

**STRESS RESPONSE AND ADAPTATION TO SEAFARING:
A STUDY OF THE CORTISOL RESPONSE TO AWAKENING**

by Jonathan Liberzon

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Abstract

Study of the Hypothalamus-Pituitary-Adrenal (HPA) Axis has allowed for a greater understanding of the neurobiological systems that control the adaptive hormonal response to stress. In order to investigate the effect of diet, work, professional experience and sleep on HPA reactivity in healthy populations, data on the cortisol response to awakening (CRA), an indicator of HPA reactivity, was collected along with a suite of psychological metrics and survey instruments. A six-week voyage of work and study aboard an oceangoing sailing vessel was selected as a model for prolonged stressors, providing opportunities for both response and adaptation to novel and challenging conditions. Baseline data were collected on shore prior to boarding. Results confirmed that CRA profiles among all subjects were altered by the experience of seafaring, and that inexperienced students' profiles were altered differently from those of professional crew. Students exhibited larger CRAs on shoreside working days than on shoreside weekend days. Working days at sea were marked by intermediate CRAs in the student samples, and CRAs for shore-leave days fell between at-sea working day and onshore weekend levels. In contrast, professional crew exhibited increasing CRAs over the course of the voyage. Profiles for experienced scientific staff did not differ from those of inexperienced students. Also, greater reported meat consumption predicted smaller weekend CRA and lower subjective stress. This is the first study to examine CRA, diet and experience in the context of seafaring.

1. Introduction

1.1 Background

For more than a century, stress has been understood as both a physiological and psychological process resulting from exposure to adverse or challenging stimulus. Early research identified reactive “fight or flight” mechanisms important for initial response to danger (Cannon 1914). These mechanisms respond to perceived threats by preparing the organism for instantaneous response. Threat recognition in the amygdala is followed by elevated heart rate and arousal, hyper-alertness and secretion of catecholamines (including adrenaline) from the adrenal gland. These prepare the organism for the demands of an aggressive encounter or flight from danger. Also, a second stress response system has been identified. The Hypothalamus-Pituitary-Adrenal (HPA) Axis is a neuroendocrine chemical cascade responsible for long-term adaptation to threatening or stressful stimulus (McEwin and Wingfield 2003). Activation of the HPA axis has been implicated in reallocation of metabolic resources and facilitation of immune response (Dhabar and McEwen 1999) following stress and injury, as well as ‘learning’ and retention of novel threat information (Rooszendaal 2000). The HPA-axis has been described as operating in response to allostatic loading (McEwin and Wingfield 2003), whereby an organism responds to stressors by shifting energy investment away from non-essential functions in order to maintain homeostasis and overcome immediate survival challenges. During normal allostasis, dynamic HPA regulation allows the organism to alter glucose production and boost immune-system function in response to a stressor, and then return to normal levels when survival demands are relaxed. In some cases, however, continual allostatic loading can cause an overloaded HPA-axis to lose the ability to properly regulate adrenocortical hormones.

1.2 Neurobiology of the HPA-axis

Initial mounting of the HPA stress response is controlled by the paraventricular nucleus (PVN) of the hypothalamus. Activation of the PVN appears to follow two main pathways: Immediate threats to physiological homeostasis, such as hypotension, hypoxia and hemorrhage may activate the PVN through a relatively direct pathway of processing and catecholamine secretion from the brainstem. Stressor-specific threats that must be assessed

in relation to previous experience (e.g. restraint), are recognized in the PVN through neuronal input from the limbic system (Herman and Cullinan 1997). Threat recognition for this pathway occurs in the amygdala and bed nucleus of the stria terminalis, a part of the limbic forebrain. Several other brain regions, such as the locus coeruleus, have been implicated in PVN activation as well, though these remain controversial.

During HPA response, activation of the PVN results in hypothalamic secretion of corticotropin releasing hormone (CRH), which signals the production of adrenocorticotropin-releasing hormone (ACTH) in the pituitary. Free circulating ACTH, in turn, triggers production of the hormone cortisol, the final component of the HPA activation cascade, in the adrenal cortex. Cortisol has long been established as an end product of HPA activation, and accordingly as an integral measure of neuroendocrine stress function in humans. Though cortisol is secreted in high concentrations in response to stress, free cortisol is constantly maintained at basal levels within the bloodstream. In addition to HPA activation, HPA inhibition is also necessary for maintenance of basal tone. Glucocorticoid (e.g. cortisol) receptors in the PVN directly inhibit HPA activation, though generalized inhibition may also depend on GABAergic sensitivity in the PVN. Additionally, neuronal feedback from the hippocampus, pre-frontal cortex and lateral septum has been implicated in inhibition of stressor-specific (limbic) responses (Herman and Cullinan 1997). Basal cortisol is regulated on a 24 hr circadian cycle, in which concentrations begin to rise shortly after midnight and peak in mid-morning, declining thereafter until the next trough is reached near midnight (Keller et al. 2006). This cycle is maintained independently of bedtime, awakening time or total hours slept.

As a negative feedback agent within the HPA axis, cortisol is secreted together with catecholamines in a dose-dependant manner during responses to physical stressors such as hypoxia or drops in blood pressure (Selye 1976), but may also be secreted independently in response to stressors requiring higher-order threat processing (e.g. conditioned fear; Herman and Cullinan 1997). Cortisol response to major stressors has been shown to correlate with greater psychological response to stressors (Alpers et al. 2003), and altered cortisol function has been demonstrated in a variety of mental illnesses, including reduced and elevated free cortisol levels in Post-Traumatic Stress Disorder and Major Depressive Disorder, respectively (Yehuda 2004). Additionally, a number of other factors have been shown to

alter cortisol function in-vivo, including changes in diet and sleep patterns. Among the salient modifiable environmental factors, free circulating cortisol has been found to decrease as a result of changing to a lactovegetarian diet from a meat-rich diet (Remer et al. 2004).

1.3 The Cortisol Response to Awakening

The cortisol response to awakening (CRA), a relatively simple and non-invasive measure of HPA activity, is defined as the change in free cortisol measured from the time of wakeup to some time immediately afterwards (usually between 30 and 90 minutes). The CRA occurs as part of the cortisol circadian cycle and has been characterized in healthy adults as a 50-160% rise in salivary free cortisol in the first thirty minutes after awakening (Clow et al. 2004). After this peak, free cortisol concentrations return to basal levels within 1-2 hours of waking. More dynamic than overall 24 hr basal cortisol secretion, the morning CRA has been shown to correlate with daytime HPA-axis activation as stimulated by injection of ACTH or through experimentally-induced stress (Schmidt-Reinwald et al. 1999). Therefore, the CRA may be used as a sensitive measure of HPA-axis reactivity and response to stress. The measure shows high intra-subject stability and is independent of adult age, weight, smoking status and alcohol consumption (Pruessner et al. 1997). Some studies have found that CRA depends on time of awakening (Federenko et al. 2004), but others have found no relationship between CRA and wakeup time, sleep duration or sleep quality (Pruessner et al. 1997). Williams et al. (2004), found that CRA was greater for workers on early shift days than on late shift days, but this finding was not significant after controlling for subjective stress and sleep disturbance. More data is therefore necessary to determine the effect of sleep and awakening time (independent of stress) on the CRA.

Recent studies, however, have supported the sensitivity and reliability of the CRA as an index for HPA reactivity (Clow et al. 2004). The CRA has been shown to be altered in some pathologies normally associated with HPA dysregulation, such as Post-Traumatic Stress Disorder (Wessa et al. 2006) and major depressive disorder (Huber et al. 2004). In healthy adults, the cortisol response to awakening is also affected by workload and perceived work overload, socioeconomic status, gender, and recently the CRA was found to be smaller on weekend days than on working days (Kunz-Ebrecht et al. 2004, Schlotz et al. 2004). Additionally, self-reported perceived chronic stressors such as worry and social stress

have been found to predict higher CRAs in healthy subjects (Wüst et al. 2000). Thus, this measure can be useful for determining relative differences in neurophysiological response to stress between variable stress periods and between individual subjects.

This study used a six-week voyage onboard an educational sailing vessel as a model for a long-term social and physical stressor. Subjects were sampled to observe subjective stress and cortisol response to the rigors of life at sea. The cortisol response to awakening was used to compare the neurobiological effects of the living/working environment on cohorts with variable levels of work experience and variable dietary habits. The study was designed to investigate the effects of diet, sleep, experience and workload on HPA responsivity to prolonged stressors in a healthy population.

1.4 Hypothesis

This study was originally devised as an investigation of the effect of variable meat and caffeine intake on the cortisol response to awakening. Specifically, we sought to investigate the effect of vegetarianism on CRA as a measure of HPA reactivity, since free cortisol has been found to decrease as a result of changing to a lactovegetarian diet from a meat-rich diet. (Remer et al. 2001). Consequently, we hypothesized that HPA reactivity (as indicated by the CRA) would be lower in lactovegetarians than in meat-eaters. Unfortunately, manipulating the diet of the cohort was not possible, and it was determined after recruitment that there would not be enough variability in subjects' diet to rigorously analyze the effect of this variable, however other important variables could be tested. We hypothesized that work experience, sleep duration and perceived control would correlate negatively with the CRA and subjective measures of stress and anxiety.

1.5 Experimental Model

The sea voyage was selected as a good model for testing stress reactivity because the experience of seafaring and work at sea presumes a number of stressors that have been shown to elicit cortisol secretion or elevated HPA activity such as increased risk to life, a combination of mental and physical labor (Schlotz et al. 2004), workplace stress (Kunz-Ebrecht et al. 2004), repeated examination and performance evaluations (Martinek et al. 2003, Ng et al. 2003, Lindahl et al. 2005) and social stress, while at the same time

controlling variables such as time of awakening, physical activity, work type and frequency of social contacts. To comply with ship protocols, subjects had to adhere to a 72 hour sleep rotation on board, and thus no clear diurnal sleep cycle could be established. Subjects were tested and evaluated based on performance, both on work-related tasks and on oral presentations. All subjects remained together for the entire course of the voyage, performing similar types and quantities of labor and sharing the same quarters on board. Subjects did not have access to outside contacts, and could not contact friends or family during the voyage. All subjects kept an identical (though offset) work schedule and took their morning samples at the same time of day for at least three of the six time points in this study (see fig 2.31). These presented excellent conditions for testing the effect of diet on stress response and habituation.

2. Methods

2.1 Cohort

Data were collected from 31 subjects enrolled in or instructing class 195A of the Sea Education Association SEA semester program, based in Woods Hole, MA. The SEA semester program included six weeks of studying on shore, followed by six weeks of work, study and research aboard the SSV Corwith Cramer, a brigantine sailing vessel. Subjects ranged from 18 to 38 years of age. Out of thirty-one, 18 were female and 13 were male. There were 23 enrolled students participating in the study, along with 4 professional science staff and 4 professional crew (three ship's mates and an engineer.) Subjects were recruited either at the SEA campus in Woods Hole or on board the SSV Corwith Cramer. All subjects were given written and verbal instructions about the study and all gave informed consent. The study was approved by the University of Michigan Institutional Review Board for Behavioral Sciences.

2.2 Demographic and Psychological Measures

Prior to departure on the sea voyage, subjects provided demographic data including: age and gender, height, weight, smoking status, alcohol, caffeine consumption and dietary habits specific to consumption of meat, fish, eggs and dairy. Subjects also completed a single round of psychological surveys including: Beck Depression Index (Beck and Steer 1984), Taylor-Manifest anxiety scale (Hoyt and Magoon 1954), Perceived Stress scale (Cohen et al. 1983), Spielberger Trait Anxiety Index (Spielberger et al. 1969) and Marlow-Crowne scale of social desirability (Fisher 1967). These surveys were completed prior to the ship's departure. Participants were instructed to complete one 7-point Lykert scale of perceived stress and one of perceived control concomitant with their saliva sampling on each of the six sampling mornings. Stress was reported based on the prompt "How stressed do I feel?" and control was reported based on the prompt "How in-control do I feel?" Subjects also recorded sleep duration and time of awakening preceding each sampling time.

2.3 Neuroendocrine Measures

Each subject provided saliva samples on six separate mornings. Each sampling morning included three saliva samples taken at 0, 30 and 45 minutes after awakening. The sampling schedule is diagramed below in figure 1. Subjects were instructed not to eat, drink, smoke, brush teeth or rinse their mouths until after completion of the 45 minute salivary sample. Two mornings were sampled on shore, prior to boarding the ship. One shore sample was taken on a working day (OSWD) during a normal school week at the campus of the Sea Education Association. All subjects were sampled on the same morning for the OSWD sample. The second shore sample was taken the morning of a pre-boarding break day during the week prior to departure. The specific date of sampling was not controlled within this week. This sample (OSWE) was intended to simulate an average weekend morning, since subjects did not have to work or prepare for exams on the day of their OSWE sample. Subjects slept off-campus the night of the OSWE sample, and some slept at home. Two science staff members took the OSWD and OSWE samples, but other science staff members did not provide OSWD or OSWE samples, and no ship's crew were sampled on shore.

Prior to boarding, subjects were divided into three groups or "watches" which adhered to a 72 hour watch rotation. Each group's work schedule was offset on the time scale in relation to the preceding "watch" such that one watch would always be on duty (at all hours of every day on board.) Within each 72 hours, the total amount and the type of shifts (morning, evening and night shifts) were equal for the three "watches". As a result, no two watches would wake up at the same time each day. To control for waking time, the three watches were sampled on consecutive mornings, always following their 5:30 a.m. wakeup call. For example, 'A' watch was sampled after wakeup calls on day 4, 'B' watch was sampled at the same time on day 5 and 'C' watch was sampled at the same time on day 6; days 4, 5, and 6 were then grouped as the 'Sea-1' sample during analysis. Each subject provided samples on three separate mornings at sea (Sea-1, Sea-2, Sea-3) and one more sample on a shore-leave day (M-2). The M-2 sample was taken on-board, on the second morning of a two-day shore leave, the second of two shore leaves during the voyage. This sampling date was chosen in an attempt to control for the novelty of shore leave, and was intended to approximate a "weekend" at sea. Subjects were instructed to complete subjective stress/control rating and sleep data cards while chewing on the first (0 min) salivette. Saliva

samples were frozen on-site and assayed using Diagnostic Products Coat-a-Count cortisol radioimmunoassay kit.

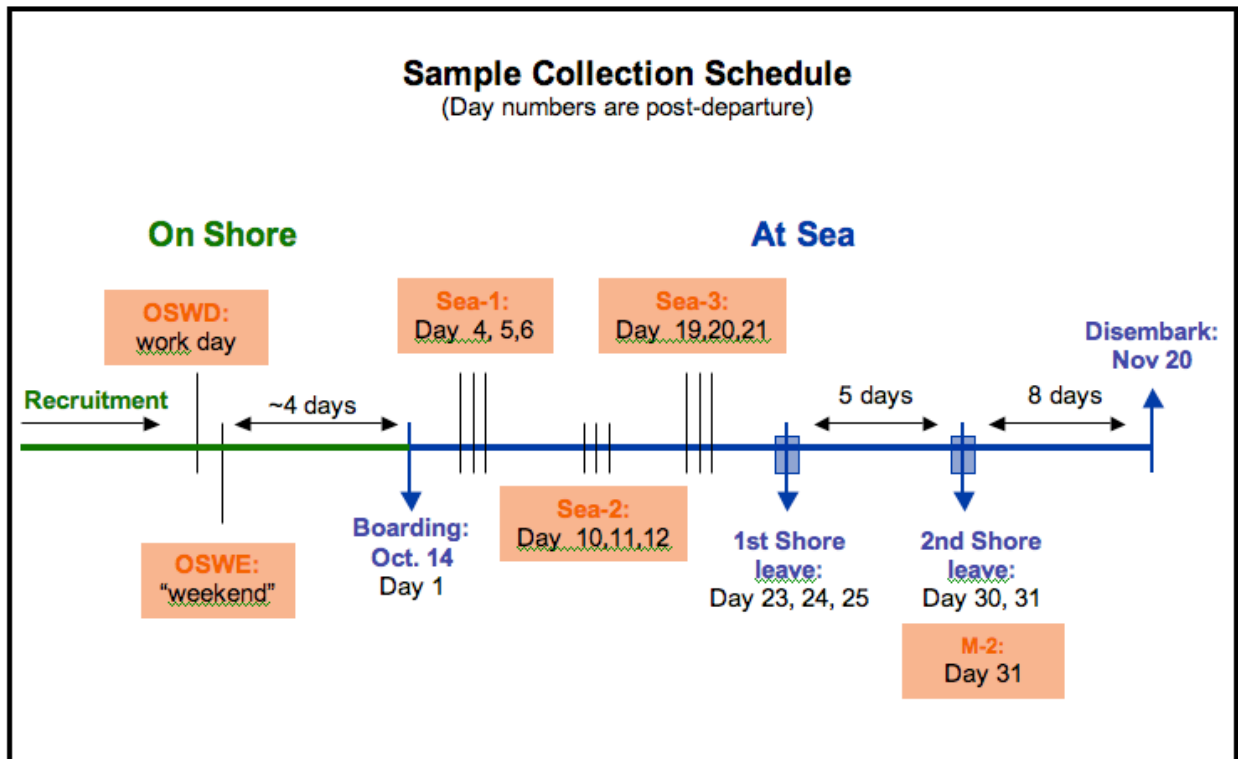


Figure 1: Collection schedule for morning cortisol response to awakening (CRA) and subjective stress and control samples. Orange boxes denote sampling mornings. Days numbers are days post-departure.

2.4 Data Analysis

A three-point response curve was generated for each morning sample by grouping wakeup, 30 min and 45 min data points within subjects (Fig. 5). In order to compare cortisol response profiles between subjects, three measures were used. Cortisol maximum response was calculated by subtracting the waking (0 min) cortisol value from either the 30 or 45 minute value, whichever was greater. Area under the curve (AUC) was also calculated, both as total area and as AUC of response, defined as the area of response above the baseline waking cortisol level (0 min value). Some AUC values could not be calculated because of missing data. Missing data points were due to subject non-compliance or sampling error (insufficient saliva volume). Excel was used for all data manipulations.

Statistical tests were performed using Statview 5.0.1 and SPSS 10.0. Change over time was tested using repeated measures ANOVA. Fisher post-hoc tests were used to compare individual time points. Group differences between males and females, and between students and crew were also examined using these tests. Some subjects were excluded from repeated measures analyses because of missing AUC data on certain time points. In order to prevent the exclusion of one ship's crew member from analyses, cortisol and subjective stress values were interpolated from the group mean for this subject at timepoint Sea-1. The Pierson test was used to investigate correlations between cortisol response to awakening and a number of reported measures, including subjective ratings of stress and control, psychological survey results, body composition, dietary habits and alcohol and tobacco use.

3. Results:

3.1 Demographic, Dietary and Psychological Findings

No differences were found between men and women for any of the variables investigated. Subjective ratings of perceived stress and control showed a strong and significant negative correlation across sampling days, meaning that less perceived control predicted more perceived stress in this cohort. Students reported lower perceived stress during onshore weekend (OSWE) morning than during onshore workday (OSWD) morning or any at-sea mornings (Fisher, $p < .001$), but no significant difference was found in perceived stress between onshore workday and at-sea workday mornings. Perceived control did not correlate significantly to any other measures. Mean ratings of perceived stress and control are summarized by duty in table 1. Average reported stress in student and ship's crew cohorts for the duration of the study is displayed in figures 2a and 2b. No correlations were found between cortisol responses and subjective ratings of stress or control. Also, no group differences were found between students, ship's crew and scientific crew in these measures.

None of the participants scored on psychological battery in ranges indicating psychiatric disease, though two were taking antidepressants. Table 2 lists mean scores of psychological survey tests for students, crew and scientific staff. None of the psychological measures correlated significantly with biological measures collected in this study, though subjective ratings of stress for the M-2 sample correlated positively with Beck Depression Index (Pearson, $p = .004$), Perceived stress scale (Pearson, $p = .001$) and Taylor Manifest Anxiety Scale (Pearson, $p = .009$). Otherwise, reports of perceived stress and control did not correlate with other psychological measures.

Time of awakening and total sleep time did not correlate with cortisol variables, except for waking cortisol (0 min) values, which correlated with time of awakening in the shore leave sample (M-2). This correlation confirms that subjects' diurnal cortisol modulation was functioning predictably at the time of sampling and does not provide information on awakening response. Also, mean perceived stress demonstrated an inverse correlation with total sleep time on the workday shore sample. Data on time slept and time of awakening for the student and ship's crew cohorts is summarized in table 3.

Table 4 shows average meat and caffeine consumption in student and ship's crew cohorts. Since variation in diet for this sample was small, we were not able to compare discreet groups of vegetarians and non-vegetarians as originally planned. By analyzing reported weekly meat consumption as a continuous variable, however, several trends were found. Significant correlations between diet and cortisol were found in OSWE maximum CRA and total AUC of cortisol, which correlated negatively with reported meat consumption (Pearson, $R^2 = .5064$, $p = 0.001$ and $p = .009$, Fig. 3). This correlation did not remain significant when testing area under the curve of response. When analyzing subjective stress and control, a significant negative correlation was found between reported meat consumption and weekend (OSWE) subjective stress ratings (Pearson, $p = .006$). 35.4% of the variance in reported stress on this day could be explained by reported meat consumption. No other diet-related correlations were found. It is likely that small variance in subjects' dietary habits contributed to the paucity of correlations or group differences in these factors.

Mean morning self-reports of subjective stress and control

		OSWD	OSWE	Sea-1	Sea-2	Sea-3	M-2
Students	Stress (0-7)	3.5	1.2	3.3	3.2	3.0	2.8
	SD	1.2	0.7	1.3	1.5	1.5	1.8
Ship's Crew	Stress (0-7)	No Data	No Data	2.2	2.4	2.6	2.5
	SD			1.1	2.0	1.7	1.6
Scientific Staff	Stress (0-7)	3.4	2.4	2.6	3.3	2.1	2.2
	SD	2.0	0.4	1.1	1.4	0.9	1.9
		OSWD	OSWE	Sea-1	Sea-2	Sea-3	M-2
Students	Control (0-7)	4.7	5.4	4.0	4.1	4.4	4.8
	SD	1.0	1.2	1.1	1.4	1.2	1.4
Ship's Crew	Control (0-7)	No Data	No Data	5.6	4.6	5.2	5.0
	SD			0.4	1.9	1.0	1.0
Scientific Staff	Control (0-7)	5.1	3.4	5.6	4.5	4.9	5.3
	SD	0.4	2.2	0.5	1.3	1.1	0.8

Table 1: Subjective ratings of stress and control were obtained for all morning samples based on a 7-point Lykert scale asking the question “How stressed do I feel?” and “How in control do I feel.?” “SD”s are standard deviations from the mean.

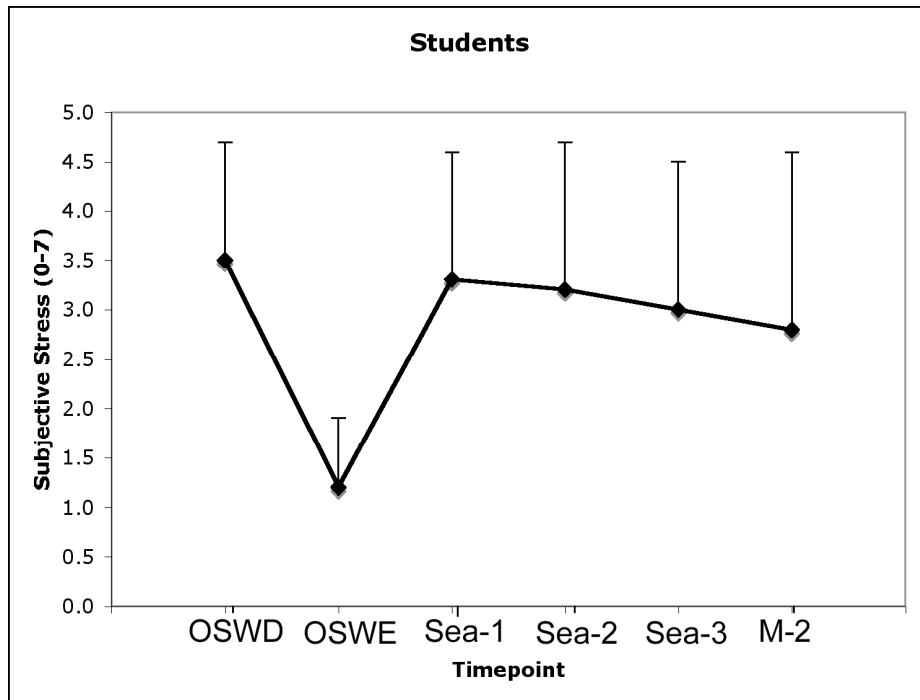


Figure 2a: Mean 7-point Lykert ratings of subjective stress in the student cohort. Error bars represent +1 standard deviation from the mean. Timescale represents all six sampling mornings in this study: onshore workday (OSWD), onshore weekend (OSWE), three at-sea workday samples (Sea-1, Sea-2, Sea-3) and second shore leave (M-2). OSWE stress was found to be significantly lower than OSWD or at-sea working days stress (Fisher, $p < .001$).

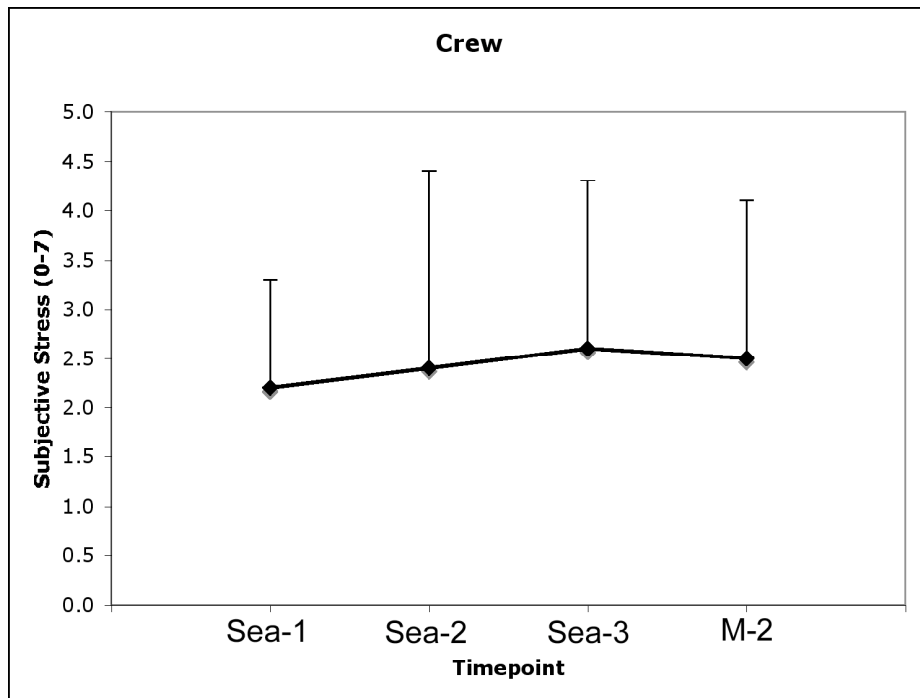


Figure 2b: Mean 7-point Lykert ratings of subjective stress in the ship's crew cohort. Error bars represent +1 standard deviation from the mean. Timescale represents 4 at-sea mornings: three at-sea workday samples (Sea-1, Sea-2, Sea-3) and second shore leave (M-2). No significant differences were found between mornings.

	Ship's Crew (N=2)		Students (N=15)		Scientific Staff (N=4)	
		SD		SD		SD
BDI	2.57	3.89	2.87	4.36	2.50	3.00
PSS	20.76	5.37	20.73	6.06	21.50	4.20
STAI Trait	46.90	5.32	47.47	5.54	46.75	5.74
TMAS	15.71	8.20	18.33	8.14	8.25	4.03
Marlow-Crowne	17.43	5.38	16.87	5.89	17.25	3.10

Table 2: Mean scores of psychosocial survey tests for ship's crew (N=2), students (N=15), and scientific staff (N=4). Survey instruments included Beck Depression Index (BDI), Perceived Stress Scale (PSS), Spielberger Trait Anxiety Index (STAI), Taylor-Manifest Anxiety Scale (TMAS) and the Marlow-Crowne scale of social desirability (Marlow-Crowne). SD column shows standard deviation of the means.

Timepoint	Mean time slept (minutes)	SD	Mean Wakeup Time (min after 00:00)	SD
Students				
OSWD	378	102	407	45
OSWE	447	253	431	251
Sea-1	341	79	338	14
Sea-2	363	34	331	10
Sea-3	320	130	289	114
M-2	378	125	335	107
Crew				
OSWD	No data		No data	
OSWE	No data		No data	
Sea-1	241	162	249	166
Sea-2	344	61	327	9
Sea-3	357	47	361	61
M-2	335	226	374	9
Scientific Staff				
OSWD	223	258	200	232
OSWE	239	277	228	270
Sea-1	233	172	210	158
Sea-2	225	169	323	18
Sea-3	268	179	335	7
M-2	500	85	425	58

Table 3: Means and standard deviations for time slept and time of awakening throughout study course. Table includes all data collected for students (N=24), ship's crew (N=4) and scientific staff (N=2 for OSWD and OSWE, N=4 for all others).

	Average Age	SD	Average Caffeine Consumption (times per week)	SD	Average Meat Consumption (times per week)	SD
Ship's Crew	27.0	1.8	5.3	10.5	1.0	2.0
Students	20.3	1.0	9.3	11.3	5.7	3.8
Science Crew	29.0	11.4	14.9	10.1	5.0	6.6

Table 4: Age and dietary habits for students (N=24), ship's crew (N=4), and scientific staff (N=4). SD shows standard deviations of the mean.

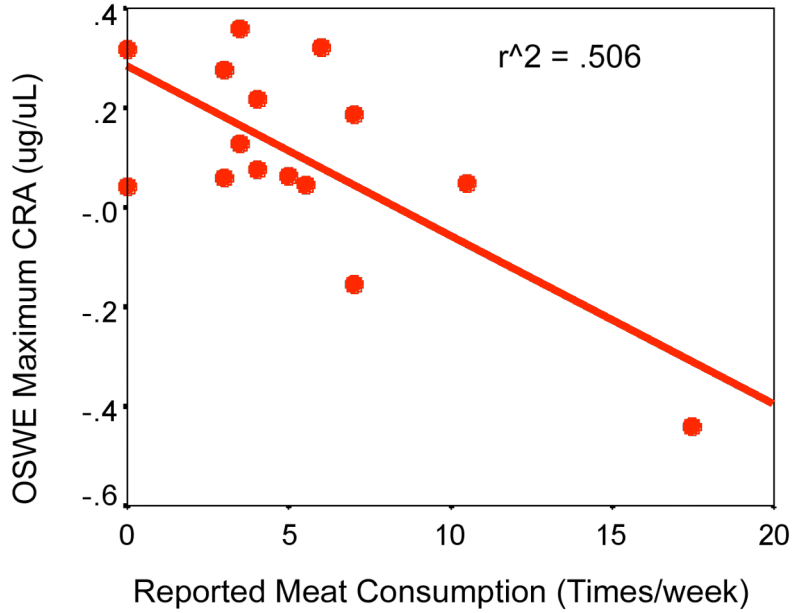


Figure 3: Correlation between maximum cortisol response to awakening during onshore weekend morning (OSWE) and reported weekly meat consumption. Pearson, $R = -.712$, $p = .001$. Data include all students and two scientific staff, $N = 15$.

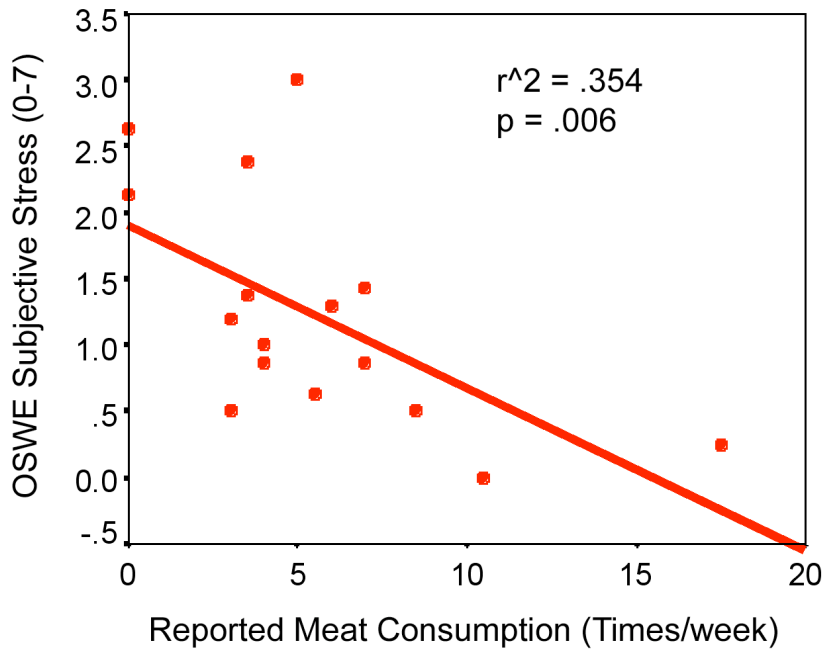


Figure 4: Correlation between subjective ratings of stress during onshore weekend morning (OSWE) and reported weekly meat consumption. Pearson, $R = -.595$, $p = .006$. Data include all students and two scientific staff, $N = 17$.

3.2 Cortisol Response to Awakening

A clear increase in salivary cortisol was detected after awakening in the majority of samples. For example, figure 5 shows the increase in mean raw cortisol over the first 45 minutes after awakening for the student cohort at timepoint OSWD. Table 5 lists mean cortisol values for 0, 30 and 45 minutes after awakening at each timepoint. Mean intra-assay coefficient of variation for cortisol radioimmunoassays was 0.02.

**Morning Cortisol Values
($\mu\text{g}/\mu\text{L}$)**

	Time	OSWD	OSWE	Sea-1	Sea-2	Sea-3	M-2
Ship's Crew	0 min	No Data	No Data	1.295	0.410	0.563	0.509
	SD	No Data	No Data	1.024	0.133	0.447	0.235
Ship's Crew	30 min	No Data	No Data	0.986	0.723	1.256	1.188
	SD	No Data	No Data	0.379	0.693	0.905	0.758
Ship's Crew	45 min	No Data	No Data	0.843	0.832	0.829	1.072
	SD	No Data	No Data	0.300	0.555	0.334	0.739
Students	0 min	0.393	0.443	0.428	0.549	0.367	0.469
	SD	0.201	0.217	0.229	0.303	0.067	0.187
Students	30 min	0.836	0.561	0.747	0.704	0.764	0.651
	SD	0.385	0.248	0.328	0.172	0.193	0.226
Students	45 min	0.931	0.556	0.730	0.721	0.716	0.724
	SD	0.391	0.305	0.326	0.172	0.256	0.299
Scientific Crew	0 min	0.459	0.524	0.447	0.406	0.358	0.452
	SD	0.270	0.285	0.143	0.310	0.097	0.261
Scientific Crew	30 min	0.909	0.766	0.578	0.720	0.633	0.490
	SD	0.434	N/A (n=1)	0.215	0.301	0.082	0.163
Scientific Crew	45 min	0.889	0.646	0.928	0.793	0.740	0.515
	SD	0.279	0.011	0.599	0.160	0.259	0.210

Table 5: Mean raw cortisol values for students (N=24) and ship's crew (N=4), with standard deviations.

Analysis of area under the curve (AUC) for cortisol response to awakening revealed an interaction effect with time, indicating that subjects' CRAs changed over the course of the study (repeated measures ANOVA, $p = .0009$, Fig. 6a). This effect remained significant when analyzing total AUC of cortisol (repeated measures ANOVA, $p =$, Fig. 6b) and cortisol maximum response (repeated measures ANOVA, $p = .0003$, Fig. 6c), suggesting that subjects' CRAs changed depending on working/living conditions at the time of sampling. In post-hoc analysis, Fisher tests showed smaller mean AUC of response on the weekend shore morning compared to the workday shore morning (OSWD) (Fisher, $p < .0001$), Sea-1 morning (Fisher, $p = .0124$) and Sea-3 morning (Fisher, $p = .0005$), suggesting that the weekend day elicited smaller CRAs than working days on shore or at sea. Figure 7 shows the difference in mean raw cortisol curves between OSWD and OSWE samples in the student cohort. At the same time, mean AUC of response was greater on the workday shore sample compared to the Sea-2 sample (Fisher, $p = 0.0225$) and the shore leave (M-2) sample (Fisher, $p = 0.0069$), suggesting that CRAs were smaller on some sea mornings (of both working and break days) than on the morning of a workday on shore. Lastly, mean AUC of CRA was greater for the Sea-3 sample compared to the M-2 sample (Fisher, $p = 0.0369$), suggesting that CRAs decreased from the third at-sea working day sampled to the shore leave day, on which subjects were not scheduled to work.

Analysis of total AUC for cortisol resulted in similar results. Mean total AUC for the weekend shore sample was lower than mean AUC of workday shore sample (Fisher, $p = 0.0032$). The workday shore sample also showed a higher mean AUC for cortisol than the Sea-1 (Fisher, $p = 0.0485$), Sea-2 (Fisher, $p = 0.0445$) and M-2 (Fisher, $p = 0.0149$) samples. When analyzing the cortisol maximum response, the workday shore sample (OSWD) showed a significantly greater response than the weekend shore sample (Fisher, $p < 0.0001$) and the Sea-2 sample (Fisher, $p = 0.0190$). Maximum response measures also revealed a significantly greater mean response on the Sea-3 sample compared to the M-2 sample (Fisher, $p = 0.0348$). Significant differences in means are summarized in table 6. A number of significant correlations were found within subjects between various sampling times.

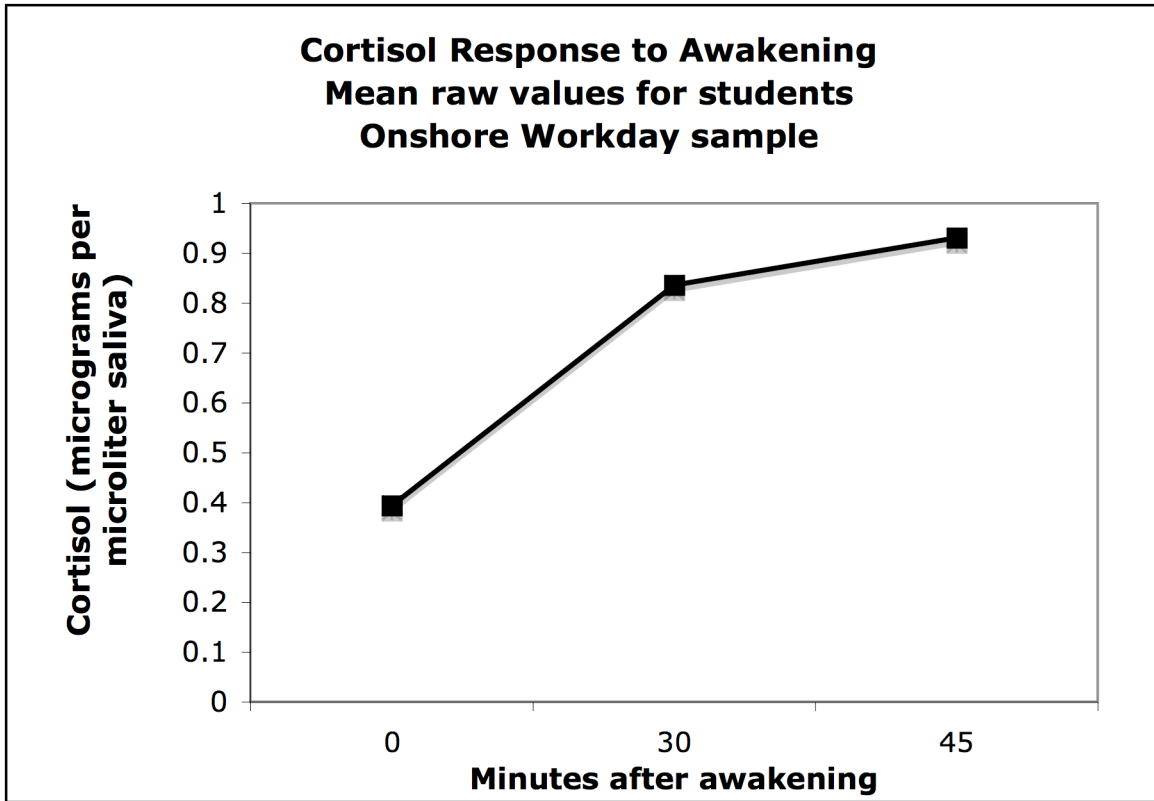


Figure 5: Cortisol Response to Awakening was measured as a three-point curve comprised of raw salivary cortisol levels at 0, 30 and 45 minutes after awakening. CRA was then quantified using three metrics: height of maximum increase over baseline (CRA Max Response), total area under the curve (Total AUC), and baseline-subtracted area under the curve (AUC of response).

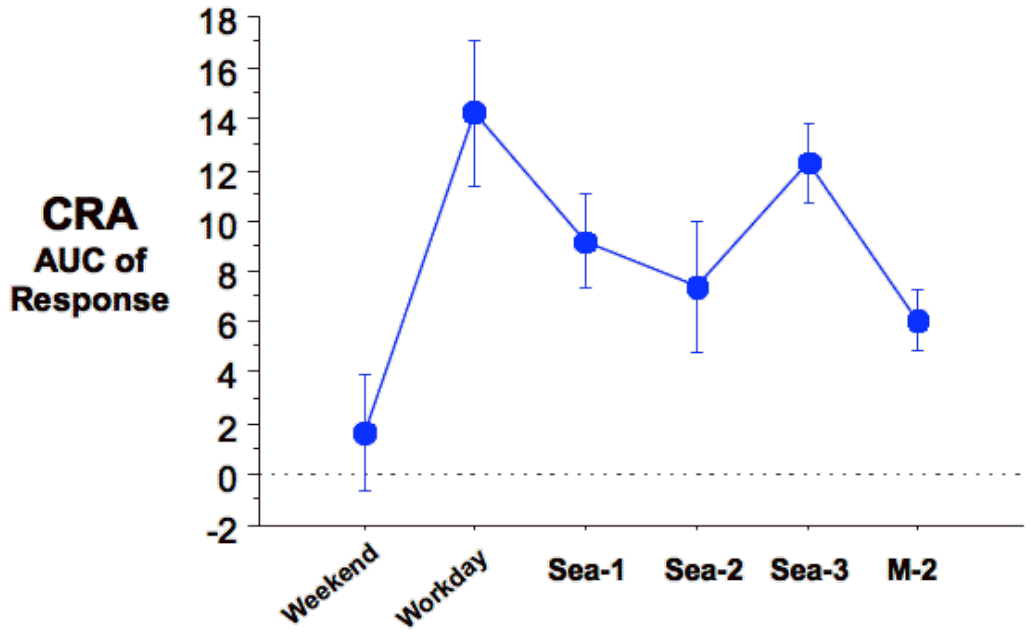


Figure 6a: Mean (\pm standard error) cortisol response to awakening over time, measured in $\mu\text{g}/\mu\text{L}$ as area under the curve of maximum increase in cortisol over baseline. A significant interaction effect with time ($p=.0009$) was found. Data includes students and two science staff over entire course of study ($N=15$).

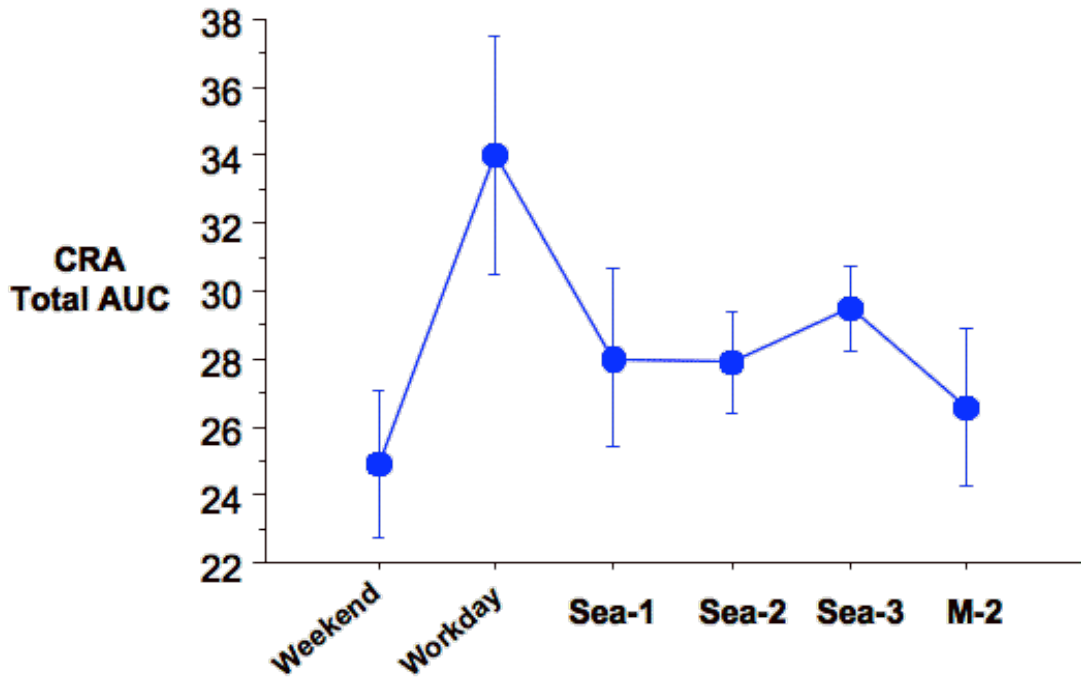


Figure 6b: Mean (\pm standard error) cortisol response to awakening over time, measured in $\mu\text{g}/\mu\text{L}$ as total area under the curve (for 0 minutes, 30 minutes and 45 minutes after awakening). A significant interaction effect with time ($p=$) was found. Data includes students and two science staff over entire course of study ($N=15$).

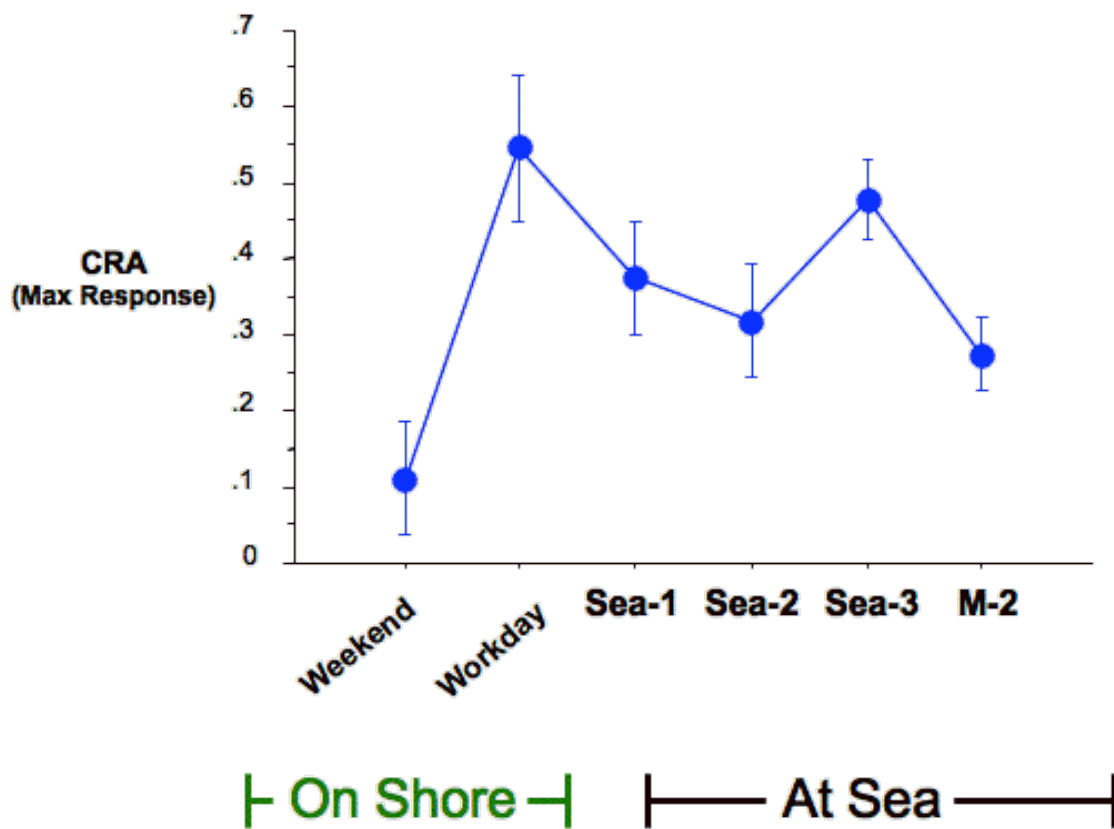


Figure 6c: Mean (\pm standard error) cortisol response to awakening over time, measured in $\mu\text{g}/\mu\text{L}$ as maximum increase in cortisol over baseline. A significant interaction effect with time ($p=.0003$) was found. Data includes students and two science staff over entire course of study ($N=15$).

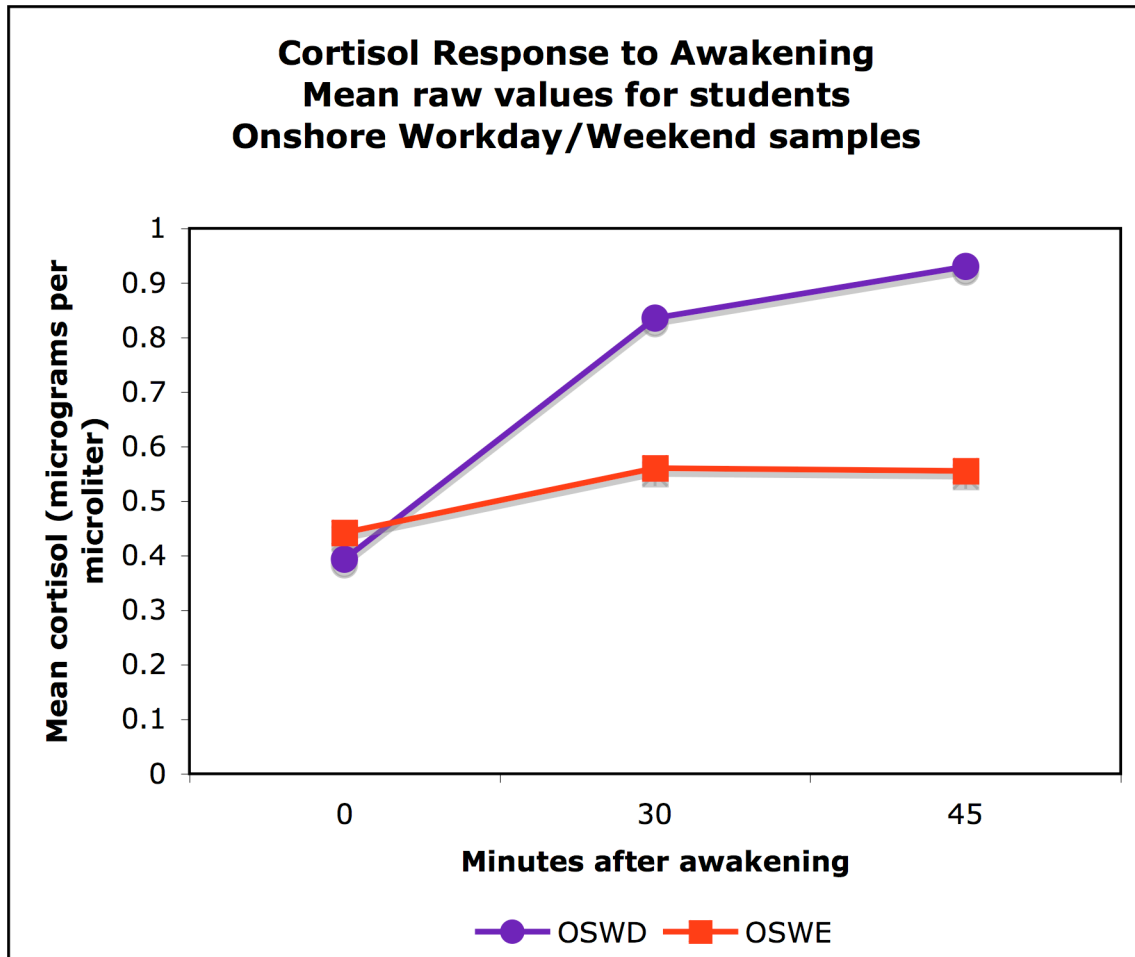


Figure 7: Mean raw cortisol values for students during onshore workday (OSWD) and onshore weekend (OSWE) samples. Data show raw value differences in CRA curves. These differences were significant in Fisher post-hoc test of total area under the curve ($p < .0001$).

Measure	Difference in sample means	p-value
AUC of Response	Weekend < Workday	<.0001
	Weekend < Sea-1	0.0124
	Weekend < Sea-3	0.0005
	Workday > Sea-2	0.0225
	Workday > M-2	0.0069
	Sea- 3 > M-2	0.0369
Total AUC for cortisol	Weekend < Workday	0.0032
	Workday > Sea-1	0.0485
	Workday > Sea-2	0.0445
	Workday > M-2	0.0149
CRA maximum response	Weekend < Workday	<.0001
	Workday > Sea-2	.0190
	Workday > M-2	0.0055
	Sea-3 > M-2	.0348

Table 6: Significant differences in means between samples: results of Fisher tests on all subjects N = 15.

3.3 *Group differences in duty*

Because professional crew and some science staff could not provide workday or weekend on-shore samples, analysis of group differences included only the four shipboard samples (Sea-1, Sea-2, Sea-3 and M-2). Analysis of group differences between students and professionals (crew and science staff, N=8) showed no significant differences (Fig. 8a). When comparing only ship's crew (N=4) and students, however, there was a main effect of time and a significant interaction effect with time for maximum cortisol response (repeated measures ANOVA, $p=.0004$), and AUC of response (repeated measures ANOVA, $p=.0002$), indicating that the CRA of students and ship's crew changed differently over time (Fig. 8b). Additionally, splitting the cohort revealed that AUC of response for the ship's crew was significantly greater in the Sea-3 sample compared to the Sea-1 (Fisher, $p=.0087$) or Sea-2 samples (Fisher, $p=.0169$). When the students were analyzed separately, there were no significant differences found between the four shipboard samples in students' mean AUC of response. No significant differences were found between students and crew in subjective ratings of stress or control.

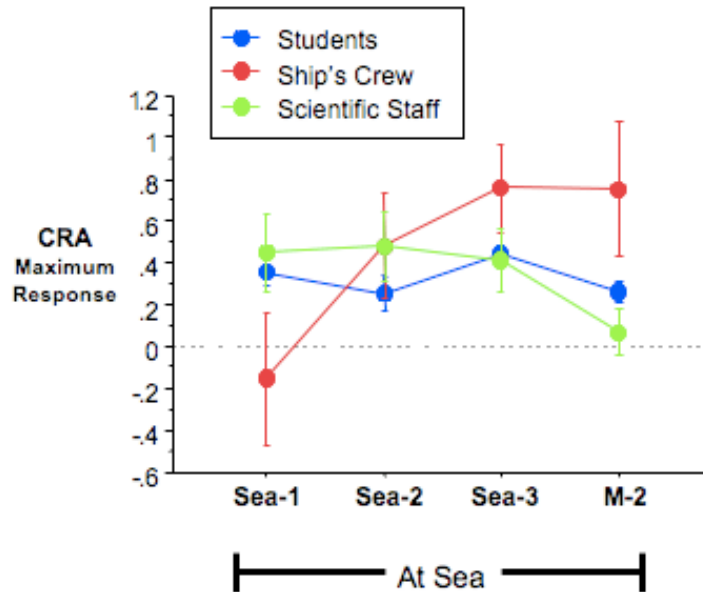


Figure 8a: Differences in cortisol response to awakening between students (N= 20) , scientific staff (N=4) and ship's crew (N=4): At-sea samples. CRA is measured in $\mu\text{g}/\mu\text{L}$ as maximum increase in cortisol over baseline (\pm standard error). Notice lack of differentiation in CRAs of students and scientific staff. Ship's crew show significant difference in interaction effect, as well as differences in the means of some timepoints.

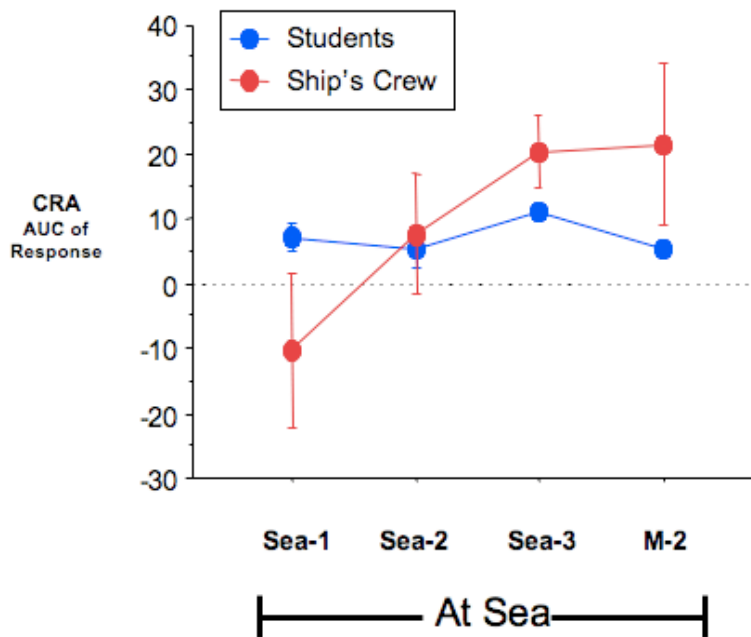


Figure 8b: Differences in cortisol response to awakening between students (N= 20) and crew (N=4): At-sea samples. CRA is measured in $\mu\text{g}/\mu\text{L}$ as area under the curve of maximum increase in cortisol over baseline (\pm standard error).

4. Discussion

Results of this study confirmed two major predictions. The cortisol response to awakening was shown to change depending on study conditions, and students were shown to respond differently from crew to the experience of seafaring. The considerably larger cortisol response to awakening on the OSWD versus the OSWE sample is consistent with other studies (Kunz-Ebrecht et al. 2004, Schlotz et al. 2004) in which CRA was higher on weekdays than on weekends. This validates our measure, but also establishes a dynamic onshore baseline for comparisons with samples taken at sea. For instance, the experience of shore leave (a non-work day of personal time on shore) appears to successfully attenuate the CRA during seafaring voyages in a manner similar to the normal weekend effect. This finding may help shed some light on weekday/weekend differences in the CRA, because while shore leave retains the weekend anticipation of low stress, free time and extra sleep, frequency and relatedness of social interactions were controlled during shore leave. In this study, mean time of awakening was somewhat higher for OSWE and M-2 samples than for the working-day samples, but since individuals' times of awakening were not found to correlate with any cortisol measures, late wakeup can be ruled out as a cause of smaller non-working day CRA. Other sleep measures, including total sleep duration and time of awakening, did not demonstrate an independent effect on the CRA. This is consistent with Federenko et al.'s (2004) study, which found no correlation between CRA and either sleep duration, sleep quality or time of awakening. Furthermore, this study did not find a relationship between subjective stress ratings and either CRA or awakening time, meaning that subjects' CRAs were not affected by waking time-dependant stress as in Williams et al. (2004).

4.2 Stress, CRA and diet

Since this study could not adequately examine the effect of diet on CRA, future studies are required to better understand the effect of diet on glucocorticoid regulation. Though variation in diet for this sample was small, this study's findings correlating meat consumption with both subjective stress and CRA should peak the interest of researchers interested in investigating the role of diet (specifically meat consumption) in modulating

cortisol function. Though Remer et al. (1998) found that experimentally reducing meat and protein consumption in a cohort also reduced 24-hr cortisol secretion, our data suggest that long-term (lifestyle-scale) reduced meat consumption may predict greater adrenocortical and psychological response to stressors. More research is necessary to determine the relative influence of diet on the HPA axis and to establish the mechanism for these interactions.

4.3 Reduced CRA during work at sea

We were surprised to find that despite the taxing, high-pressure conditions of working onboard a sailing vessel, subjects' CRAs were smaller onboard the ship relative to an average workday on shore. After removing the ship's crew from the analysis, this trend became even more significant (ship's crew members showed increasing CRA during the three on-duty sea samples.) We offer a number of possible explanations for this reduction in CRA. First, it is possible that the physical experience of seafaring or the oceanic environment influence CRA through undetermined pathways. To explore this possibility, further studies would need to manipulate the living environment while controlling other factors known to influence the CRA. Another potential explanation for diminished responses at sea involves the anticipation of challenge in a self-selecting cohort of young students. It is possible that individuals who would choose (in fact, who would pay) to undergo training at sea may perceive those challenges more positively than the stressors of everyday life. This challenge-seeking attitude or excitement may be responsible for reducing anticipatory stress and attenuating the CRA. Lindhal et al. (2005) have found that students who applied the confidence building procedure "I say to myself: I can solve this task" in a school test situation showed lower CRA the morning of testing when compared to other students. Also, Lai et al. (2005) have shown that the CRA is lower in individuals with relatively higher positive affect and optimism. These findings suggest that a combination of personality traits such as challenge-seeking self-confidence and positive affect resulting from the holistic experience of seafaring may have attenuated stress-related effects on the HPA-axis in our student cohort.

Another possibility is that increasing social bonding over the course of the program acted to reduce social stress and subsequently buffered or offset reactivity of the HPA-axis. Perceived social stress and lack of social recognition have been shown to elevate CRA

(Wüst et al. 2000) and loneliness was found to correlate positively with CRA (Steptow et al. 2003), therefore the effects of progressive social bonding on the HPA warrant further study. In past studies, prolonged exposure to stress was found to diminish cortisol response as a result of allostatic overload (McEwin and Wingfield 2003), but this seems an unlikely explanation for diminished CRA in the current study, because ship's crew, who experienced identical conditions on board, exhibited an increasing trend in CRA while at sea. Also, subjects did not self-report the high levels of stress that would normally be associated with allostatic overload. One potential confound was the effect of seasickness on participants. Klosterhalfen et al. (2005) have found that nausea and vomiting can alter cortisol secretion, but seasickness could neither be prevented nor controlled in this study, as subjects demonstrated a wide range of symptoms, and some participants reported no seasickness at all.

4.4 Enhanced CRA in experienced crew

Along with the surprising lack of increase in CRA at sea, higher CRAs in ship's crew than in students or scientific staff were similarly unforeseen; we had originally hypothesized that experience (i.e. lack of novelty) would attenuate the cortisol response to seafaring in the same way that repeated exposure has been shown to reduce adrenocortical response to novel stressors in animals (Hennessey and Levine 1979). Conversely, the highly experienced crew not only exhibited larger CRAs, but also appeared to follow a trend towards increasing CRA over the course of the working-day sea samples (Sea-1, Sea-2, Sea-3). There was no significant difference between the reported subjective stress of ship's crew and other subjects, implying that this difference may not reflect perceivably higher stress among crew. One possible explanation for these findings is that crew CRA may be responding to a turnover of ship's duties to students. During training voyages, students begin with few responsibilities on board, and are provided with increasing duties as they learn new skills. These duties, once the responsibility of ship's crew, are taken on by inexperienced students under supervision. This process requires the crew to relinquish control of some operations, which may result in greater HPA reactivity or CRA-boosting anticipatory stress. Analyses of subjective ratings of control did not produce a trend, but variation in the ratings

was very low. Also, there may have been a response bias against reporting low perceived control among the ship's crew.

In light of this data, new questions have arisen regarding the role of the living and working environment in HPA function. Further study is required to assess the role of challenge, landscape and perceived control in HPA reactivity. In conclusion, this study found that the cortisol response to awakening changed over time in response to the experience of seafaring, depending on work load, and that the CRAs of students changed differently from those of experienced crew.

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