ANX_{rep}$  group in immersions 4 ( $p = .007$ ) and CON2 ( $p = .041$ ) and nearing being statistically different in immersion 5 ( $p = .053$ ). Importantly,  $f_R$  was not different between groups when anxiety levels were manipulated to be increased in both groups ( $p = .159$ ). The  $\dot{V}_E$  data mirrored these effects being higher, on average, in the  $CWI-ANX_{rep}$  group ( $F_{(1,21)} = 4.928$ ,  $p = .038$ ) and as the immersion series ensued (interaction effect:  $F_{(1,21)} = 171.468$ ,  $p = .001$ ). The  $\dot{V}_E$  was not higher in CON1 (i.e. the first immersion) but was consistently higher in the  $CWI-ANX_{rep}$  group in immersions 4 ( $p = .049$ ), 5 ( $p = .011$ ) and CON2 ( $p = .016$ ) but were not different when the anxiety levels were manipulated to be increased in both groups ( $p = .083$ ).  $V_T$  was

not different between group (no main effect for group:  $F_{(1,21)} = 1.531$ ,  $p = .230$ ) or as the immersion series ensued (no interaction effect:  $F_{(1,21)} = 1.425$ ,  $p = .294$ ) suggesting the differences in  $\dot{V}_E$  were achieved largely by changes in  $f_R$  rather than tidal ventilation. The differences in the cardiorespiratory responses between group across the immersion series are summarised in Figure 5.

\*\*\*INSERT FIGURE 5 NEAR HERE\*\*\*

#### 4.0 Discussion

This study tested the hypothesis that the concomitant experience of increased anxiety maintained throughout a series of immersions that would ordinarily have induced an habituation of the CSR [13-15,17], would prevent the habituation occurring ( $H_1$ ). Our data show that the habituation of the CSR did not occur when participants' anxiety levels were kept high and the CSR did not reduce in the CWI-ANX<sub>rep</sub> group even when high anxiety levels were not induced in a subsequent immersion (i.e. CON2; Figures 2 & 3); we therefore accept the experimental hypothesis. We contrasted these new data to our previous study where we showed that low(er) levels of state anxiety are permissive of CSR habituation. In our previous study we showed that when high(er) levels of state anxiety were induced *after* habituation the CSR was partially reversed but this effect was largely evident in the  $f_c$  component of the response. Collectively we suggest that anxiety levels are integrally linked to habituation of the CSR and the degree of anxiety experienced either accentuates or attenuates components of the CSR and could effect the likelihood that habituation will be achieved or retained.

We are not the first to suggest that habituation of the CSR is linked to the concomitant experience of anxiety. Indeed, Glaser et al [19] showed that habituation of the cold pressor

response can be reversed or modified by other afferent impulses, for example by acute anxiety caused by being observed by an audience, arriving from sensory organs at the same time as the thermal stimulus. Yet we are the first to show that habituation of the CSR is abolished when acute anxiety is increased throughout a series of whole body immersions; Glaser and colleagues' [19] observations were restricted to hand immersion. We are also the first to confirm that it is the concomitant stimulus of lowered anxiety ratings *combined* with the cold water stimulus that culminates in CSR habituation rather than a largely independent contribution by each. This interpretation has been supported by a more recent study where acute anxiety associated with the immersion scenario *per se*, in the absence of a repeated cold-water stimulus, culminated in only minor reductions in the CSR [18]. A study where state anxiety is abolished prior to repeated CWI would confirm the additive contribution state anxiety makes to the CSR; although this may be difficult to undertake experimentally.

The present study's evidence that at least one of the cardiorespiratory components (i.e.  $f_c$ ,  $f_R$ ,  $V_T$ , &  $\dot{V}_E$ ) of the CSR did not change across the immersion series, in line with changes in anxiety level, is inconsistent with our previous findings [17]. Based on the differences seen between our past and present studies we can now suggest an important mediating role for anxiety levels and attentional focus in permitting the occurrence of habituation as we believe that this is the distinguishing feature between our methods. In the present study we show that anxiety, and speculate that attention allocated to the distracting maths task, was maintained at an artificially higher level than would be seen in our previous study [17] thereby preventing any habituation of any of the components of the CSR. In our previous study we see that during the habituation immersions, when attention could be allocated to relevant cues in the environment and anxiety levels were allowed to fall, the majority of cardiorespiratory components of the CSR also reduced. When anxiety levels were increased *after* habituation

had taken place only the cardiac component of the CSR increased significantly [17] as learned control of ventilation had been partially achieved. Yet, in a separate group of participants, we showed that *both* cardiac and respiratory components of the CSR increase when anxiety levels are increased *before* habituation had taken place as voluntary control of ventilation has not yet been achieved in this scenario [17]. We therefore suggest that the respiratory component of the CSR is learned over the series of immersions particularly during the latter stages of each immersion where the thermal input from peripheral cold receptors is likely to be reducing [24]. Collectively this leads us to the idea that attention and anxiety levels reducing over a series of habituation immersions is permissive of learning to control breathing after which only the  $f_c$  component of the CSR, which is under lesser voluntary control, responds to increases in anxiety. There is a sound anatomical basis for this suggestion as heart rate is not subject to the same extent of voluntary control as ventilation. Indeed, heart rate is primarily regulated by autonomic nervous system pathways by contrast to ventilation which includes a central brain structure that enables partial voluntary influence [16, 25].

As a consequence of these data we can refine our model of the thermal, physiological and psychological mechanisms that may prevail to induce habituation. Tipton and colleagues [13] demonstrated habituation of the CSR in the right hand side of the body when they repeatedly immersed the left hand side of the body (on separate days) thereby confirming that habituation is caused after central rather than peripheral integration of the thermal afferent information. Primary neural candidates for the central site of habituation include the reticular formation and the frontal and prefrontal cortices [26,27]. We now add the important mediating role that anxiety and attention plays in this habituation. The physiological response to acute anxiety is thought to be mediated primarily by the dorsomedial hypothalamus

(DMH) with previous experience of a given stimulus and emotional valence (e.g. threatening or non-threatening) of that stimulus interpreted and conveyed to the DMH through the amygdala and medial prefrontal cortex [28,29]. Clearly there are common and potential anatomical pathways by which anxiety could interfere with the occurrence of habituation. It is important to include the mechanism that may dictate the strength of the signal caused by differences in perceived threat in the immersions scenario. Indeed, current stress theory [30] indicates that one's appraisal of the immersion scenario as threatening (as recurrently manipulated in the present study) as opposed to increasingly less threatening (as in previous studies [17,18]) would lead to different anxiety responses. The anxiety response is fundamentally determined by the primary (i.e., predictability, novelty) and secondary (i.e., coping resources) appraisal of the immersion scenario [30]. A novel and unpredictable situation coupled with a perceived imbalance between the coping requirements and coping resources available would lead to a highly anxious response requiring a large amount of attentional resource especially if the valence (i.e. the positive or negative perception) of this situation was viewed as negative. We suggest that we repeatedly induced this threat imbalance and negative valence in the present study and this prevented habituation from being achieved.

The cognitive relationship between state anxiety and habituation of the CSR is clearly worthy of further investigation, not least to confirm the hypothesis that the voluntary control of ventilation is learned during habituation, particularly during the latter stages of CWI. The voluntary control that is ordinarily seen as a consequence of habituation [13-15,17] was abolished by state anxiety in the present study. In previous studies we have shown that psychological skills training (PST) can improve the voluntary control of ventilation, as evidenced by extending maximal breath hold time, on CWI [14,16]. The cognitive bases of

components of PST have recently been elucidated. Wallace et al [31] showed that motivational self talk (MST), which is a component of PST, improved executive function and the learned ability to counteract psychological context-specific demands throughout an exercise task performed in a demanding environment. It would clearly be advantageous, by way of stopping water entering the airway and improving survival prospects, to facilitate rapid learning of respiratory control. Low levels of anxiety may permit this to occur. Identifying the cognitive architecture by which this can be achieved may enhance the precision of survival training and advice for those at daily risk of CWI.

This study is not without limitation some of which continue from our previous studies [17,18]. It is possible that our semi-structured interview included leading questions with regard to water temperature but we were bound by way of consistency with that included in our original investigations. However, a revision in line with this issue may be included in future studies. The present and previous cohorts may be open to a self-selection bias towards those experiencing lower levels of daily anxiety given that the anxiety inducing, unpleasant and demanding nature of the study's tasks may stimulate avoidance in study participation in highly anxious individuals. Our questionnaire assessment of the present cohort of participants suggests this to be true with state-trait anxiety scores recorded that are towards the lower end of the STAI assessment scale (i.e. mean [SD] 40 [8]; range 29 to 56 on an 80 point scale). It remains possible that participants with a comparatively high trait anxiety rating (e.g. those with a general anxiety disorder) would not habituate to cold water, which would place them a higher risk should accidental CWI occur. It is also possible that we have been premature in concluding that repeated anxiety, as induced in the present study, prevents habituation of the CSR taking place. It is not likely that we are underpowered in making this observation since we have tested more participants over a similar number of immersions than reported in

previous studies, all of which demonstrated an habituation of the CSR takes place after 4 ( $n = 10$ ; Barwood et al [14]) or 5 immersions (i.e.  $n = 8$ ; Tipton et al [13]). However, it remains possible that we simply *delayed* CSR habituation taking place although visual inspection of our data (see figure 5 ANX-CW<sub>rep</sub> group) provides no consistent indication across cardiorespiratory variables that habituation of the CSR is beginning to occur. Lastly, the water temperature used in the present study (15°C) is towards the upper end of the range of temperatures that is suggested to maximally evoke the CSR (i.e. 5–15°C; [32,33]). It is possible that acute anxiety may not influence the CSR seen at lower water temperatures because the CSR is already maximally evoked. Nevertheless, it was for ethical and safety reasons that we selected the present and preceding water temperatures.

Our data have important practical implications for the administration of survival training and for advising those who are at daily risk of immersion. Our series of studies clearly show the importance in lowering situation specific anxiety in order that a beneficial physiological response is seen whether it is before or after CWI habituation has taken place [17]. Lowering situation specific anxiety can even confer a benefit in the absence of repeated cold-water stimulation [18]. Failing to address the anxiety-inducing nature of impending CWI will prevent or delay habituation of the CSR taking place and may culminate in a failure to learn to exert some control over the respiratory component of CSR, particularly towards the end of an acute immersion event. Accordingly, it is prudent to suggest that survival training or any preparatory training for those at daily risk of accidental CWI includes an evidence-based psychological intervention that can be tailored to address the cognitive demands of a situation in order to facilitate perceptual and physiological control. For individuals at risk who experience high levels of trait anxiety such interventions might be particularly important given our speculation that habituation is less likely to be achieved in these individuals.

Moreover, it is possible that repeated CWI to induce an habituation of the CSR, may also prove to be a therapeutic treatment for those individuals with trait anxiety disorders as repeated immersion clearly lowers anxiety levels [17,18]. Such an experiment would reveal whether this reduction is situation specific.

We conclude that high levels of anxiety prevent or delay habituation of the CSR. Practical steps must be taken to address the extent of situation-specific anxiety if habituation is to be effectively administered as a protective strategy to reduce the risk of death by drowning should accidental CWI occur.

## 5.0 Acknowledgements

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## Figure Legends

Figure 1. Experimental design completed for each of the two immersion groups. Group 1 ( $n = 16$ ) i.e. repeated anxiety throughout CWI-ANX<sub>rep</sub>) includes participants who experienced increased anxiety during their habituation CWIs (immersions 2 to 5), group 2 ( $n = 10$ ; i.e. acute anxiety after habituation immersions; from Barwood et al., [17]) includes participants who did not undergo anxiety inducing manipulation during their habituation CWIs (immersions 2 to 5) but experienced an acute increase in anxiety after habituation (CWI-ANX<sub>ac</sub>); \* indicates counter-balanced conditions within group.

Figure 2A-B. Mean [SD] anxiety responses prior to and on immersion in the habituation immersions (CWI-ANX<sub>rep</sub> immersions; panel A.) and the experimental immersions (panel B.); # denote differences between CON1 & CON2, \* indicates difference between CWI-ANX<sub>rep</sub> & CON2 ( $n = 15$ ); \* in axis title indicates conditions were counter-balanced.

Figure 3A-D. Mean [SD] cardiorespiratory ( $f_c$  panel A,  $f_R$  panel B,  $V_T$  panel C,  $\dot{V}_E$  panel D) responses to immersion in the experimental immersions across time; \* in axis title indicates conditions were counter-balanced,  $n = 15, 16, 16, 16$  respectively.  $p > 0.05$  indicates no difference between equivalent time points for each immersion.

Figure 4. Mean [SD] averaged anxiety responses across the experimental (i.e. CON1, CWI-ANX<sub>rep</sub> or CWI-ANX<sub>ac</sub> [INT] & CON2) and habituation (i.e. IMM2 to IMM5) immersions in groups 1 and 2. Figure 1 denotes the order of the experimental conditions and intervention (i.e. INT) manipulations in each group; \* in axis title indicates conditions were counter-balanced; # denote differences between two groups ( $n = 25$ ).

Figure 5A-D. Mean [SD] averaged cardiorespiratory responses ( $f_c$  panel A,  $f_R$  panel B,  $V_T$  panel C,  $\dot{V}_E$  panel D) across the experimental (i.e. CON1, CWI-ANX<sub>rep</sub> or CWI-ANX<sub>ac</sub> &

CON2) and habituation (i.e. IMM2 to IMM5) immersions in groups 1 and 2. Figure 1 denotes the order of the experimental conditions and intervention (i.e. INT) manipulations in each group; \* in axis title indicates conditions were counter-balanced; # denote differences between two groups (n = 26, 24,23 & 23 respectively).

### Tables

See separate file with Table 1.

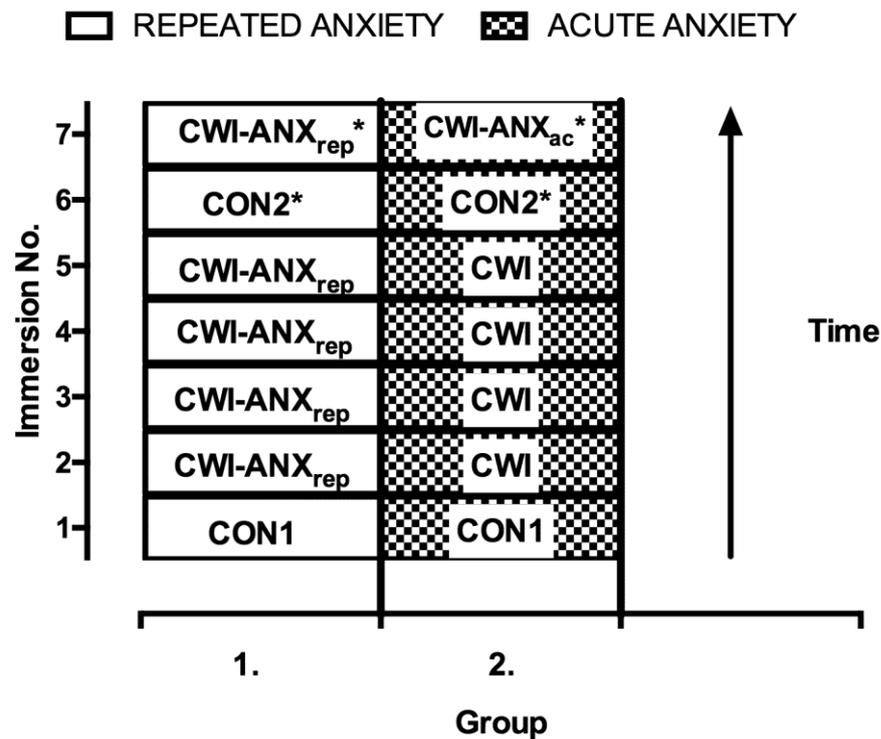


Figure 1

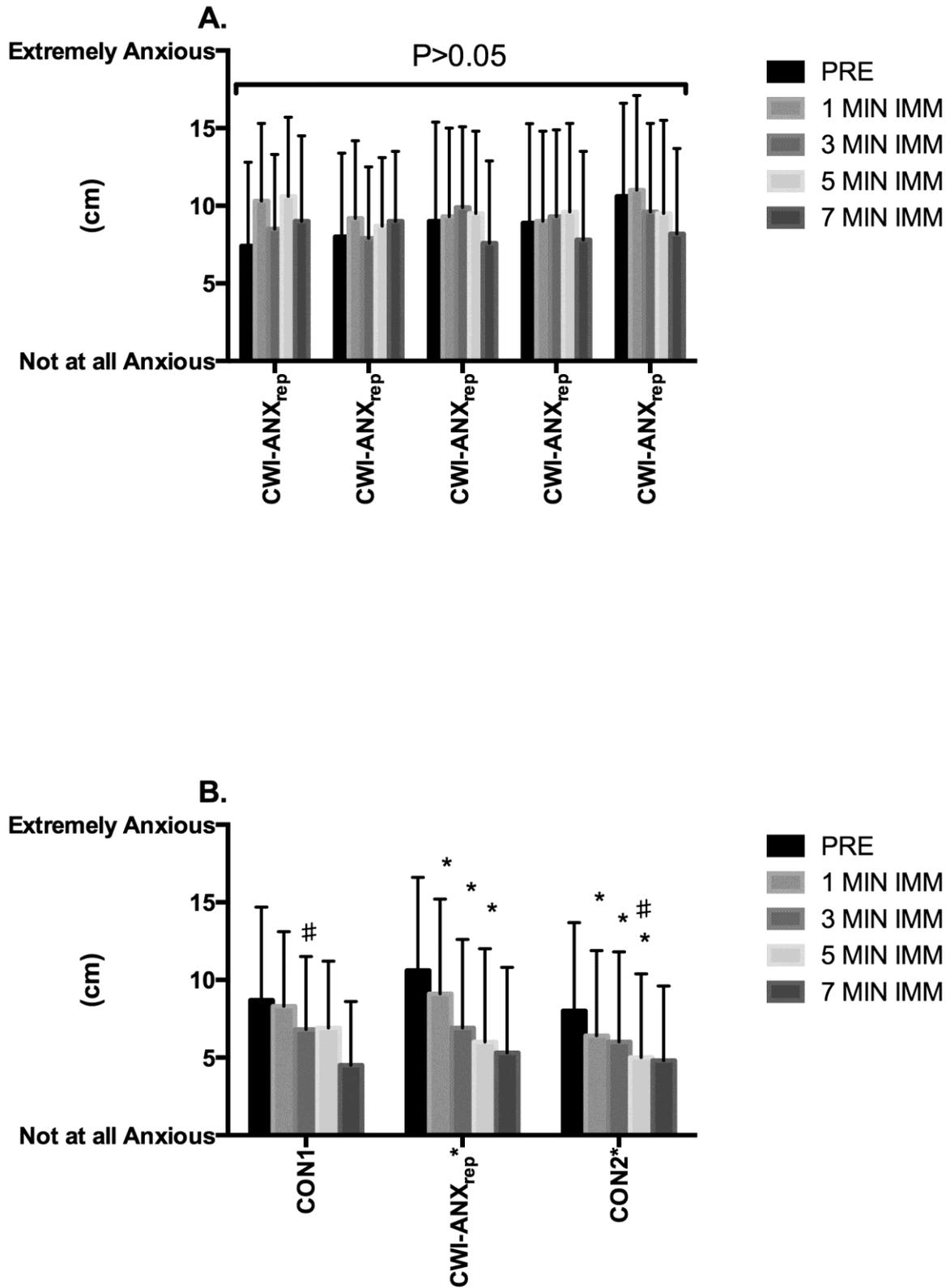


Figure 2

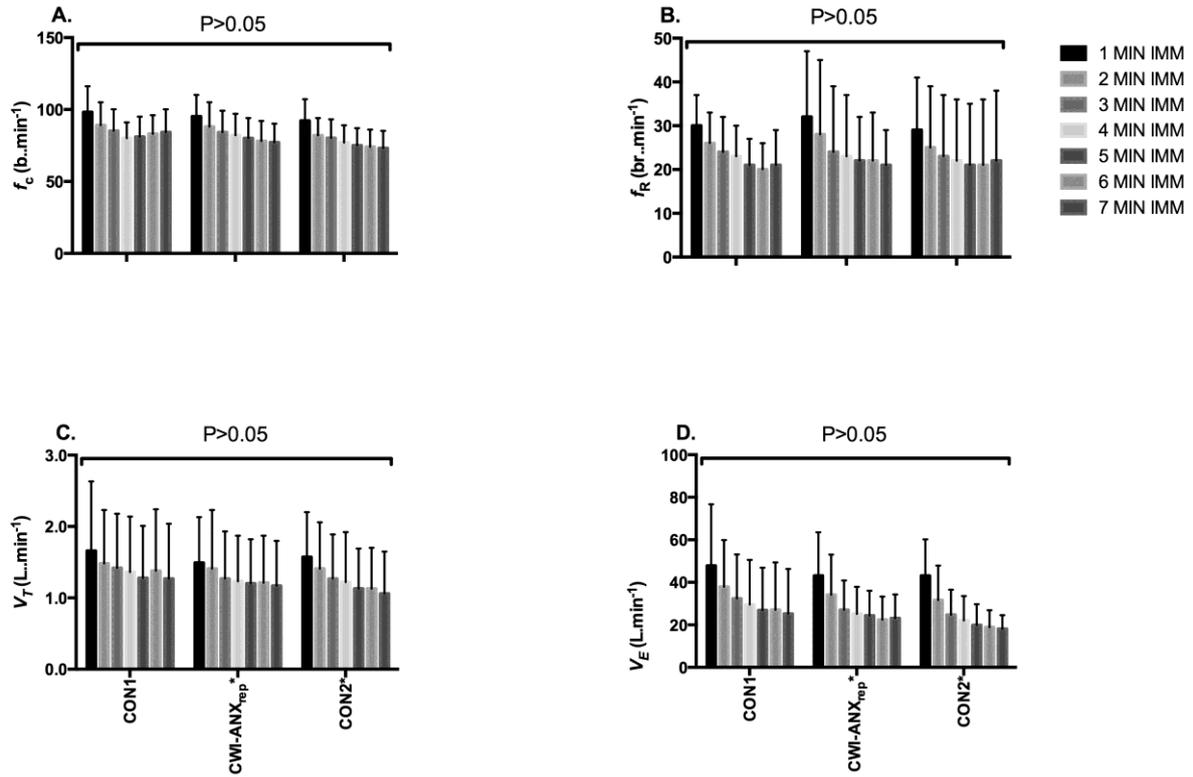


Figure 3

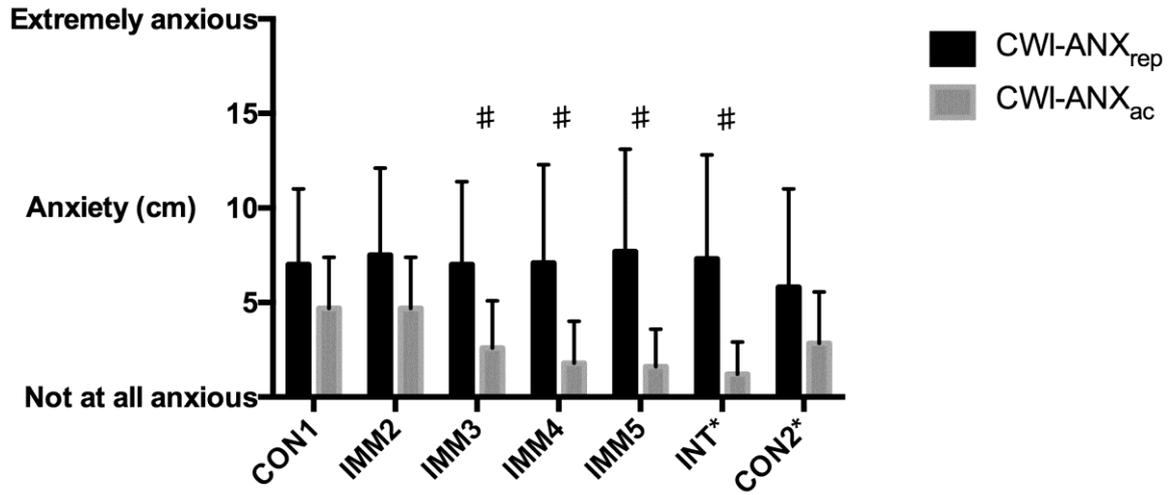


Figure 4

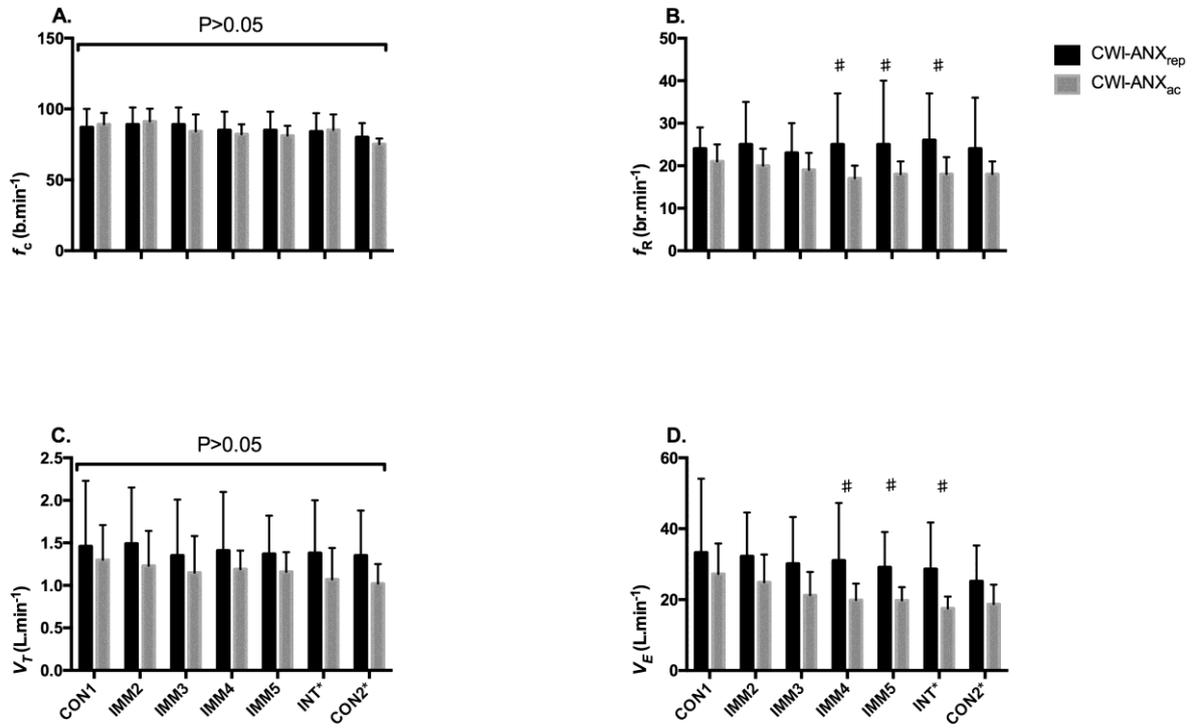


Figure 5

Table 1. Mean [SD]  $f_c$  ( $n = 15$ ),  $f_R$  ( $n = 14$ ),  $V_T$  ( $n = 13$ ),  $\dot{V}_E$  ( $n = 14$ ) responses on immersion across the four habituation immersions where anxiety was manipulated across the seven-minute period ( $n = 17$ ); \* =  $p < 0.05$  between consecutive conditions.

		1 MIN	2 MIN	3 MIN	4 MIN	5 MIN	6 MIN	7 MIN
$f_c$ (b.min <sup>-1</sup> )	CWI- ANX <sub>rep</sub> 1	100 [20]	92 [13]	88 [15]	81 [11]	85 [13]	83 [12]	82 [13]
	<sup>1)</sup> CWI- ANX <sub>rep</sub> 4	99 [20]	89 [15]	85 [15]	80 [12]	80 [13]	74 [12]	78 [12]
$f_R$ (br.min <sup>-1</sup> )	CWI- ANX <sub>rep</sub> 1	28 [10]	24 [10]	23 [9]	21 [9]	19 [6]	19 [6]	22 [11]
	<sup>1)</sup> CWI- ANX <sub>rep</sub> 4	30 [11]	25 [11]	23 [11]	20 [9]	20 [7]	18 [7]	19 [7]
$V_T$ (L.min <sup>-1</sup> )	CWI- ANX <sub>rep</sub> 1	1.8 [0.6]	1.7 [0.7]	1.5 [0.7]	1.5 [0.7]	1.4 [0.8]	1.4 [0.8]	1.4 [0.9]
	<sup>1)</sup> CWI- ANX <sub>rep</sub> 4	1.7 [0.6]	1.6 [0.6]	1.4 [0.6]	1.4 [0.6]	1.3 [0.5]	1.2 [0.6]	1.2 [0.5]
$\dot{V}_E$ (L.min <sup>-1</sup> )	CWI- ANX <sub>rep</sub> 1	51.6 [21.6]	40.6 [17.0]	33.3 [14.6]	30.0 [15.5]	26.3 [11.6]	26.4 [12.4]	26.6 [14.9]
	<sup>1)</sup> CWI- ANX <sub>rep</sub> 4	52.1 [20.0]	39.4 [17.8]	30.5 [13.9]	26.6 [10.1]	24.2 [8.3]	22.0 [8.8]	21.8 [9.2]

**Highlights**

- Accidental cold water immersion evokes the life-threatening cold shock response
- Repeated cold water immersion can induce a beneficial habituation (reduction) in the magnitude of the response
- This habituation may be disrupted by the coincidental experience of high levels of anxiety; we tested this hypothesis
- High levels of anxiety prevented or delayed habituation occurring
- At risk individuals may not be protected by habituating to cold water if they experience high levels of anxiety during or following cold water habituation
- Safety and survival training should consider measures to lower situation specific anxiety to maximize the beneficial effects of habituation