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The mission of AAUS is to facilitate the development of safe and productive scientific divers through education, research, advocacy, and the advancement of standards for scientific diving practices, certifications, and operations.

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Measurement of Fatigue following 18 msw Open Water Dives Breathing Air or EAN36

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Abstract

SCUBA divers often report feeling fatigued upon conclusion of diving activities. Post-dive fatigue is thought to be induced by increased energy demands of submersion in a hyperbaric environment and decompression stress. Anecdotal reports indicate a reduction in post-dive fatigue when using enriched-air nitrox (EAN). The purpose of this double-blind study was to compare subjective fatigue levels experienced by SCUBA divers after two repetitive air dives and two repetitive EAN36 dives on separate, nonconsecutive days. Eleven male participants completed pre- and post-dive fatigue assessment using the Multidimensional Fatigue Inventory and a Visual Analogue Scale, while general health was assessed using the Diver Health Survey. Divers did tend to be more fatigued after diving; however, breathing gas mixture exhibited no statistically significant effect. Participants did have significantly lower Diver Health Survey scores upon the conclusion of EAN36 test sessions, possibly indicative of reduced decompression stress.

Keywords: decompression stress, nitrox, post-dive fatigue, SCUBA

Introduction

Many SCUBA divers use a breathing gas mixture, enriched-air nitrox (EAN), which contains a higher percentage of oxygen and lower percentage of nitrogen compared to air (20.93% O_2, 78.08% N_2). Since nitrogen is inert in metabolic respiration, lowering the fraction of nitrogen in a SCUBA diver's breathing gas effectively reduces the relative decompression stress experienced when compared to an air dive of similar depth and duration. The two most common EAN mixes contain 32% or 36% oxygen, denoted as EAN32 and EAN36. These have been established as standard EAN mixes in the National Oceanic and Atmospheric Administration Dive Manual and are often available from retail dive shops (Joiner, 2001).

Anecdotal reporting suggests that breathing EAN during a dive helps reduce post-dive fatigue (Charlton, 1998; Lang, 2001). The mechanism by which this may occur is not clearly understood; however, it has been purported that post-dive fatigue might be a result of decompression stress (Lang, 2001). Under this assumption, reducing inert gas levels in a breathing mixture reduces decompression stress as relatively lower concentrations of inert gas are absorbed and eliminated throughout the course of a dive. A diver would, thereby, surface with a noticeable reduction in post-dive fatigue. The only known study to test this premise found no significant difference between fatigue levels following air and EAN dives (Harris et al., 2003). Harris et al. acknowledged that the single, dry chamber dive profile used in their study may not have induced enough decompression stress to observe a difference between air and EAN36 post-dive fatigue levels.

It is known that greater levels of decompression stress are experienced during repetitive dive profiles due to incomplete equilibration toward normobaric inert gas saturation between dives (Marroni et al., 2000). Open water environments elicit increased energy costs of repetitive SCUBA dives; specifically, adaptations to thermoregulatory demands that would be difficult to simulate in a dry chamber. If decompression stress is associated with post-dive fatigue, assessment following repetitive dives in an open water environment may provide the combined stimuli to produce a significant difference between air and EAN post-dive fatigue.

The purpose of this double-blind study was to compare subjective fatigue levels experienced by SCUBA divers after two repetitive air dives and two repetitive EAN36 dives on separate, nonconsecutive days. The open water environment and repetitive dive profile required increased energy expenditure and decompression stress relative to a single, dry chamber dive, thereby creating a potential for increased differentiation between post-dive fatigue levels following air and EAN dives.

Methods

Participants

Eleven certified male SCUBA divers, aged 18-35 years, volunteered to participate in two test sessions separated by a minimum of 48 h. Separating test sessions in this manner minimized any effect related to multiday diving. Participants had active cold water dive experience and a minimum of 12 dives in the past year, ensuring they were accustomed to the thermoregulatory demands and the need for adequate thermal protection (typically a 7 mm wetsuit or drysuit). The minimum 12 dive requirement was consistent with active diving standards established in scientific diving manuals (American Academy of Underwater Sciences [AAUS], 2006). In addition, participants were certified as either AAUS scientific divers or dive leaders (i.e., Divemaster, Assistant Instructor, or Instructor) from nationally recognized dive agencies. Individuals meeting these qualifications have experience with task-related dive protocols requiring mastery of buoyancy control. The specified age range was selected to minimize risk of age-related health complications. The number of participants was deemed appropriate based on similar methods used by Harris et al. (2003). Females were excluded from this study to control for potential variability related to decompression incidence and the menstrual cycle (Lee et al., 2003).

Instrumentation

Fatigue was assessed using the Multidimensional Fatigue Inventory (MFI-20) and a 100 mm Visual Analogue Scale (VAS). The MFI-20 is a 20 item questionnaire consisting of five subscales measuring different aspects of fatigue. It is a validated tool that has been used to evaluate fatigue after SCUBA diving and physical activity (Smets et al., 1995; Harris et al., 2003). In an effort to direct participant responses to acute fatigue levels, the instruction set of the MFI-20 was modified slightly; participants were asked to view questions in terms of how they were feeling "right now" instead of how they had been feeling "lately." The VAS is a reliable tool for measuring general fatigue and has been utilized to compare pre- and post-dive fatigue levels (Grant et al., 1999; Harris et al., 2003). Perceived workload and thermal comfort were measured using a subset of questions from the Diver's Alert Network Project Dive Exploration questionnaire. The Diver Health Survey (DHS) was used to monitor a participant's general wellbeing after a test session. The DHS has been validated for assessing general symptoms indicative of decompression illness (Doolette, 2000).

Participants provided their own primary and backup regulator, submersible pressure gauge (SPG), compass, timing device, and depth gauge or computer. Cochran DDR-200 data recorders were attached to each diver to document depth, temperature, dive time, and ascent rates. Dive teams were provided a waterproof slate with the dive plan written on it, and two SCUBA cylinders containing either air or EAN36. All other dive equipment, including appropriate thermal protection (7 mm wetsuit or drysuit), was provided by participants. Participants were required to use the same gear configuration for all dives.

Professional Association of Dive Instructors repetitive air dive tables and National Association of Underwater Instructors EAN36 dive tables were utilized to design safe dive profiles. A 20 m transect tape was used to mark a fixed underwater course along an 18 msw depth contour. Test session data consisting of start and end times, surface interval time, beginning and ending cylinder pressure, and cylinder test set designator were recorded for each dive.

Procedures

The methods employed in this study were approved by an Institutional Review Board in accord with the ethical principles for research involving human subjects at San Jose State University. Participants were informed that they were being asked to participate in an underwater research project assessing post-dive fatigue levels after breathing either air or EAN36. Inherent risks and potential benefits of this study were discussed in detail with each participant. Participants read and signed a consent form. In the unlikely event of a diving accident, a certified Diving Medical Technician was available for consult during all test sessions.

Participants completed two test sessions separated by a minimum of 48 h. The test sessions occurred at approximately the same time each day to maintain consistent sleeping patterns and to avoid offsetting circadian rhythms. Participants were asked to refrain from heavy exercise, smoking, SCUBA diving, nonprescription drugs, and drinking alcohol or caffeinated beverages for 24 h prior to testing.

Prior to the beginning of a test session, environmental assessments were made to determine whether ocean conditions were safe. Participant feedback was part of the assessment; both the researcher and participant could cancel a test session if either individual felt conditions were hazardous. Swell height and period, wind speed and direction, and water craft advisory data were retrieved the morning of each test session from the National Weather Service Coastal Waters Forecast for Monterey Bay, California.

At the beginning of each test session, fatigue was assessed using the MFI-20 and VAS, in random order. Participants completed tests in a quiet environment to minimize distractions from other divers. After pretests were completed, test session dive plans were discussed, with participants informed that they would be breathing either air or EAN36 for two repetitive dives. The oxygen fraction of 0.36 was selected to provide the largest differential between oxygen concentrations of common EAN mixes and air. Both participant and researcher were unaware of which test session utilized EAN36. Decompression and oxygen loading parameters were analyzed for both breathing mixtures to ensure participants of safe dive profiles that fell within recommended no-decompression stop limits. Underwater signals and safety protocols were discussed in detail.

A test session dive plan consisted of two, 30 min square profile dives at a depth of 18 msw, separated by a one hour surface interval. Participants dived in teams of two, maintaining close proximity (within 1 m of each other). Either participant could abort a dive at any time for any reason. If divers became separated at depth, they were to stop and search for each other for 1 min. If they were unable to reconnect underwater, the divers were to ascend at a rate of 9 m/min and reconvene at the surface.

Participants were transported by motor boat to the test site. Prior to all test session dives, beginning cylinder pressure was recorded. Dive teams descended down a fixed line. Descent rate was controlled by the participants' ability to equalize pressure in their ears. The descent was expected to take approximately 1 min. Participants swam laps along a 20 m transect tape at a depth of 18 msw. A Timex Expedition digital watch was used to monitor and maintain a swimming rate of 18.29 $m \cdot min^{-1}$. Dujić et al. (2005) used a similar rate of 17 m/min to assess the effects of low intensity underwater exercise (approximate oxygen consumption rate of 13 $mL \cdot kg^{-1} \cdot min^{-1}$) on decompression stress in fit divers. Participants were to begin an ascent at a rate of 9 $m \cdot min^{-1}$ when their total bottom time reached 25 min. If a problem occurred or a dive team member's SPG read 700 psi (49.2 $kg \cdot cm^{-2}$) or lower, participants were instructed to abort the test protocol and ascend safely. Dive teams conducted a 3 min safety stop at 5 msw, and then proceeded to the surface at a rate of 9 $m \cdot min^{-1}$. The total ascent time including safety stop was 5 min. Dive time, maximum depth, ending cylinder pressure, and water temperature were recorded. Participants prepared dive gear for a second dive, completed a questionnaire rating perceived workload and thermal comfort, reported problems experienced, if any, and then rested for the remainder of the surface interval. Water, granola bars, bagels, yogurt, and bananas were available at each test session. Participants were not required to consume these items, but were asked to be consistent in snack choice between test sessions. The second dive followed the same protocol as the first.

Upon conclusion of the second dive, participants were instructed to refrain from heavy exercise, smoking, additional SCUBA dives, nonprescription drugs, alcohol consumption, caffeinated beverages, and napping for 90 min. After 90 min, participants were asked to complete the MFI-20 and VAS. The 90 min post-dive interval for fatigue assessment was implemented in an effort to account for continued decompression stress beyond the duration of the actual dive (Radermacher et al., 1990; Marroni et al., 2000). A test session concluded with participants completing the DHS 24 h after the second dive.

Cylinders were filled by a designated, certified, compressed gas fill station operator. The fill station operator was provided four balanced, randomized templates specifying gas order for each participant. One template was randomly selected by the fill station operator to be used throughout the study. Participants and researcher did not know which template was used. A participant ID was assigned and recorded on the fill template to ensure each participant received both treatments. Cylinder fill pressure and percentage of oxygen were recorded on a spreadsheet. All EAN36 fills contained between 35-37% O_2.

Statistical Analyses

The independent variable in this study was breathing gas (air or EAN36). The dependent variables were fatigue, workload, and thermal comfort. The general fatigue (GF), physical fatigue (PF), and mental fatigue (MF) subscales of the MFI-20 were scored. Distance from the 'no fatigue' anchor to the participant's subjective fatigue marking on the VAS was measured to the nearest millimeter. The effect of breathing gas on post-dive fatigue levels was analyzed using a two-way repeated measures analysis of variance (ANOVA). A paired t-test was performed to examine DHS responses. Pearson product-moment correlations were used to examine relationships between breathing gas volume consumed, thermal comfort, workload, temperature, and swimming speed. A paired t-test was used to assess perceived workload relative to breathing gas treatment. Sigma Stat version 3.5 (Systat Software, Inc.) was used for all statistical analyses. Dive profile data were acquired using Professional Analyst 4.01 (Cochran Consulting, Inc.).

Results

Eleven participants completed two test sessions with minimal complications. Three test sessions were cancelled and rescheduled due to unsuitable diving conditions (e.g., strong currents, large swell); mechanical issues with the motor boat led to the rescheduling of another. No other test sessions were cancelled or aborted. The average interval between test sessions for participants was 25 ± 5 days, mean (*M*) ± standard error of mean (*SEM*). Seven days was the minimum; 148 days was the maximum. No equipment problems were reported; however, one participant's data recorder failed during a test session, yielding irretrievable dive profile information. For this case, the individual's profile was based on the second team member's data recorder. Descriptive data for test session dive profiles are outlined in Table 1.

Table 1. Test Session Dive Profiles

	Water Temperature (°C)	Depth (m)	Time Underwater (min)	Time Swimming (min)	Speed ($m \cdot min^{-1}$)	Distance Swam (m)	Gas Used (L)
M	12.0	17.3	30.2	21.1	17.2	362.5	1626.7
SEM	0.2	0.1	0.3	0.3	0.2	7.0	30.9

Note. Values are means (*M*) ± standard error of the mean (*SEM*). Distance does not include descent, ascent, or movement during safety stops.

Figure 1 shows the expected dive profile and an actual profile retrieved from a data recorder. The descent and ascent rates were 15 ± 0.4 $m \cdot min^{-1}$ and 6.7 ± 0.2 $m \cdot min^{-1}$ (*M* ± *SEM*), respectively. These were slightly slower than the prescribed rates.

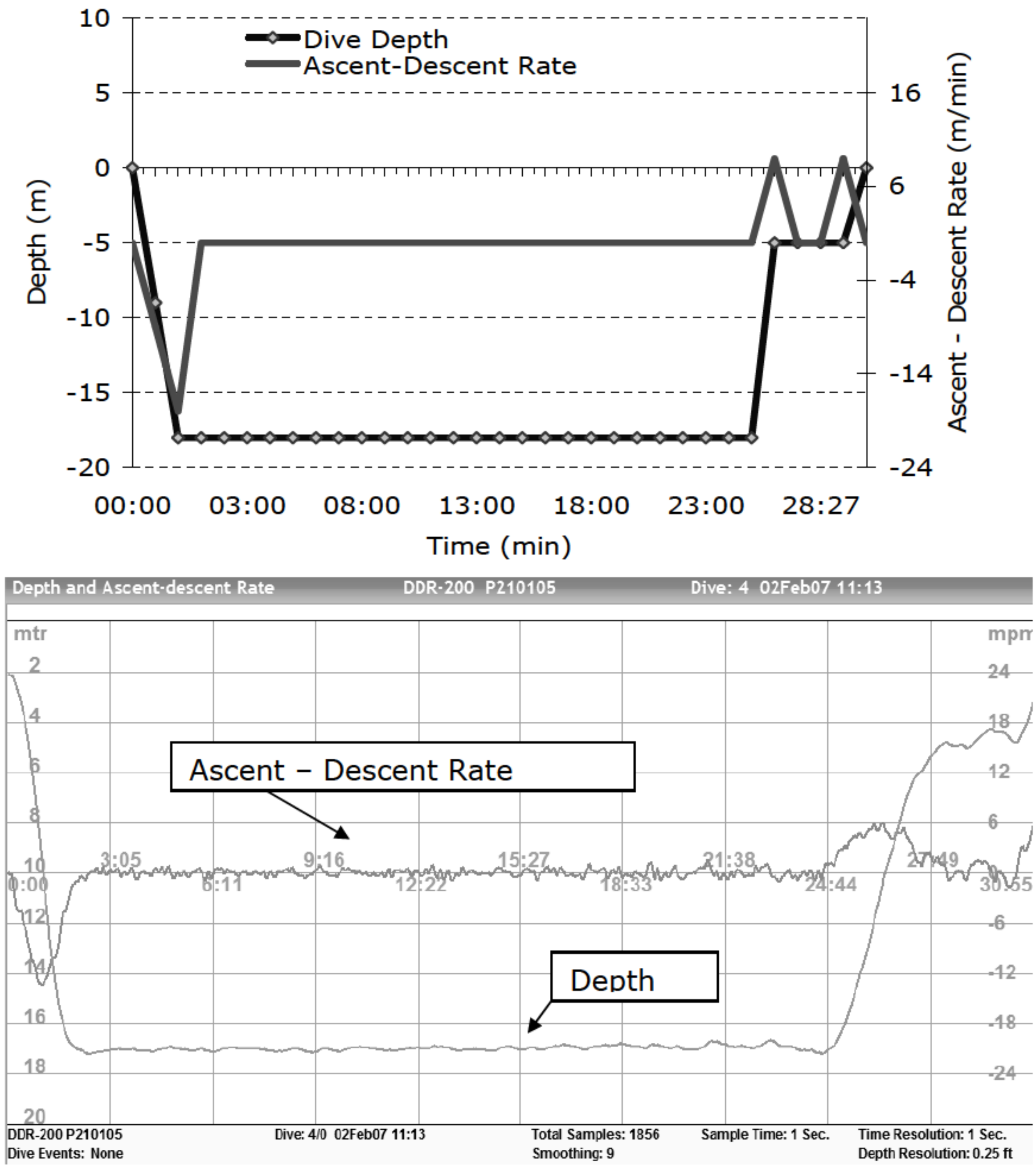

Figure 1. Planned profile (top) and actual data retrieved from the Cochran DDR-200 (bottom).

Visual Analogue Scale

There were no statistically significant differences between breathing air or EAN36 on fatigue ratings (30.8 ± 3.7 vs. 34.7 ± 4.6 mm, p>0.05). Although not statistically significant, post-dive fatigue levels, 39.2 ± 4.3 mm, tended to be greater than pre-dive ratings, 26.4 ± 3.6 mm (*M* ± *SEM*), independent of breathing gas mixture ($F_{1,\ 10}$ = 4.675, p=0.056). There was no statistically significant interaction between breathing gas mixture and pre- and post-dive fatigue ratings

MFI-20

There were no statistically significant differences between breathing air or EAN36 on fatigue ratings. There were statistically significant differences in post-dive fatigue compared to pre-dive ratings, independent of gas mixture, for the GF ($F_{1, 10}$ = 6.115, p=0.033) and MF ($F_{1, 10}$ = 11.658, p=0.007) subscales. The PF scores did not yield a statistically significant result when comparing pre- and post-dive fatigue ratings (see Figure 2). There were no significant interactions between breathing gas mixture and pre- and post-dive fatigue ratings. ANOVA data are summarized in Table 2.

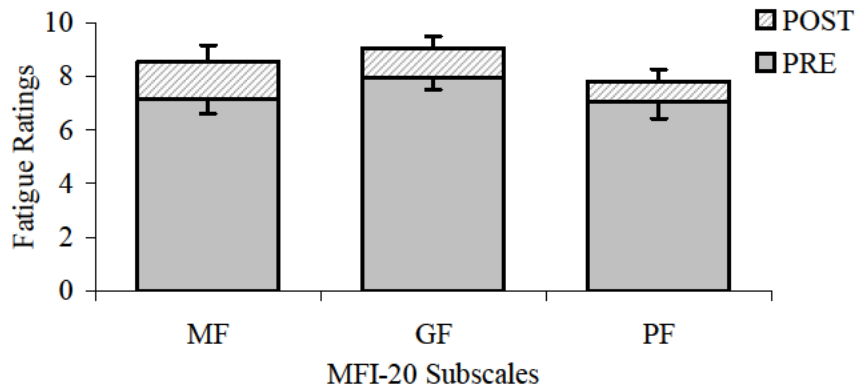

Figure 2. Least squares mean fatigue responses for mental fatigue (MF), general fatigue (GF), and physical fatigue (PF) subscales of the MFI-20 pre- and post-dive, independent of breathing gas mixture.

Table 2. MFI-20 Two-Way Repeated Measures ANOVA summary

Subscale	Source of Variation	df	SS	MS	F	p
MF	Participant	10	216.182	21.618		
	Factor A	1	20.455	20.455	11.658	0.007
	Factor A x Participant	10	17.545	1.755		
	Factor B	1	0.091	0.091	0.012	0.914
	Factor B x Participant	10	74.909	7.491		
	Factor A x Factor B	1	4.455	4.455	2.279	0.162
GF	Participant	10	101.500	10.150		
	Factor A	1	13.091	13.091	6.115	0.033
	Factor A x Participant	10	21.409	2.141		
	Factor B	1	0.364	0.364	0.117	0.740
	Factor B x Participant	10	31.136	3.114		
	Factor A x Factor B	1	0.818	2.668	0.307	0.592
PF	Participant	10	166.045	16.605		
	Factor A	1	6.568	6.568	1.101	0.319
	Factor A x Participant	10	59.682	5.968		
	Factor B	1	6.568	6.568	3.029	0.112
	Factor B x Participant	10	21.682	2.168		
	Factor A x Factor B	1	0.205	0.205	0.102	0.756

Note. MF is mental fatigue, GF is general fatigue, and PF is physical fatigue. Factor A is time of test (PRE or POST); Factor B is breathing gas (AIR or EAN).

Post-Dive Health Analysis

Diver Health Survey responses were evaluated after each test session to determine if participants were experiencing signs or symptoms of decompression sickness. One participant did not submit a DHS after his second test session. Although no responses warranted further inquiry into a participant's post-test health, a paired t-test yielded a significant difference between air and EAN36 posttest responses ($t = 2.60$, $p=0.032$). The mean scores for air and EAN36 test sessions were 2.89 ± 0.68 and 1.44 ± 0.29 (*M* ± *SEM*), respectively (both considered low risk for developing health complications from a previous day's dive—30 was the maximum possible score).

Environment and Workload Analyses

Thermal comfort was rated on a scale of 1 (cold) to 5 (warm), and workload was rated 1 (light) to 5 (hard). Table 3 shows the Pearson product-moment correlation coefficients between these variables. Small, positive correlations were found between gas consumed and workload ($r = 0.320$ $p=0.034$) and thermal comfort and water temperature ($r = 0.404$, $p=0.007$). No other statistically significant correlations were found. No significant difference was found in perceived workload relative to breathing gas treatment ($t = 1.678$, $p=0.108$).

Table 3. Pearson Product-Moment Correlation Coefficients

	Thermal Comfort	Workload	Temperature	Swimming Speed
Gas Consumed	-0.198 p=0.198	0.320 p=0.034	-0.087 p=0.573	0.174 p=0.259
Thermal Comfort		0.076 p=0.622	0.404 p=.007	-0.073 p=0.673
Workload			-0.118 p=0.447	0.185 p=0.229
Temperature				-0.029 p=0.851

Note. Gas consumed was measured in psi, temperature in ºC, and swimming speed in m/min. Thermal comfort was rated on a scale of 1 (cold) to 5 (warm), and workload was rated from 1 (light) to 5 (hard). Samples = 44 (11 participants, two test sessions, two dives each).

Results from the subjective fatigue tests showed that divers were fatigued following repetitive SCUBA dives. The data did not indicate reduced fatigue following EAN36 dives compared to air dives of similar depth and duration. Scored responses for the DHS were significantly higher following air dives compared to EAN dives. Divers breathed more gas during dives with higher perceived workloads and reported more thermal discomfort when the water temperature was colder.

Discussion

The purpose of this study was to compare fatigue levels between two test sessions consisting of two repetitive dives breathing either air or EAN36 in an open water environment. It was hypothesized that the reduced nitrogen level and subsequent higher oxygen levels would lead to decreased fatigue following two repetitive EAN36 dives. Analyses of reported fatigue failed to support this premise; however, scores on the Diver Health Survey did exhibit a significant decrease following dives using EAN36 compared to air.

The results of the current study are consistent with the findings of previous research by Harris et al. (2003) who observed no discernable differences in fatigue levels following single, dry chamber, air and EAN36 dives. Harris et al. suggested the dive profile used in their study may not have induced the necessary decompression stress to distinguish between air and EAN36 post-dive fatigue. It was postulated that increased decompression stress might induce greater subjective fatigue ratings, possibly with significant differences between air and EAN36 dives. The open water, repetitive dive profile used in the current study was designed to accomplish this task. However, it may not have induced the necessary decompression stress to produce a statistically significant difference in fatigue levels. Charlton (1998) reported generally lower levels of fatigue and more energy among a group of research divers performing a series of repetitive, multiday dives using EAN. The benefits of EAN in reducing post-dive fatigue may only be realized over a series of dives carried out over multiple, continuous days. Further research is warranted to compare fatigue following a series of multiday, repetitive air and EAN dives.

The number of participants used in this study may have been too small to determine a trend or significant effect of breathing gas mixture on fatigue. With the fatigue levels and variability found in the current study, power analyses showed that over 100 participants would have been necessary to establish significance, a number which would be impractical for controlled, open water assessment using the current study's design.

Although the MFI-20 was used in previous research to assess acute, dive-related fatigue, the original intent of the tool was to measure fatigue in cancer patients. This tool may be inappropriate for measuring fatigue associated with SCUBA diving. The results did detect one expected outcome: divers were more fatigued after diving. The VAS results also suggested increased fatigue after diving. It was not apparent whether post-dive fatigue was induced through decompression stress or energy expenditure. It may be that the fatigue induced in the current study was primarily due to workload and thermoregulation. The question remains as to whether a smaller, more subtle difference in fatigue existed based on breathing gas mixture than could be detected by the MFI-20 or VAS.

The 90 min post-dive assessment may not have been the ideal time period for measuring fatigue. One measure of decompression stress experienced during a dive is the presence of venous gas emboli (VGE). It is known that VGE can circulate for hours upon conclusion of a dive (Dick et al., 1984; Radermacher et al., 1990). Although not a direct measure of post-dive fatigue, participants did respond with significantly higher DHS scores following air dives. This was in contrast to previous research that found no difference in DHS scores following EAN and air dives (Harris et al., 2003). This result supported the contention that multiple dives would induce a greater degree of decompression stress than a single exposure; however, it did not necessarily imply reduced fatigue following EAN36 dives. In response to question six of the Diver Health Survey, "How much of the time since your last dive have you felt full of energy?," 3 of 10 participants indicated less energy following test sessions using air. However, after his EAN test session, one participant remarked, "Although I didn't feel too fatigued within an hour of diving yesterday, by 3 pm I was feeling fatigued and wasn't feeling up to working on the computer." Future studies might consider multiple measurements beyond a 90 min period to compare changes in fatigue between air and EAN dives over a prolonged period. The difficulty would lie in controlling for other post-dive activities, such as caloric intake.

Three participants felt they would be able to determine which gas they were breathing during a test session. Only 1 of 3 guessed correctly. One commented that his breathing rate was better during one test session and, therefore, he assumed he was breathing EAN36. The data did not support this conjecture; the diver actually consumed 249.75 L (8.82 ft^3) more gas during the EAN test session. The diver perceived the same workload across test sessions, but reported a higher level of thermal comfort during the EAN test session.

Results from this study did not support the contention that using EAN36 as a breathing gas reduces post-dive fatigue. To date, research has indicated that there is no difference in fatigue levels between air and EAN36 dives. This conclusion has been supported by research using single, dry chamber and this study, which used repetitive, open water dive exposures. However, the conclusion from this study using a repetitive, open water dive protocol should be viewed with caution due to the low power of the design.

Development of a more suitable test tool for measuring acute changes in SCUBA-related fatigue may be warranted. Future research might consider studying individuals who report feeling less post-dive fatigue when using EAN to determine possible trends in dive profiles. A qualitative study exploring the feelings and common themes reported by divers affected by post-dive fatigue may be necessary. A comparison of venous gas emboli using Doppler ultrasound could provide more insight into the level of decompression stress exhibited following air and EAN dives. Fatigue induced through repeated hyperbaric exposure may be more chronic in nature and present itself over a prolonged period of time. Multiple assessments of post-dive fatigue, beyond the 90 min protocol used in the current study, could help determine if there is delayed onset. This may entail repeated measurements following repetitive dives or possibly assessing post-dive fatigue after repetitive, multiday profiles.

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Invertebrate Communities Associated with Various Substrates in the Nearshore Eastern Aleutian Islands, with Emphasis on Thick Crustose Coralline Algae

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Abstract

In the Aleutian Islands, crustose coralline algae can be widespread in the low intertidal and shallow subtidal regions, and cover most available hard substrates. Subtidal habitats dominated by crustose coralline algae are often associated with sea urchin-barren grounds and regarded as supporting limited invertebrate communities, especially compared to the adjacent kelp forests. *Clathromorphum nereostratum* is one of the most abundant crustose coralline algae found in the Aleutian Islands. Although the surface of this crustose alga exhibits little structural complexity, it can develop into crusts dozens of centimeters thick that provide microhabitats for a variety of cryptic invertebrates. Despite the omnipresence of this alga throughout the nearshore Aleutians, very little is known about its associated faunal community. In the summer of 2006, a benthic survey was conducted throughout the Eastern Aleutian Islands as part of the Alaska Monitoring and Assessment Program. Twenty-three sites, with depths <20 m, were randomly selected between Tigalda and Yunaska Islands. The habitat encountered at each site was categorized as soft sediment, macroalgae-dominated, or crustose coralline-dominated. At each site, scuba divers scraped clear three 25 x 25 cm (0.06 m^2) quadrats and an airlift dredge was used to collect samples. All preserved benthic invertebrates were later indentified to the lowest taxonomic level possible. Invertebrate communities associated with thick crustose coralline algae were compared to soft-sediment and macroalgae-dominated communities based on faunal abundance and diversity. Despite the desolate appearance of crustose habitats, this study suggests that crustose environments support faunal communities as diverse and abundant as those found in rich macroalgal habitats.

Keywords: *Clathromorphum*, EMAP, hard substrate, kelp holdfast, soft sediment, urchin barren

Introduction

The nearshore environment of the Aleutian Islands provides very diverse habitats ranging from soft sediments to bedrock and from sea urchin barrens to dense kelp forests. In many marine systems, highly heterogeneous habitats have been shown to support highly diverse floral and faunal communities (McCoy and Bell, 1991; Coleman and Williams, 2002; Steller et al., 2003). Tropical coral reefs are recognized as the most productive and diverse marine ecosystem and support the highest number of species per unit area (Knowlton, 2001; Groombridge and Jenkins, 2002; Roberts et al., 2002). In temperate and sub-boreal regions, kelp forests (North, 1971; Foster and Shiel, 1985; Graham, 2004), and rhodolith beds (Foster, 2001; Steller et al., 2003; Kamenos et al., 2004) also sustain high invertebrate biodiversity. Those rich and diverse ecosystems all provide highly complex dense k2( ) -5(a very diverse fish and invertebrate communities (Feder et al. 1974; Hicks, 1980; Ebeling and Laur, 1985; Bodkin, 1988). The holdfasts of brown algae in particular harbor a great variety of organisms (Ghelardi, 1971; Smith et al., 1996; Anderson et al. 2005).

Urchins are often found inhabiting kelp forests, but when urchin densities grow unchecked, urchin grazing pressure can remove most sessile organisms, including the prominent kelp, and transform lush kelp forests into urchin barrens. Urchin barrens are often considered the alternate state of kelp forests and, by removing the structural complexity offered by macrophytes, are believed to generate low biodiversity (Paine and Vadas, 1969; Lawrence, 1975; Steneck, 1986). Urchin barrens are also frequently associated with red crustose coralline communities, and urchin grazing may in fact help maintain the presence and dominance of crustose communities (Paine and Vadas, 1969; Steneck, 1986). In contrast to macrophytes and rhodoliths, crustose algae are very low-relief features that provide very little structural complexity and heterogeneity (Smith, 1944; Lebednik, 1973; Steneck and Dethier, 1994). At first glance, crustose coralline habitats resemble barren grounds and encrusting coralline algae are often referred to as primary substrate because of their bare rock appearance (Menge, 1976; Rowley, 1989).

During the summers of 2006 and 2007, the Alaska Monitoring Assessment Program (AKMAP) conducted coastal surveys of the Eastern and Western Aleutians Islands, respectively. AKMAP is a collaborative investigation between the School of Fisheries and Ocean Sciences at the University of Alaska Fairbanks and the Alaska Department of Environmental Conservation (ADEC). AKMAP is part of the Environmental Monitoring and Assessment Program (EMAP), a national research program led by the US Environmental Protection Agency's Office of Research and Development (EPA-ORD) (Jewett et al., 2008). Several types of habitats were encountered during the assessment, but kelp forests were a prominent sight along the Aleutian Chain. Soft sediments, urchin barrens and crustose coralline habitats were also commonly present.

In the Aleutians, the two most common red crustose coralline species are *Lithothamnion* sp. and *Clathromorphum nereostratum*. *Lithothamnion* sp. forms a thin layer of crust that conforms to the substrate (Smith, 1944; O'Clair and Lindstrom, 2001). In contrast, *C. nereostratum* forms extensive pavement-like deposits that can be 0.5 m in thickness and over 1 m in diameter (Lebednik, 1976). Upon further observations during the sampling efforts, we recognized that the thick crustose coralline, despite its bare and barren appearance, harbored a great number of invertebrates. Despite the ubiquitous presence of *C. nereostratum* throughout the Aleutian Chain, very little is known about the faunal communities associated with this thick crustose coralline.

The objective of this investigation was to assess and compare the invertebrate communities associated with the thick crustose coralline *Clathromorphum nereostratum*, with communities commonly found in other Aleutian coastal habitats (soft sediments and kelp holdfast). The data collected during the AKMAP research cruise of 2006 along the Eastern Aleutian Islands were used to address this objective, in terms of community assemblages, invertebrate abundance and species diversity.

Methods

Study Area

AKMAP conducted coastal surveys of the Eastern and Western Aleutians Islands during the summers of 2006 and 2007, respectively. In 2006, 23 sites were randomly selected between Tigalda Island, near the Alaska Peninsula, and Yunaska Island, west of the Islands of Four Mountains (Figure 1), a distance of approximately 500 km. Sites were located both on the Pacific and Bering Sea sides of the islands and in various types of habitats. The sites were located on soft substrates (mud and sand) and hard substrates, ranging from gravel to bedrock beneath kelp forests, urchin barrens, and crustose coralline habitats.

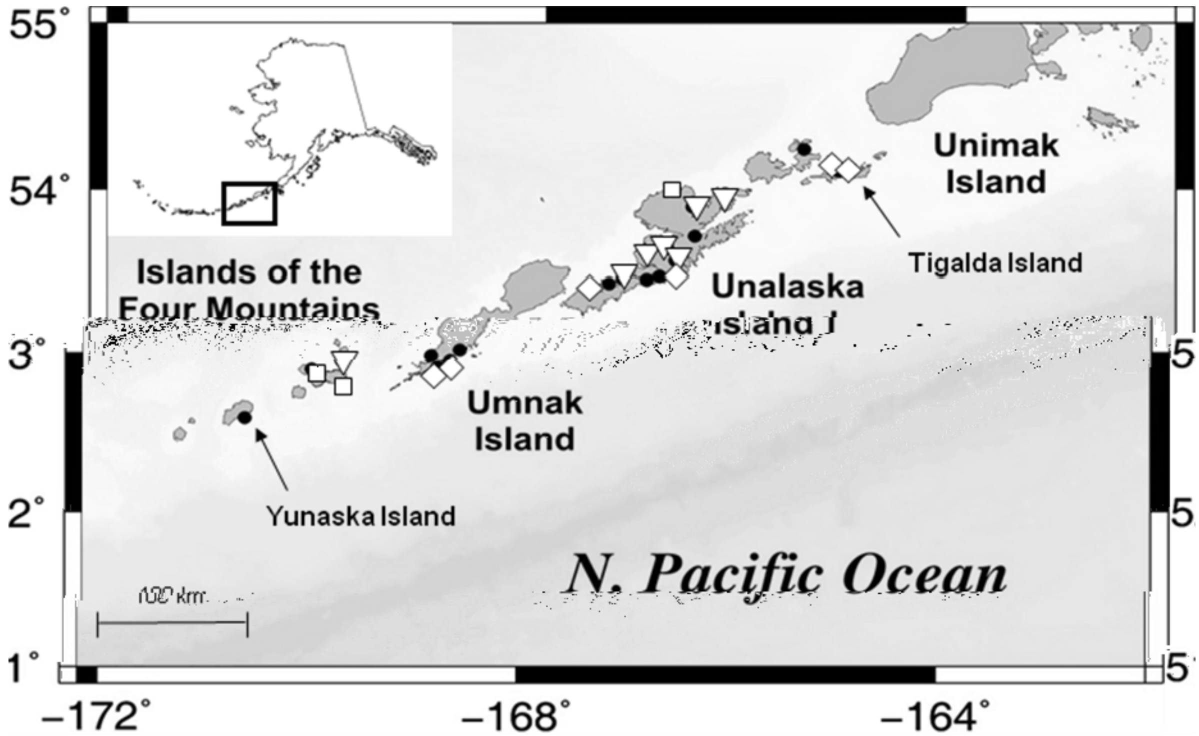


Figure 1. Map of the study sites in the Eastern Aleutian Islands, Alaska. AKMAP surveyed 23 nearshore sites between Tigalda and Yunaska Islands in 2006. Invertebrate samples were collected from three 25 x 25-cm (0.06 m^2) quadrats at each site. The sites that contained quadrats categorized as Soft, Thick Crustose Coralline, or Holdfast habitat are marked with white triangles, squares, or diamonds, respectively. The black dots represent surveyed sites that were categorized as Mixed habitat and were not included in the analysis.

Kelp forests in the study area were dominated by two canopy-forming species *Nereocystis luetkeana* and *Druehlia fistulosa.* The understory kelp often formed very dense mats and the dominant species included *Saccharina* sp., *Agarum* sp. and *Thalassiophyllum clathrum*. Urchin barrens were also common in the study area. Crustose coralline habitats were commonly found concurrent with urchin barrens. In the shallow subtidal region of the Aleutians, extensive coralline crusts were often very ubiquitous and covered nearly all available substrate (bedrock, large boulders, and small rocks). Two types of red crustose coralline were encountered, the thin crustose coralline, mostly *Lithothamnion* sp., and the thick crustose coralline, *Clathromorphum nereostratum*. Sandy areas were also commonly found throughout the survey.

Community Sampling

A detailed description of the sampling protocol is included in Jewett et al. (2008). The sample sites were located by GPS and nautical charts, and surveyed for safety and acceptable depth range. The depth of each site was randomly selected at <20 m. A large surface float with line and anchor was deployed for the dive-skiff's moorage. At each site, a 30-m transect tape was deployed along a depth contour and three sets of nested PVC quadrats were placed at random distances along the transect.

Each set included 1 x 1-m (1 m^2), 50 x 50-cm (0.25 m^2), and 25 x 25-cm (0.06 m^2) quadrats. The percent cover of algae and sessile invertebrates, and counts for kelp stipes and understory macroinvertebrates (>2 cm) were recorded within the 1 x 1-m frame. All algal material was collected from the 50 x 50-cm frame and was placed in labeled collection bags for later identification and weighing.

The 25 x 25-cm frames on rocky substrates were scraped clear of all algae (foliose and crustose) and invertebrates were collected by an airlift suction dredge powered by a scuba cylinder. If the quadrats were on soft sediments, the sediments were dredged to a depth of 10 cm. The destructive sample was retained in a labeled fine-mesh (500 µm), drawstring bag for later processing. Once on board the ship, the individual sample bags were rinsed in separate buckets of seawater to remove adhering material and poured on to a 1-mm mesh sieve, gently transferred to labeled Whirlpacks or wide-mouth plastic containers and preserved in 10% buffered Formalin. In the laboratory, the samples were rinsed and transferred to 50% Isopropanol for later processing.

Invertebrates associated with the thick crustose coralline *Clathromorphum nereostratum* represented a challenge as they were located deep inside the matrix of the thick crust. Under a dissecting microscope, the conspicuous invertebrates were removed first from the crust surface. The remaining invertebrates were extracted by fracturing the large calcareous chunks with a hammer. Smaller coralline pieces were then broken into small gravel-size pieces using wire cutters. Counts were recorded for solitary invertebrates whereas colonial organisms were recorded as present/absent. All sorted invertebrates were identified to the lowest practical taxonomic level, enumerated and archived in labeled vials. For the present analysis, challenging groups (mostly Porifera, Hydrozoa, Bryozoa, and Ascidiacea) were only identified at the phylum or class level. *Strongylocentrotus droebachiensis* and *S. polyacanthus* were the two dominant sea urchin species. Because of the difficulty in identifying individuals to the species level, and until further taxonomic and genetic information become available, sea urchins were conservatively identified to the generic level. Specimens difficult to identify were sent to expert taxonomists for verification, further identification, and for quality assurance / quality control. Two voucher collections were created, one to be archived at UAF and a duplicate for the EPA in Washington, D.C. Approximately 2,000 hours were required to process all invertebrate samples.

Data Analysis and Quadrat Selection

A total of 23 sites and 69 - 25 x 25-cm quadrats were sampled throughout the study area. Because several types of substrate were encountered within a single site, individual quadrats along the transect were categorized into one of four habitat types: Soft, Holdfast, Thick Crustose Coralline (TCC), or Mixed. In order to standardize as much as possible our quadrat categories, any quadrats that did not match specific characteristics chosen for Soft, Holdfast, and TCC habitats were labeled as Mixed. The Mixed category encompassed very diverse and inconsistent types of substrate (coarse sediments, encrusting sponges, *Lithothamnion* sp., bedrock covered with debris, etc.); therefore Mixed quadrats were not included in our community analyses.

Quadrats categorized as Soft were mostly sand, but the grain size ranged from fine to coarse. Quadrats that contained small gravel and pebbles were not included in our Soft category and were labeled as Mixed. Accordingly, quadrats that were located within kelp beds, but did not include holdfasts, were not included as Holdfast quadrats. To be categorized as TCC, *Clathromorphum nereostratum* had to cover the majority of the quadrat surface area.

Statistical Analysis

Non-metric multidimensional scaling (MDS) analyses were used to plot community similarity patterns based on abundance of invertebrate taxa from 25 x 25-cm quadrats within Soft, TCC, and Holdfast habitats (PRIMER 6.0, Primer-E, Ltd., 2001). Colonial invertebrates (mostly sponges, bryozoans, hydroids, and ascidians) were not included in the community analysis since they were only recorded as present/absent. The data were *ln* (x+1) transformed and the Bray-Curtis coefficient was used as a resemblance parameter. The multivariate patterns, in terms of habitat type resemblances, were depicted in ordination plots. Because samples from hard-substrate habitats (TCC and Holdfast) clumped more closely together than with soft-sediment samples, a subset MDS plot was produced to more clearly visualize how taxa composition of benthic fauna differed between TCC and Holdfast habitats.

The patterns depicted on MDS plots were derived from combined abundance data from individual taxa within each sample. The presence or absence, as well as the relative abundance, of certain influential species are responsible for the gradients observed (i.e., how closely or spread out samples are plotted). In order to visualize how certain taxa influence the community patterns within each habitat type, the abundance values of selected invertebrates that typify each habitat type were superimposed over the MDS plots. The relative size of each bubble on a plot was proportional to the number of individuals collected within each quadrat.

One-way Analysis of Similarity (ANOSIM, randomization/permutation procedure; PRIMER 6.0, Primer-E, Ltd., 2001) was carried out to assess the degree of difference in benthic invertebrate assemblages between habitats. The Null hypothesis that there were no significant differences amongst groups was rejected if the significance level (p) was <0.05. The R-statistic value was used to evaluate the extent of any significant difference. R-values close to 1.0 suggested that invertebrate communities of samples within a specific habitat were more similar to each other than to samples from another habitat.

The biodiversity indices taxon richness (S), abundance of individual organisms (N), and the Shannon-Wiener Diversity Index (Shannon Index) (H', log base e) were calculated using Primer statistical software (PRIMER 6.0, Primer-E, Ltd., 2001). The statistical differences in those factors between habitat types were evaluated with ANOVA's (R 2.6.2, 2008). All diversity and abundance values were *ln* (x +1) transformed prior to testing to satisfy assumptions of normality and equal variances. Taxon richness, the number of taxa recorded within each quadrat, was based on both solitary and colonial organisms since this index is not based on the counts of individuals within each taxa. In contrast, only organisms for which actual counts could be recorded were used to obtain the indices N and H'.

Results

The mean depths at each of our habitat types varied from 10-17 m for soft-sediment habitat, 7-17 m for TCC habitat, and 6-15 m for kelp holdfast habitat. There was no significant difference in mean depth between habitat types (p=0.254). All habitat types were spread out and well distributed throughout the entire study area (Figure 1).

The survey of the 23 sites (69 - 25 x 25-cm quadrats) along the Eastern Aleutian Islands led to the collection of a total of 57,726 individual invertebrates belonging to a total of 512 taxa. Not all organisms were identified to the species levels, but the taxonomic level of identification remained consistent throughout the samples. A total of 402 species, 292 genera, 184 families, 51 orders, 27 classes, and 16 phyla were recorded. The most abundant phyla were Annelida (30,831 individuals), Arthropoda (19,614 individuals), and Mollusca (3,561 individuals). A list of the 20 most abundant families within each substrate type shows that polychaetes and amphipods are abundant in all habitat types, but certain families are often specific to each habitat (Table 1). Owenid polychaetes are the most abundant group in soft sediments but not in hard-substrate habitats, whereas accrocirid polychaetes are abundant only in TCC habitat. The amphipods families Gammaridae, Coropiidae and Phoxocephalidae are abundant in soft sediments, whereas Caprellidae and Ischyroceridae amphipods are abundant in both hard-substrate habitats. Pleustidae and Eurisidae amphipods are characteristic of TCC; and Isaeidae and Hyalidae amphipods are characteristic of holdfasts. Bivalves are abundant in both soft sediments and holdfasts, with carditids and tellinids characteristic of soft sediments, and mytilids and hiatellids characteristic of holdfasts. Some groups of invertebrates are only abundant in specific habitats. For example, Cumaceans (Diastylidae and Lampropidae) and ostracods (Philomedidae) are only found in soft sediments and strongylocentrodid urchins are only abundant in TCC habitat.

Table 1. List of the 20 most abundant families within each habitat type (Soft, TCC, and Holdfast). The taxa are listed in decreasing order of abundance.

Soft	**TCC**	**Holdfast**
Oweniidae (Polychaeta)	Spionidae (Polychaeta)	Syllidae (Polychaeta)
Spionidae (Polychaeta)	Sabellidae (Polychaeta)	Spionidae (Polychaeta)
Philomedidae (Ostracoda)	Syllidae (Polychaeta)	Ischyroceridae (Amphipoda)
Gammaridae (Amphipoda)	Acrocirridae (Polychaeta)	Sabellidae (Polychaeta)
Corophiidae (Amphipoda)	Capitellidae (Polychaeta)	Caprellidae (Amphipoda)
Munnidae (Isopoda)	Munnidae (Isopoda)	Munnidae (Isopoda)
Lumbrineridae (Polychaeta)	Terebellidae (Polychaeta)	Isaeidae (Amphipoda)
Capitellidae (Polychaeta)	Caprellidae (Amphipoda)	Spirorbidae (Polychaeta)
Uristidae (Amphipoda)	Ischyroceridae (Amphipoda)	Mytilidae (Bivalvia)
Ampharetidae (Polychaeta)	Strongylocentrotidae (Echinoida)	Terebellidae (Polychaeta)
Rissoidae (Gastropoda)	Nereidae (Polychaeta)	Hiatellidae (Bivalvia)
Phoxocephalidae (Amphipoda)	Spirorbidae (Polychaeta)	Tanaidae (Tanaidacea)
Diastylidae (Cumacea)	Janiridae (Isopoda)	Serpulidae (Polychaeta)
Syllidae (Polychaeta)	Lumbrineridae (Polychaeta)	Capitellidae (Polychaeta)
Pholoidae (Polychaeta)	Phyllodocidae (Polychaeta)	Hyalidae (Amphipoda)
Carditidae (Bivalvia)	Pleustidae (Amphipoda)	Lumbrineridae (Polychaeta)
Tellinidae (Bivalvia)	Rissoidae (Gastropoda)	Rissoidae (Gastropoda)
Orbiniidae (Polychaeta)	Hippolytidae (Decapoda)	Janiridae (Isopoda)
Amphictenidae (Polychaeta)	Eusiridae (Amphipoda)	Pleustidae (Amphipoda)
Lampropidae (Cumacea)	Cirratulidae (Polychaeta)	Epialtidae (Decapoda)

The objective of this study was to compare invertebrate communities associated with the thick crustose coralline, *Clathromorphum nereostratum*, to those found in kelp holdfasts and soft-sediment habitats. Of the 69 samples collected, 34 were classified as Mixed, 19 as Soft, 6 as TCC, and 10 as Holdfast samples. The following results pertain only to the 35 samples categorized as Soft, TCC, or Holdfast habitats.

Overall invertebrate community structure, as displayed with MDS plots, suggested discernible differences between soft-sediment and hard-substrate communities (Figure 2a). Samples collected from soft-sediment quadrats were quite spread out on the plots, suggesting great variability in invertebrate community composition within that habitat, especially compared to the samples collected from TCC and Holdfast quadrats. ANOSIM results validated the graphical observations that community compositions were significantly different between habitat types and that samples collected from similar habitats were more similar to each other than to samples from other habitat types (p=0.001; Global R-value = 0.381).

Although hard-substrate samples (TCC and Holdfast) tended to clump together and segregate from soft-sediment samples, when the MDS plot focused on hard-substrate samples only, invertebrate communities also showed partitioning between TCC and Holdfast quadrats (Figure 2b; p=0.003, Global R-value = 0.416).

Of the many taxa collected during the survey, some contributed most to the divergence observed in community composition between the different habitats. Examples of influential species that are highly typical of a particular substrate type or habitat are presented below. Good discriminating taxa are not necessarily abundant, but they tend to be consistently present (or in higher numbers) within samples characteristic of one habitat and absent (or in lower numbers) from samples from other habitats. The amphipod *Grandifoxus vulpinus* and opheliid polychaetes were found in most soft-sediment samples and were completely absent from the hard-substrate samples (TCC and Holdfast) (Figures 3a and b). In contrast, chitons (Polyplacophora), spirorbid polychaetes, and *Polydora* spp. (spionid polychaete) were predominantly found within hard substrate (Figures 4a, b, and c). Although many taxa were found in both TCC and Holdfast samples, some species were highly typical of either TCC or Holdfast habitat. The acrocirrid polychaete *Acrocirrus heterochaetus* was present in all TCC samples, but only 20 % of the Holdfast quadrats and was found in greater numbers in TCC samples (Figure 5a). Both the polychaete *Polydora* spp. and echinoid *Strongylocentrotus* spp. were present in most TCC and Holdfast samples, but were found in greater abundance within TCC quadrats (Figure 5b and c). Examples of species that are characteristic of Holdfast habitats were the bivalves *Hiatella arctica* and *Vilasina vernicosus* and the amphipod *Ischyrocerus* sp. (Figure 6a, b and c). *Hiatella arctica* was found in all Holdfast samples but only in a few (and in low numbers) TCC samples. Similarly, *Vilasina vernicosus* was present in all Holdfast samples but never encountered within TCC samples. Likewise, *Ischyrocerus* sp. was predominantly collected from Holdfast quadrats.

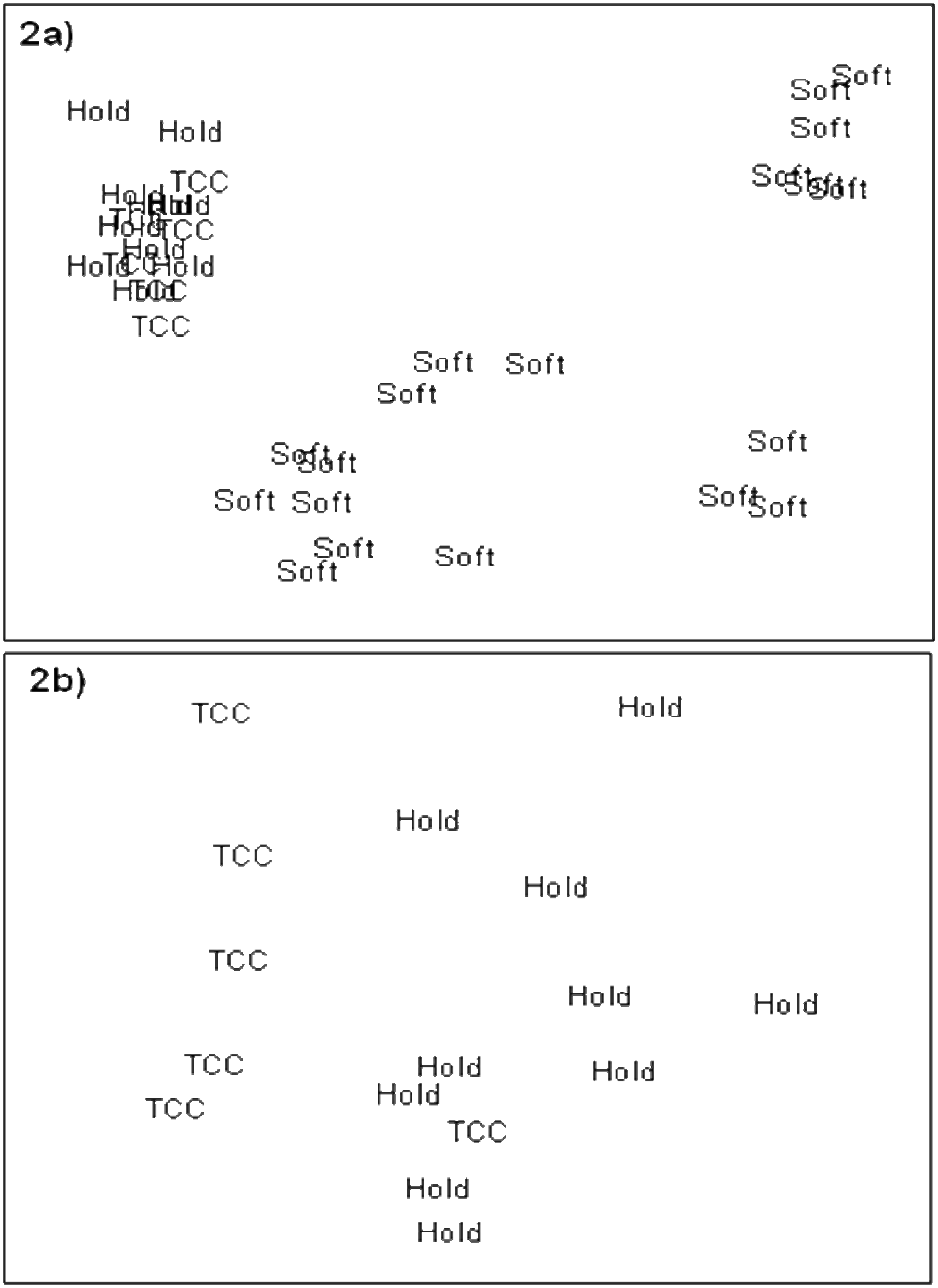


Figure 2. MDS plots of benthic fauna composition based on abundance data of solitary invertebrates within 25 x 25-cm (0.06 m^2) quadrats. The community similarity patterns are plotted by habitat types, Soft (Soft), Thick Crustose Coralline (TCC), and Holdfast (Hold). The upper plot (a) includes all 3 habitat types and the lower plot (b) focuses on hard-substrate habitats (TCC and Hold). The data were *ln* (x+1) transformed and resemblance was based on Bray-Curtis similarities (2D Stress = 0.12 in 'a' and 0.18 in 'b').

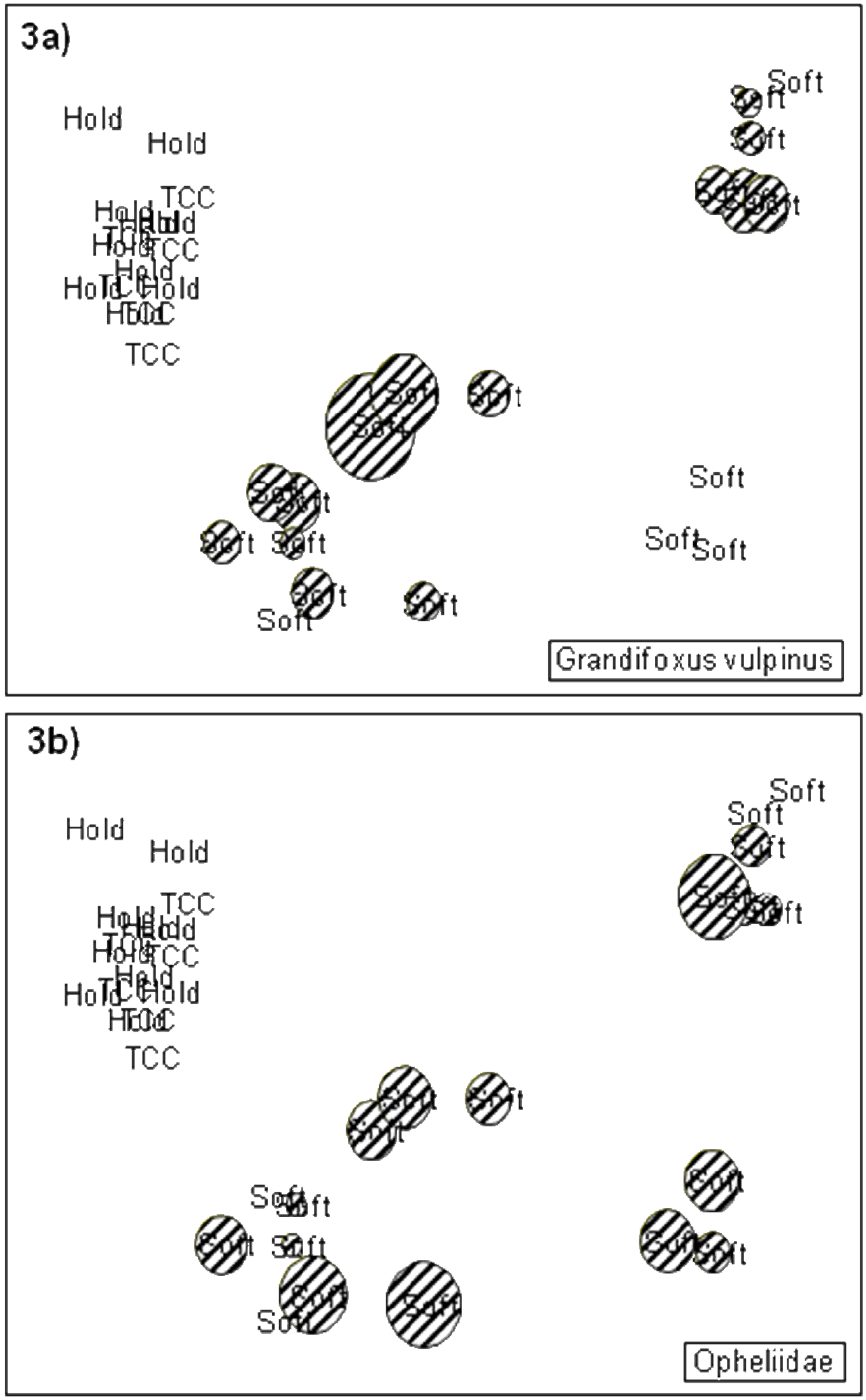


Figure 3. MDS plots based on taxa composition of benthic fauna. The community similarity patterns (same as Figure 2a) are superimposed with abundance data of a) *Grandifoxus vulpinus*, amphipod and b) Opheliidae, polychaete, that are characteristic of soft-substrate communities. The size of the density bubbles are proportional to the number of organisms collected within each quadrat. The density of *Grandifoxus vulpinus* varied between 0 and 38 individuals·0.06 m^{-2}. The density of Opheliidae varied between 0 and 13 individuals·0.06 m^{-2}.

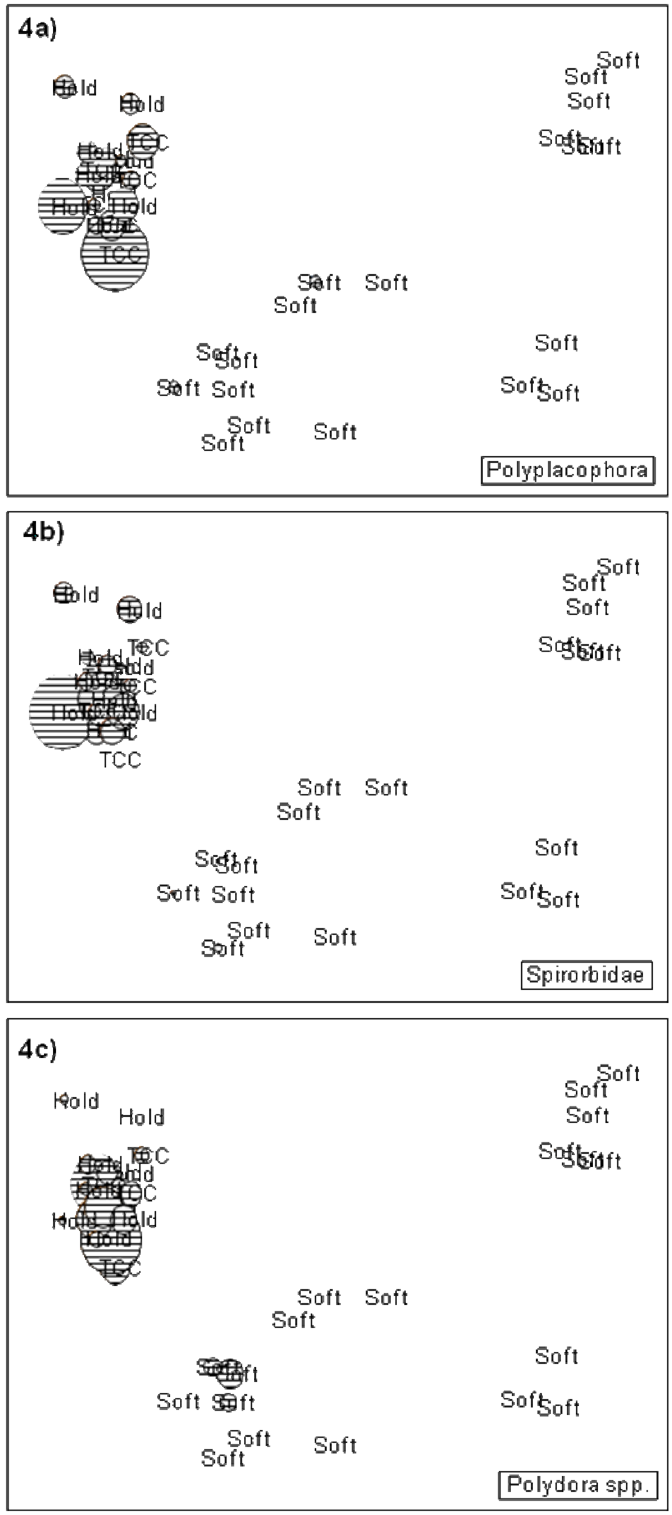


Figure 4. MDS plots based on taxa composition of benthic fauna. The community similarity patterns (same as Figure 2a) are superimposed with abundance data of a) Polyplacophora, chiton b) Spirorbidae, polychaete, and c) *Polydora* spp., polychaete, that are characteristic of hard-substrate communities. The size of the density bubbles are proportional to the number of organisms collected within each quadrat. The density of Polyplacophora varied between 0 and 38 individuals·0.06 m^{-2}. The density of Spirorbidae varied between 0 and 353 individuals·0.06 m^{-2}. The density of *Polydora* spp. varied between 0 and 1535 individuals·0.06 m^{-2}.

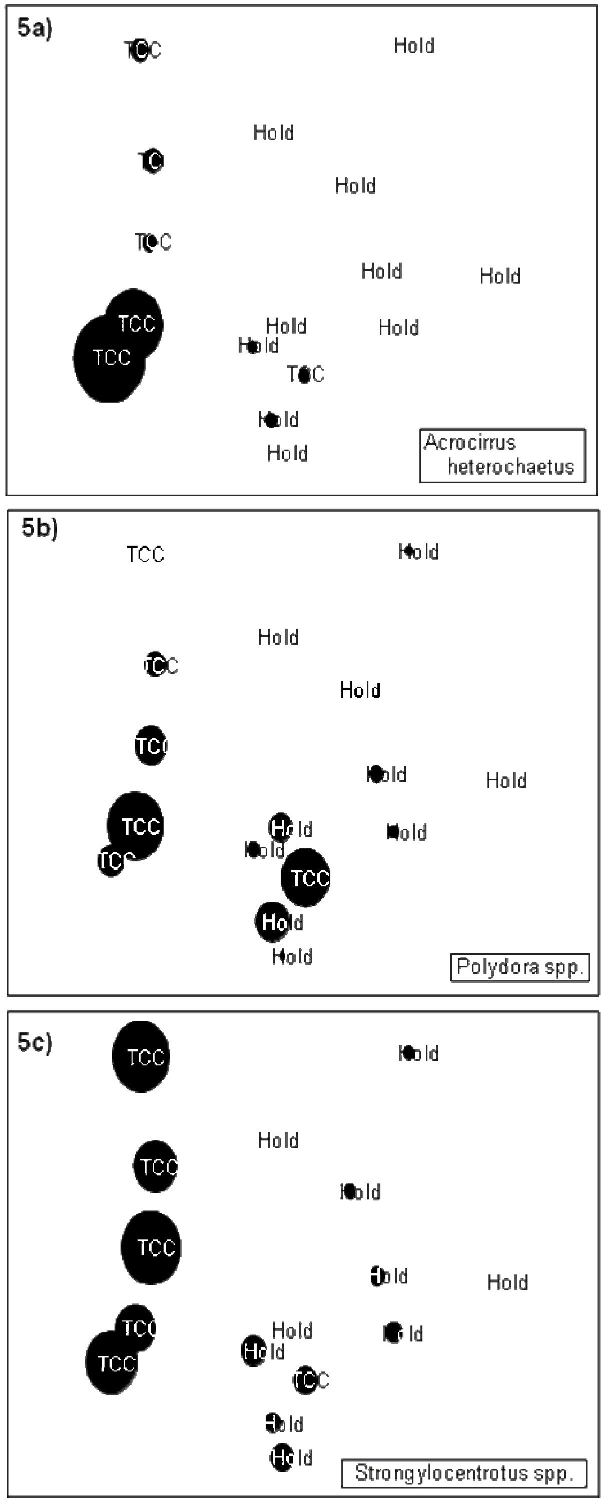
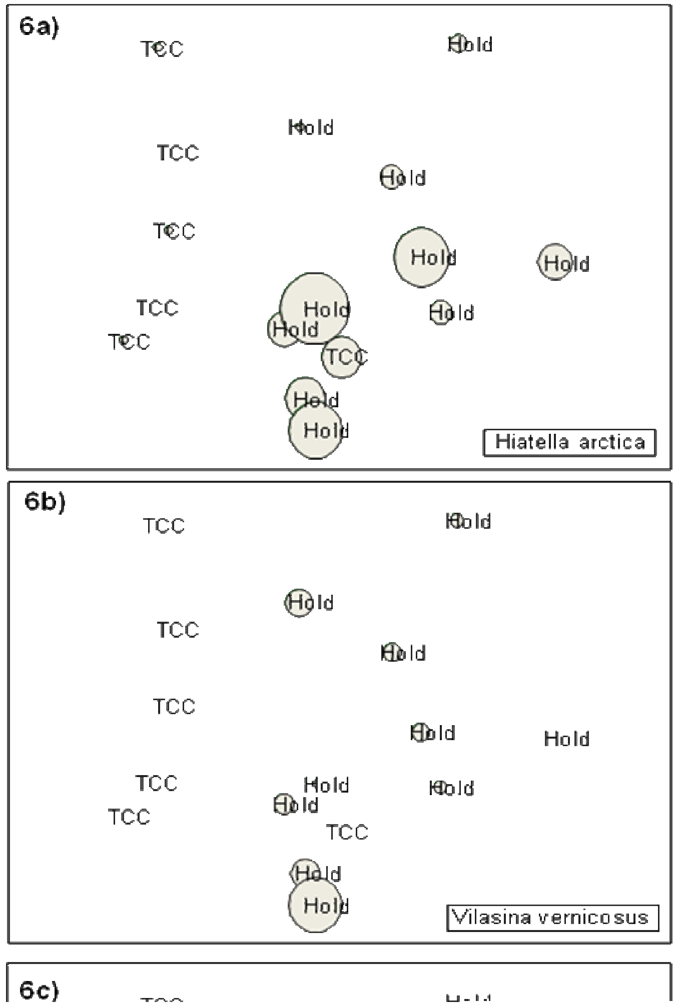


Figure 5. MDS plots based on taxa composition of benthic fauna on hard substrate. The community similarity patterns (same as Figure 2b) are superimposed with abundance data of a) *Acrocirrus heterochaetus*, polychaete, b) *Polydora* spp., polychaete, and c) *Strongylocentrotus* spp., echinoid, that are characteristic of thick crustose coralline (TCC) communities. The size of the density bubbles are proportional to the number of organisms collected within each quadrat. The density of *Acrocirrus heterochaetus* varied between 0 and 197 individuals·0.06 m^{-2}.The density of *Polydora* spp. varied between 0 and 1535 individuals·0.06 m^{-2}. The density of *Strongylocentrotus* spp. varied between 0 and 27 individuals·0.06 m^{-2}.


6a)
TCC
Hold
Hiatella arctica
6b)
TCC
Hold
Vilasina vernicosus
6c)

The number of taxa (S, based on both solitary and colonial invertebrates) collected varied greatly between samples ranging from 14 to 113 for Soft, 54 to 79 for TCC, and 49 to 112 for Holdfast quadrats (Figure 7). The mean taxon richness was significantly lower in the Soft habitat (42 ± 6.1 taxa per quadrat; mean ± SE) versus TCC (65 ± 4.6) and Holdfast habitats (79 ± 6.8) (p<0.001).

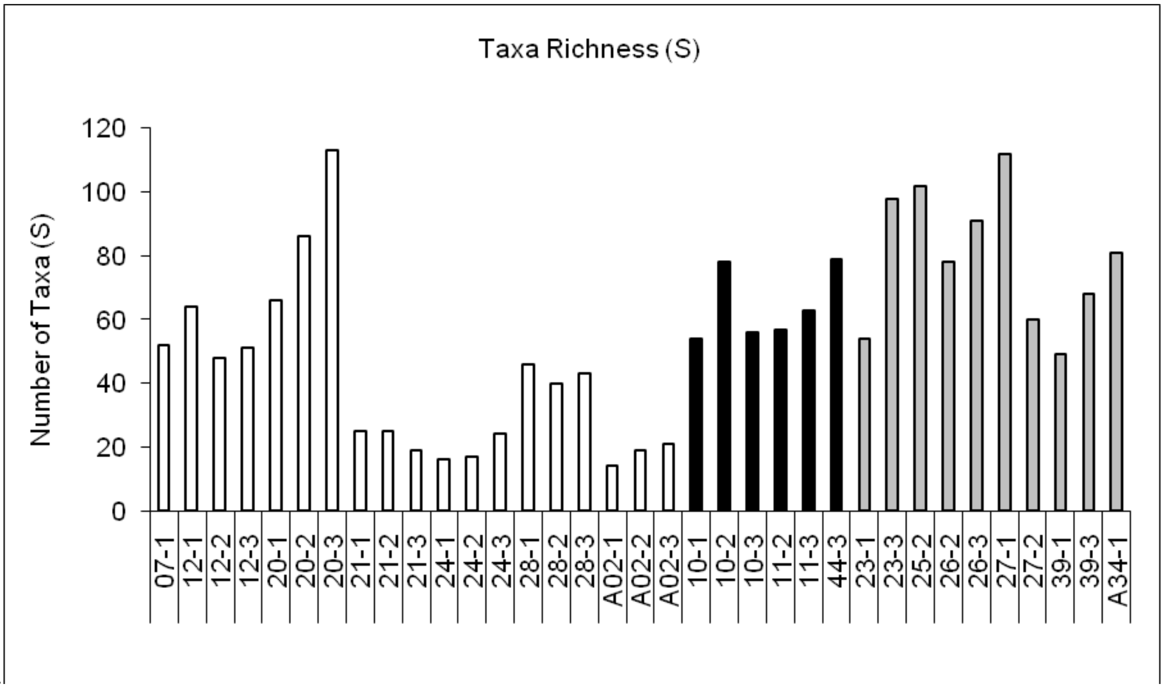


Figure 7. Taxa richness (S) based on all invertebrates (colonial and solitary) collected within individual 25 x 25-cm (0.06 m^2) quadrats in Soft, TCC, and Holdfast habitats. The mean number of taxa encountered in Soft habitats was 41 (± 6.1, SE). Taxa richness was 64 (± 4.6) taxa and 79 (± 6.8) taxa in TCC and Holdfast habitats, respectively. Mean taxa richness was significantly lower in Soft than in TCC and Holdfast habitats (p<0.001 with *ln* (x + 1) transformation).

A total of 255 taxa were encountered within the 19 Soft-sediment quadrats. The same number (255) of taxa was collected from 10 Holdfast samples, whereas 174 taxa were accumulated from the 6 TCC quadrats. When the cumulative number of individual taxa was divided by the number of quadrats per habitat, TCC habitat averaged 29 new taxa, whereas Soft-sediment samples produced only 13 new taxa. Holdfast samples provided 26 taxa per quadrat.

The number of individuals (N, based on solitary invertebrates only) counted within each sample varied greatly ranging from 44 to 5014 for Soft, 268 to 2477 for TCC, and 166 to 3380 for Holdfast quadrats (Figure 8). The mean number of individuals was significantly different between habitats (p=0.038). TCC habitat averaged the highest number of invertebrates per quadrat (1327 ± 360.4) compared to Soft (786 ± 286.9) and Holdfast (1171 ± 316.6) habitats.

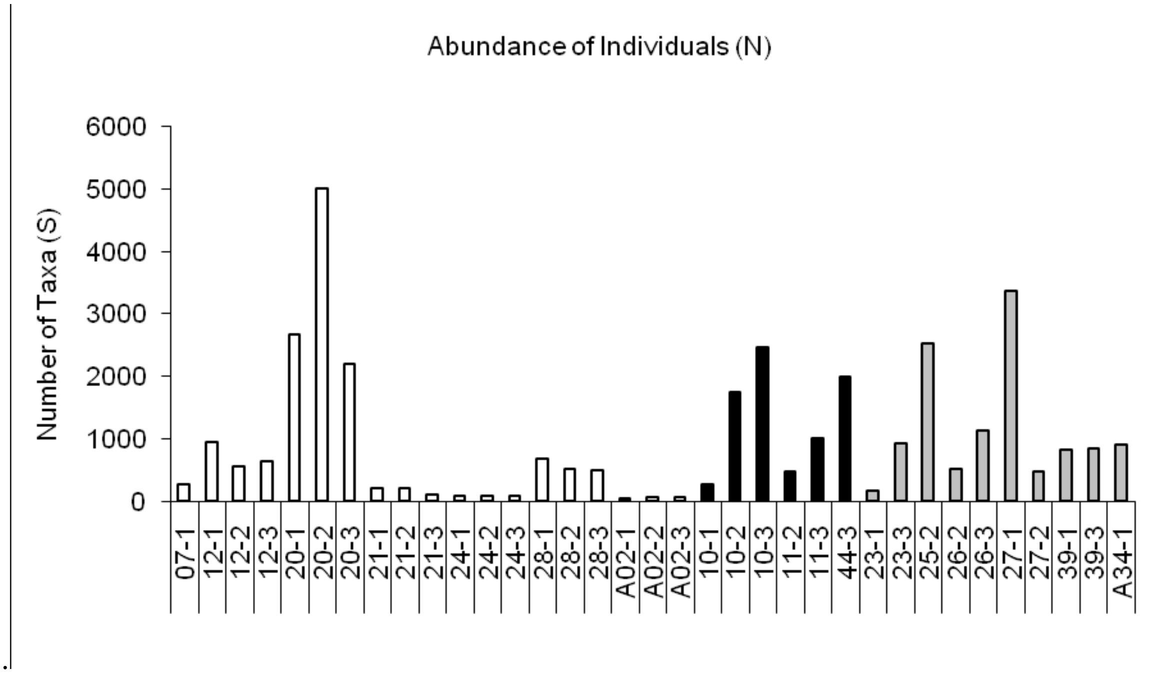


Figure 8. Abundance of individuals (N) for solitary invertebrates collected within Soft, TCC, and Holdfast habitats. The mean number of individuals encountered in Soft habitats was 786 (± 286. 9, SE) per 25 x 25-cm (0.06 m^2) quadrat. The mean number of individuals was 1327 (± 360.4) and 1171 (± 316.2) in TCC and Holdfast habitats, respectively. There was a significant difference in the mean number of individuals collected between habitats (p=0.038 with *ln* (x + 1) transformation).

The Shannon Index (H') takes into account both the number of taxa as well as the number of individuals within each taxon. Invertebrate diversity varied between 1.13 to 3.04 for Soft, 1.74 to 3.05 for TCC, and 1.92 to 3.61for Holdfast quadrats (Figure 9). Biodiversity was significantly lower within Soft habitat (2.23 ± 0.14) than Holdfast habitat (3.07 ± 0.21) (*p*=0.006). TCC samples had an H' value of 2.43 (± 0.21) that was not significantly different from Soft and Holdfast values.

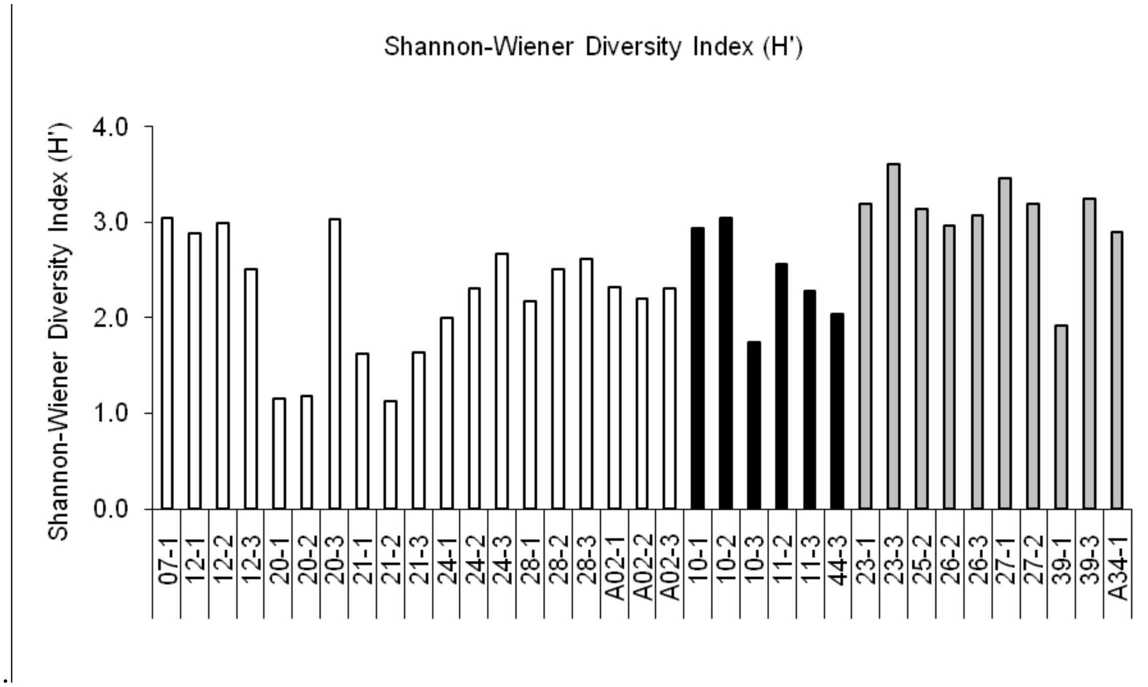


Figure 9. Shannon Index (H') for solitary invertebrates observed within individual 25 x 25-cm (0.06 m^2) quadrats in Soft, TCC, and Holdfast habitats. The mean number of individuals encountered in Soft habitats was 2.23 (± 0.14, SE). The mean number of individuals was 2.43 (± 0.21) and 3.07 (± 0.14) in TCC and Holdfast habitats, respectively. There was a significant difference in the mean taxa richness between Soft and Holdfast habitats (p=0.006 with *ln* (x + 1) transformation).

Discussion

The Aleutian nearshore region supports a very diverse benthic community. A total of 35 animal phyla have been recorded from the entire world's ocean, all habitats, depths, latitudes, and longitudes combined (Groombridge and Jenkins, 2002). During the 2006 investigation of the nearshore region of the Eastern Aleutian Islands, AKMAP encountered a total of 16 benthic marine phyla or 512 taxa of invertebrates. Considering the relatively narrow bathymetry (6-20 m) explored, short coastal distance travelled (500 km), and low number of sites surveyed (23), the species richness of this Aleutian region is high. Those numbers are also comparable to previous AKMAP investigations. A total of 14 phyla and 441 taxa were collected in Southcentral Alaska in 2002 and 14 phyla and 531 taxa were collected by AKMAP in Southeast Alaska in 2004 (unpublished data). However, those samples (n=50 from each region) were only collected from soft sediments, ranging in depth from 3.5 to 503 m, using a 0.1 m^2 van Veen grab and most organisms were identified to the species level. Once the taxonomic resolution for the 2006 samples will be achieved to the species level, the number of taxa recorded from the Eastern Aleutian Islands will surpass those from Southcentral and Southeast Alaska.

Some of the most common types of habitats encountered during the AKMAP of the Eastern Aleutian Islands were soft sediments, kelp beds, and urchin barrens and their associated crustose coralline communities. The present study reveals that the thick crustose coralline *Clathromorphum nereostratum* provides habitat to a much more diverse and abundant community of invertebrates than expected from its low-relief structural complexity.

Structural complexity is a key factor in explaining the association of high invertebrate biomass and biodiversity with certain habitats. High habitat heterogeneity provides increased microhabitats, resource partitioning and niche availability to a wide variety of organisms (McCoy and Bell, 1991; Coleman and Williams, 2002; Steller et al., 2003). Heterogeneous habitats provide refuge from predators (Taylor, 1984; Lewis and Eby, 2002) and are often nursery grounds for a wide variety of fishes and invertebrates (Ebeling and Laur, 1985; Bodkin, 1988; Kamenos et al., 2004).

Kelp forests are often considered the temperate-boreal equivalent to tropical coral reefs. They are dominant nearshore habitats that are important to the entire ecosystem because they sustain high primary and secondary productivity and are very dynamic systems (Foster and Shiel, 1985; Duggins et al., 1989; Graham, 2004). In California, *Macrocytis* kelp forests can produce 120 to 1900 $gC \cdot m^{-1} \cdot y^{-1}$ (Mann, 1982). In comparison, the annual production rates of rain and temperate forests range from 400 to 1900 $gC \cdot m^{-1} \cdot y^{-1}$ (Valiela, 1995). Kelp forests support an extremely diverse fauna including seabirds, marine mammals, fishes, and invertebrates (for review see North, 1971; Foster and Schiel, 1985). An extensive list of over 800 species of animals (ranging from protozoans to mammals) was recorded in association with the kelp beds of southern California (North, 1971). The structural complexity of macroalgae expand benthic habitats upward into the water column, thereby increasing refuge availability for juveniles (Ebeling and Laur, 1985) as well as substrate accessibility for potential prey attachment (Hicks, 1980; Bodkin, 1988).

Holdfasts are a structurally complex feature of kelp thalli; these networks of inter-twined haptera (root-like projections) form an intricate lattice and produce numerous refuge spaces for various invertebrates. Holdfasts of many different kelp species have been documented to harbor a great variety of organisms (Ghelardi, 1971; Smith et al., 1996; Anderson et al. 2005). In their study of the fauna associated with the holdfast of *Ecklonia radiata* Smith et al. (1996) reported 385 species belonging to 152 families and 10 phyla. Those results are comparable to what Anderson et al. (2005) recorded (351 taxa, from 296 genera, 213 families, 72 orders, 26 classes, 15 phyla).

The sampling protocol used by AKMAP was not specifically designed to address the biodiversity of particular habitats; however, the results from our study are comparable to others and support the idea that kelp forests sustain a rich and diverse invertebrate community. A total of 255 different taxa were collected from our 10 holdfast quadrats, which was as high as the number of taxa collected from the 19 soft-sediment samples. Only 174 different taxa were collected from the TCC samples (6 samples). However, when those numbers are normalized by the number of samples for each habitat type, TCC samples yielded even higher numbers of new taxa per quadrat than holdfast samples (29 versus 26 new taxa per quadrat). A similar trend was observed for the mean number of individuals collected from each habitat type, and although highest in Holdfast quadrats, the mean Shannon Index, also, was not significantly different between TCC and Holdfast samples. Those results suggest that crustose coralline habitats may also support an unexpectedly high diverse invertebrate community.

Sea urchins can graze off most sessile organisms (in particular kelp) and, therefore, remove most of the structural complexity that kelp provides. Nearshore habitats dominated by crustose coralline algae are often referred to as 'coralline barrens' because of their low structural complexity and their low associated diversity (Rowley, 1989; Sala et al., 1998). In early studies, encrusting coralline algae were often designated as primary substrate because of their bare rock appearance (Paine and Vadas, 1969; Menge, 1976; Rowley, 1989). Although crustose coralline communities are often referred to as barrens grounds and are frequently overlooked because of their relatively low productivity, Ojeda and Dearborn (1989) observed that in the rocky subtidal zone off the Gulf of Maine, coralline communities support a high diversity of organisms and sustain relatively high secondary productivity. In their study, the authors recorded that crustose coralline (*Lithothamnion glaciale, Lithothamnion lemoineae, Clathromorphum circumsciptum,* and *Phylmatoliton rugulosum*, all form relatively thin crusts) covered most of the available primary substrate and that the sea urchin *Strongylocentrotus droebachiensis* was the most prominent organism. In addition, a total of 60 species of macroinvertebrates, representing nine phyla, were collected from 133-0.25 m^2 benthic samples. Although our sampling efforts of TCC were very limited (only six 0.06 m^2 samples), we enumerated 174 taxa, representing 12 phyla.

The compositions of invertebrate communities found within each of the three habitats were significantly different and were dominated by different groups of organisms. The communities observed within TCC and Holdfast habitats were more similar to each other than to Soft-sediment communities. Both crustose coralline algae and kelp require hard substrate to develop and grow and both types of habitat are often found adjacent to one another. In our study, grazers such as chitons, limpets and urchins were obviously more abundant in our hard-substrate than soft-sediment samples. Urchins (*Strongylocentrotus* spp.) were the most conspicuous herbivores observed in the shallow hard-sediment habitats throughout the Eastern Aleutian Islands. Urchin barrens are often seen as an alternate state of lush kelp forests (Lawrence, 1975; Steneck, 1986). Many studies have demonstrated that in productive environments with intense herbivory, algal crusts dominate, but when herbivores are removed, larger canopy-forming macroalgae overgrow coralline crusts and diversity increases again (Paine and Vadas, 1969; Lawrence, 1975; Steneck, 1982, 1986).

Most herbivores are physically deterred by the resistance presented by the calcified coralline tissues (Steneck and Walting, 1982; Steneck, 1986; Steneck and Dethier, 1994), and because of their low caloric values coralline algae are not very nutritious (Littler and Littler, 1980). Calcified coralline algae are relatively resistant to grazing pressures, especially compared to the faster-growing fleshy algae (Steneck and Dethier, 1994). Urchin grazing may actually help perpetuate the presence of crustose communities by removing fast-growing micro and macro algae that have the potential to overgrow and out-compete the slow-growing red crusts (Paine and Vadas, 1969; Steneck, 1982). Several studies suggest that some invertebrate species are found almost exclusively in association with crustose coralline algae. The limpet *Tectura testudinalis* shares a symbiotic relationship with the crustose coralline *Clathromorphum circumscriptum* (Steneck, 1982). Similarly, the lined chiton, *Tonicella lineata*, is found almost exclusively on crustose coralline algae (Barnes and Gonor, 1973). Many invertebrates seem to preferentially settle and metamorphose on crustose coralline algae (chitons, Barnes and Gonor, 1973; abalone, Morse et al., 1979; limpets, Steneck, 1982; and urchins, Pearce and Scheibling, 1988; Rowley, 1989; Lambert and Harris, 2000). Nevertheless, coralline algae seem to inhibit the recruitment of certain invertebrates, such as some species of polychaetes, bryozoans, and amphipods (Breitburg, 1984). There are still debates and controversies about the processes and factors involved in triggering preferential invertebrate settlement and metamorphosis on coralline algae. Some investigators suggest that the inducers are produced by the algae (Morse and Morse, 1984), while others propose that the chemical cues responsible for inducing invertebrate settlement and metamorphosis are bacterial in origin (Johnson et al., 1991).

Although the smooth crustose surface was often entirely dominated by sea urchins or seemed relatively denuded of conspicuous fauna, we found a surprisingly rich cryptic fauna hiding underneath the crust or within the calcareous matrix itself. Individual *Clathromorphum nereostratum* thalli can be wider than 50 cm in diameter, but adjacent plants can form extensive pavements that cover most of the available substrate and dominate the benthic floral community (Lebednik, 1976; personal observations). Mature specimens can form crusts that are almost 1 m thick (Lebednik, 1976). In contrast to the thin crustose corallines, the thick crust of *C. nereostratum* does not conform to the substrate and forms a plethora of crevices, nooks, crannies, and cavities, providing valuable hiding places for a vast variety of cryptic invertebrates. In addition, the calcite nature of the skeleton of *C. nereostratum* allows boring invertebrates to drill tunnels and burrows through the calcified matrix itself. The deep burrows inside the thick calcareous matrix of the TCC offer boring organisms shelter from predation and extreme environmental conditions (Sato-Okoshi, 1999).

The assortment of invertebrates we found in association with *Clathromorphum nereostratum* ranges from sponges, hydrozoans, bryozoans, polychaetes, echiurans, sipunculids, mollusks, ophiuroids to ascidians. Some of the invertebrates found hidden between the primary substrate and the crust were of relatively large size. Sabellids, nereids, sipunculids, echiurans, bivalves (*Hiatella arctica*), and ophiuroids (*Ophiopholis aculeata*), were often found underneath the thick crust.

The most abundant groups of organisms found within the calcareous matrix itself were the spionid polychaetes (mostly *Polydora* spp.). Boring polychaetes belong to the families Eunicidae, Lumbrineridae, Dorvilleidae, Cirratulidae, Sabellidae, and Spionidae (Blake, 1969; Hutchings, 1986), are predominantly suspension-feeders and surface deposit feeders (Jumars and Fauchald, 1977; Blake, 1996; Sato-Okoshi, 1999). Some species, in particular, are infamous in aquaculture as they infest shells of economically-valuable mollusks and can cause great damage to stocks (Blake and Evans, 1973). Blake and Evans (1973) mentioned that at least five species of *Polydora* and three *Boccardia* (both spionids) bore or nest into coralline algae (*Lithothamnion* sp., *Lithophyllum* sp., *Prolithion* sp.) and a total of 26 spionid species have been reported to only occur in calcium carbonate substrates. Sato-Okoshi (1999) examined 28 different types of calcareous substrate (i.e., coralline algae, barnacle tests, mollusk shells) and found a total of 13 boring species of spionids. Although some species of spionids are found in calcareous as well as non-calcareous substrates, most are always found within their self-excavated burrows and were only encountered in calcareous substrate (Blakes and Evans, 1973; Sato-Okoshi, 1999). Most boring spionid species are unable to move, relocate, or form a new burrow if dislodged; therefore, the stability of the thick crustose coralline is essential to the survival of individual spionids (Sato-Okoshi, 1999). The boring mechanisms are still not well understood but Blake and Evans (1973) propose that the spionid *Polydora* sp. uses a chemical process by secreting acid to dissolve the calcareous substrate, uses its modified setae on the 5^{th} setiger to mechanically erode the substrate, or uses a combination of chemical as well as mechanical process. Liu and Hsieh (2000) suggest that the burrow of *Polydora villosa* consists of two parts that are formed at different stages. *Polydora villosa* begins by actively boring into the coral and forming a U-shaped burrow, but as the coral grows, the spionid passively keeps elongated the tunnel. However, boring behaviors appear to be specific to each spionid species (Sato-Okoshi, 1999).

Crustose coralline algae seem to be a key factor in supporting a rich community in the shallow subtidal of the Aleutians Islands by providing substrate, refuge, and food to a wide variety of infaunal (e.g., spionids) as well as epifaunal invertebrates (e.g., sea urchins). In return, infaunal borers may play a crucial role in maintaining the rich diversity of the entire community. Bioerosion has been reported to be extremely important in the dynamics of coral reefs. Polychaetes are some of the initial coral colonizers and are thought to make the substrate more attractive to other boring organisms. By creating burrows and tunnels, boring organisms also increase the habitat available to non-boring fauna (for review, see Hutchings, 1986). Bioerosion of the crustose coralline algae by spionids and other boring organisms tends to make the calcareous matrix more fragile (personal observations) and susceptible to physical disturbances (e.g., storms). When large pieces of coralline crust break off and detach, more of the primary substrate becomes available for recolonization. This erosion process is essential for the entire community, as many of the cryptic organisms that were hiding within or underneath the crust are now exposed and provide food to many predators. Most fauna associated with *C. nereostratum* seem out of reach of predators. However, when large chunks of the thick crust were broken off during sampling, a plethora of food became available and attracted invertebrate predators such as urchins, sea stars and fishes (personal observations).

In addition to playing a crucial role as a community former, *Clathromorphum nereostratum* has the potential to be used as a paleothermometer. Because red crustose coralline algae are long-lived and slow-growing, they can act as climate recorders, recording past temperatures and environmental variations in their skeleton (Foster, 2001; Frantz et al. 2005; Halfar et al., 2007; Kamenos et al., 2008). Red nongeniculate coralline species grow at extremely slow rates, as low as 0.01 $mm \cdot y^{-1}$ (Halfar et al., 2000; Foster, 2001; Rivera et al., 2004). Frantz et al. (2005) estimated the growth rate of a *C. nereostratum* specimen collected from Adak Island to be 0.30 ± 0.03 $mm \cdot y^{-1}$. Based on U/Th procedures, a live specimen of *C. nereostratum* collected at Attu Island was dated at 850 ± 28 years cal BP (Before Present), making it the longest-lived marine organism known (Halfar et al., 2007). *C. nereostratum*, in particular, can be an extremely valuable 'climate archive' because of its boreal distribution (Halfar et al., 2007). Halfar et al. (2007) reconstructed a 117-year annual marine climate record of the Western Bering Sea region based on $\delta^{18}O$ time series and suggested a significant warming and potential freshening of surface waters over the past decades. Kamenos et al. (2008) determined that sub-monthly Mg and Sr records in rhodoliths make them unique globally distributed paleothermometers which may help refine regional climate histories during the Holocene.

The longevity and slow growth-rate of coralline algae also make them vulnerable to major disturbances, including anthropogenic disturbances (Steller et al., 2003). In addition, crustose coralline algae form by depositing magnesian calcite, a type of calcium carbonate ($CaCO_3$) that is very sensitive to acidification. These algae are believed to be at increased risk from global ocean acidification. As atmospheric concentrations of CO_2 increase, the pH of the ocean decreases. As a result, organisms that utilize $CaCO_3$ will have increasing difficulties in forming their shells (e.g., coccolithophorids, foraminiferans, mollusks) and growing calcified thalli (coralline algae). Organisms that precipitate $CaCO_3$ in the form of aragonite or magnesian calcite, in particular, will be the first to suffer ocean acidification as those forms of calcium deposits are more soluble than normal calcite (used by corals) (Feely et al., 2004; Orr et al., 2005). Because of the important role crustose coralline algae plays in the nearshore ecosystem of the Aleutian Islands, the repercussions could be dramatic for the entire invertebrate communities that find shelter and food in TCC habitats.

In summary, the smooth surface of crustose coralline algae exhibit very little structural relief and gives a deceptive notion that very little life is associated with these algae. However, the majority of the invertebrates associated with the thick crustose coralline hides underneath or within the crust. In terms of the number of species and individual invertebrates found in association with *Clathromorphum nereostratum*, it is not significantly different from the diversity associated with kelp holdfast and slightly higher than the invertebrate community found in soft-sediment habitats. We do believe that the actual biodiversity associated with TCC may have been underestimated because the AKMAP survey was not specifically designed to assess TCC diversity. For example, the TCC quadrats were not always 100 % covered by *C. nereostratum*; therefore, lessening the actual density of individuals and taxa measured per quadrat. Also, biodiversity indices (H' and N) were based only on solitary invertebrates. Preliminary analyses that include colonial organisms reveal the predominance of organisms such as sponges, hydroids, bryozoans, and ascidians on hard substrate versus soft sediment, suggesting the underestimation of TCC biodiversity in this study.

This study is only a snapshot of the composition of invertebrate communities in the Eastern Aleutian Islands. Data collected from the Western Aleutian Islands in 2007 will be added to improve our spatial resolution. Much more research is needed to better understand the role of the TCC and its associated invertebrate communities in the foodweb of the nearshore region of the Aleutian Islands. Considering the major function *Clathromorphum nereostratum* may play in supporting a rich and diverse fauna and the dramatic implications global warming could have on its health, we believe that this habitat deserves greater scientific consideration.

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What Can the U.S. Navy Contribute to Scientific Diving?

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Abstract

The U.S. Navy began supporting scientific diving in 1971 through its partnership with NOAA and the State University System Institute of Oceanography by sponsoring the Scientist in the Sea Program (SITS). Graduate students from around the U.S. were trained in Navy diving equipment and procedures, and scientific diving methods. Some of those students have mentored a new generation of science divers. Although the SITS program ended in the mid-seventies, it was briefly revived in 2000 by Florida State University, and was once again supported by the Navy; the Navy Experimental Diving Unit (NEDU) and the Navy Diving and Salvage Training Center. NEDU continues support to science divers by assessing rebreathers for the NOAA diving program, by supporting both the certification and manning of the Aquarius habitat, supporting the Smithsonian Polar Diving Workshops for Science Divers, and providing support for the Florida State University's Advanced Science Diving Program and their Underwater Crime Scene Investigation Program. Computer simulations of semiclosed-circuit UBA allow the user to explore the safety of various UBA and dive profiles. Computer simulations of CO_2 scrubber canister dynamics have led to patented methods for monitoring canister expenditure. Decompression research on topics such as "deep-stops" has obvious implications for those involved in decompression diving, and the generation of new oxygen limits for repetitive 1.3 PO_2 diving are relevant for those considering the use of rebreathers.

Keywords: cold water regulators, CO_2 scrubbers, deep stops, oxygen toxicity, scientist-in-the-sea, semiclosed rebreathers,

Introduction

Although most science divers have some notion of what U.S. Navy diving is about, few are likely to understand how Navy diving is relevant to scientific diving. That is the purpose of this paper, to cover the broad scope of Navy diving and diving research, and to discuss their applicability to scientific research diving.

The many types of Navy divers can be divided into two broad categories: divers who wear fins, and those who don't. Booted, non-finned divers are, simply put, the manual laborers of the diving world — the bottom dwellers, the salvors. Their jobs are to fix what is broken, to salvage debris on the bottom, and to assist in eliminating obstacles to navigation. They do not need fins to get to their worksite; they are carried to their workplace by an underwater elevator, and they walk to work. Even in the case of saturation divers, whose home may be on the seabed itself, booted divers comprise the most historically significant group: they have assisted in rescuing trapped submariners, and in collecting human remains and debris from crashed aircraft and spacecraft, and even collapsed bridges. Many excellent books chronicle the work done by salvage divers (Bartholomew, 1990).

Navy divers with fins use the water as a means of getting to a job site that may be floating in the water column, anchored to the sea floor, or across the beach (Lonsdale, 2005). They often use the water to conceal themselves as they travel to a hostile area for potential enemy contact. The enemy might be as cold-blooded as mines, or as fanatical as guerilla insurgents. These divers place themselves in mortal danger — in "harm's way" as they say — each time they dive.

Discussion

Scientist-In-The-Sea Program

The father of saturation diving, Captain George Bond, a U.S. Navy physician, not only developed the concept of saturation diving that made possible the SEALAB series of habitats, but also fostered the education of underwater scientists (Figure 1). He had a moral conviction that the tools and techniques developed for military purposes could be used for civilian underwater science. To that end, he promoted the Scientist-In-The-Sea Program (SITS), a cooperative training program for graduate students with an interest in diving as a means of conducting scientific research. In a sense, Capt. Bond saw science divers in the same light as military divers: diving was simply a means to an end, a way to get scientists to their work site in a potentially hostile environment.

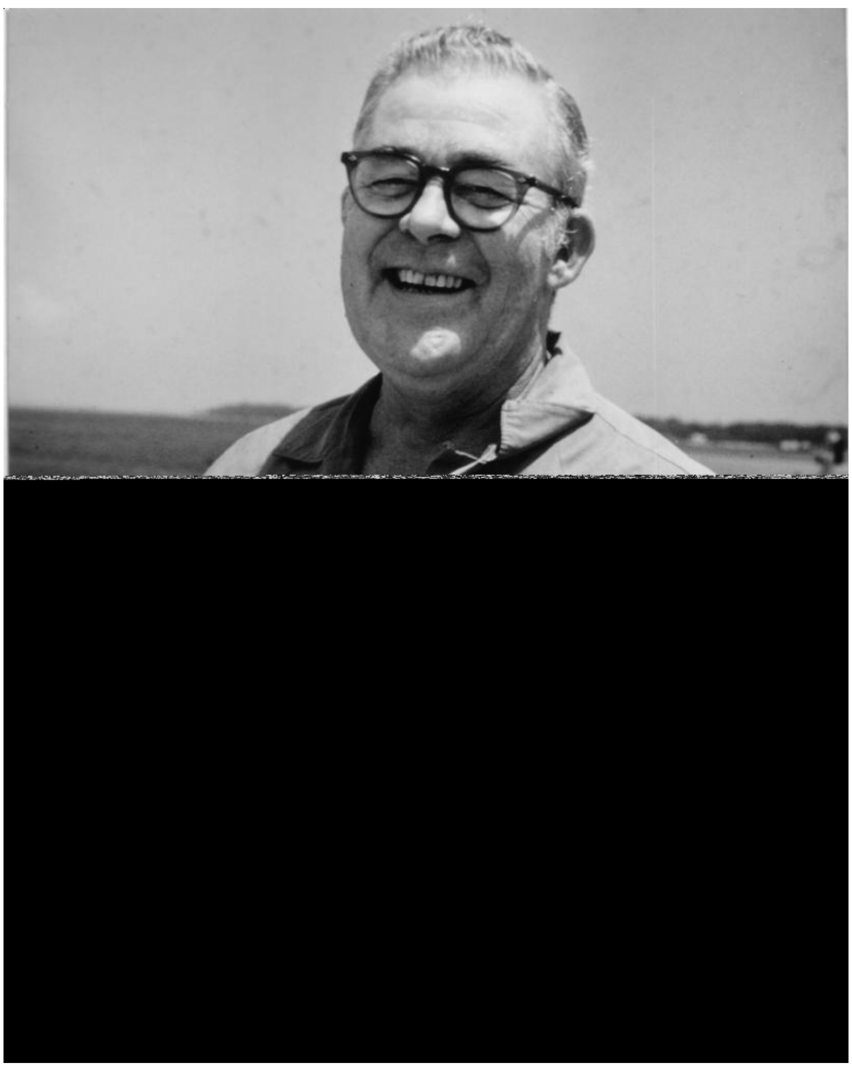

Figure 1. Captain George "Pappy" Bond, U.S.N., M.D. Photo credit: John R Clarke.

The SITS program was a joint venture with the U.S. Navy providing a training site and diving equipment, the National Oceanic and Atmospheric Association (NOAA) Sea Grant program providing financial support, and the State University System of Florida providing administrative support. Over a six-year period, sixty students were trained in the methods of scientific diving. A good review of the SITS program, including its brief reprise in 2000, is available in the 20th Annual AAUS Symposium Proceedings (Stanton, 2000).

Semiclosed UBA

Science divers are always looking for ways to extend their missions with a minimum of encumbrance and expense. Scuba is on one end of the expense-complexity spectrum, while closed-circuit, computer-controlled rebreathers are on the other end. Semiclosed rebreathers lie in the middle. Before the advent of electronically controlled, constant partial pressure of oxygen (PO_2) rebreathers, the Navy used semiclosed UBA for the SEALAB program and explosive ordnance disposal. The units were robust and safe. Recently, the Navy, and the Navy Experimental Diving Unit (NEDU) in particular, have been researching the capabilities and complications of using modern variants of the semiclosed UBA. We discovered a risk of hypoxia in the new, low-flow UBA, and developed computer models to describe the functioning of fixed flow rebreathers under all conceivable diving scenarios (Clarke, 1998; Nuckols et al., 1998).

Computer-controlled Rebreathers

The Navy developed one of the first computer controlled rebreathers, the EX-19 (Figure 2). It was designed for easy breathing to great depths, but the development program was cancelled after one of the design engineers almost died from a combination of O_2 sensor lock-out and alarm logic failure. Sensor lock-out occurs when condensed moisture prevents an O_2 sensor from responding to changing UBA O_2 levels. A constantly changing status of the EX-19's three sensors continually reset the alarm circuitry so that the alarms did not activate. The affected test diver had to be resuscitated (Clarke, *in press*).

As a result of this accident, NEDU developed a procedure for bench-level testing of the black boxes in computer-controlled rebreathers (NEDU, 1994).

Figure 2. EX 19 – early computer controlled rebreather. Photo credit: U.S. Navy

Cold-Water Regulators

For Navy divers such as those in underwater construction teams, their enemies are the elements, the ice packs of the Arctic. These Navy divers share much with science divers who explore and collect samples in the Arctic and Antarctic. For both Navy divers and civilian scientists using scuba in Arctic and Antarctic regions, the robustness and reliability of their regulators is of great interest. NEDU has developed a rigorous testing program for evaluating cold-water regulators. Every few years we update the listing of regulators authorized for Navy use, and that information is made available to the Smithsonian Diving Program supporting National Science Foundation polar research. The latest such exchange of information occurred at the 2007 International Polar Diving Workshop in Ny-Ålesund, Svalbard (Clarke, 2007).

NOAA Support

NOAA conducts science diving, and the Navy supports NOAA: it provides support to the Aquarius habitat, the world's only research habitat (Figure 3), provides testing and guidance on rebreather use for NOAA science dives, and has supported NOAA in salvaging of the Monitor, the Civil War ironclad.

Figure 3. Aquarius habitat. Photo credit: NOAA

CO_2 Scrubber Canister Kinetics

NEDU's modeling and simulation effort has for the first time allowed scientists and divers to visualize the dynamic CO_2 absorption front and carbonate deposition in scrubber canisters. The stochastic method has also been used to understand the highly dynamic processes involved in freezing and reusing scrubber canisters (Clarke, 2001).

Contaminated Water Diving

The Navy has an active research program in contaminated water diving (Leyva, 2004). This work is of obvious relevance to scientists diving in meromictic H_2S containing lakes like those of Palau (Lyons et al., 1996).

Pulmonary Oxygen Toxicity

The use of elevated PO_2 in rebreathers is convenient for minimizing decompression obligations, but it poses a risk of pulmonary oxygen toxicity that can limit repetitive diving, a mainstay of expedition diving (Shykoff, 2007). The results of NEDU research on this topic have recently been summarized in the sixth and latest version of the U.S. Navy Diving Manual (NAVSEA, 2008).

Thermal Status and Decompression Risk

NEDU recently made the surprising observation that during dives to 120 feet of seawater (fsw) with a 30-minute bottom time, and decompressions on a standard air table (91 min decompression time), diver thermal status greatly influenced the risk of decompression sickness (DCS) (Gerth et al., 2007). When divers were kept cool on the bottom and warm during decompression, no decompression cases resulted from 80 dives (95% confidence limits of 0 to 3.7%). When divers were kept warm on the bottom and cool during decompression, seven "hits" resulted from 32 dives, for an incidence of ~22% (95% confidence limits of 9.2 to 40.0%).

Deep Stops

In spite of the avowed, almost religious mantra about the efficacy of deep decompression stops, NEDU's experience with deep stops has been disappointing (Gerth et al., 2007; Gerth et al., *in press*). NEDU conducted air dives to 170 fsw for 30 min with a total stop time of 174 min. That stop time was distributed either deep, or with the traditional, relatively shallow decompression stops. With traditional stops three DCS incidents resulted from 192 dives: with deep stops there were 11 events in 198 dives. Furthermore, graded scores for venous gas emboli were considerably greater with deep stops than with traditional stops.

Dive Computers

The U.S. Navy has been slow to adopt decompression computers because it requires decompression schedules to be evaluated and tested before being released to the fleet. We are currently using Cochran dive computers with the Navy developed VVAL 18 decompression algorithm for use with air diving in the Air III computer (Gault, 2006). The Naval Special Warfare III decompression computer is used for constant PO_2 diving.

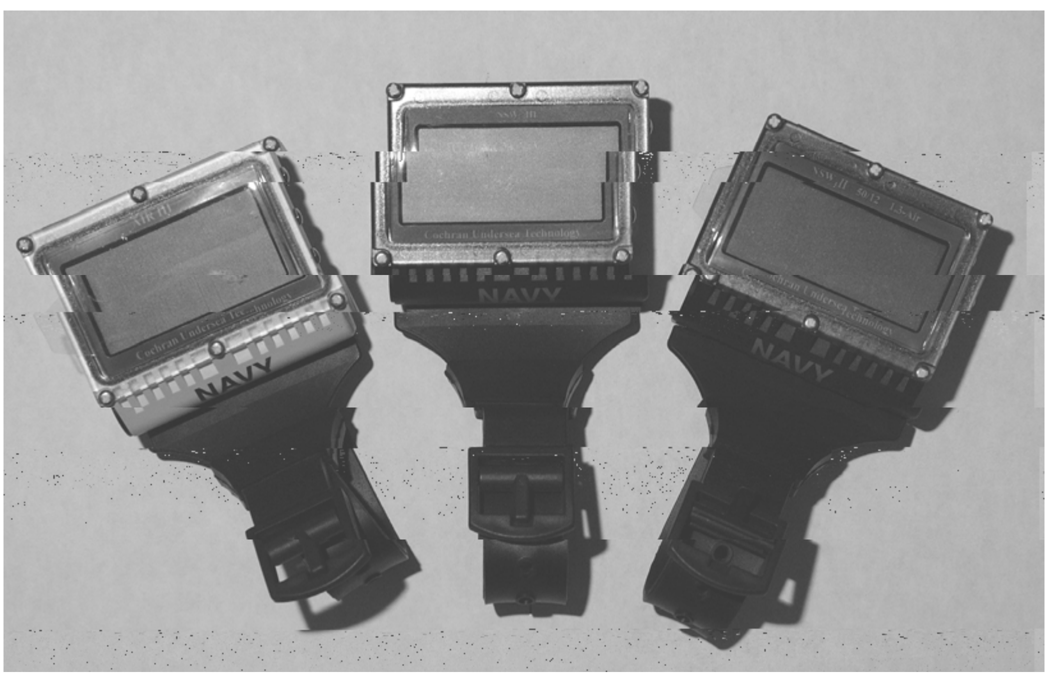

Figure 4. Cochran Navy dive computers (dive tested WAL 18 algorithms). Air III [yellow] on left; NSW III (Mk 16 rebreathers) [blue] on right. Photo credit: NEDU

The U.S. Navy has had a long history of both directly and indirectly promoting science diving. Perhaps the most recent significant trend is its cooperation with NOAA and the Smithsonian diving program to advance common goals in diving. Lately, interest in resurrecting the productive SITS Program has resurged, primarily among its former students. Those students see a need for broadly based training in science diving, a need that cannot be easily met by individual University research departments. Once again the U.S. Navy has resources than can facilitate these diver-training goals.

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Only the Strong Will Survive: Red Tides as Community-Structuring Forces in the Eastern Gulf of Mexico

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Abstract

A harmful algal bloom (red tide) and associated anoxic/hypoxic event in 2005 resulted in massive fish kills and collapse of epibenthic communities in depths less than 25 m along the central west Florida shelf. An ongoing collaborative monitoring study involving University of South Florida and Florida Aquarium science divers provided a focused time series (2005 to 2007) of natural hardbottom/ledge community succession after the red tide disturbance. Coral species, including *Oculina diffusa, Solenastrea hyades, Stephanocoenia intersepta*, and *Siderastrea spp.*, appear to have bleached during the red tide disturbance, but recovered soon thereafter. Successional stages of fish communities tend to follow a predictable progression and revert to a pre-red tide state. This is in contrast to MacArthur-Wilson mechanisms which have been used to describe species diversity of patch or discontinuous habitats as a balance between immigration and extinction rates (equilibrium). Regular occurrence of red tides, along with fluctuating sea temperatures, turbidity, hurricanes, and other disturbances in the eastern Gulf of Mexico, may prevent communities from reaching a dynamic equilibrium. These results corroborate previous predictions that the fluctuating nature of the shallow eastern Gulf of Mexico may limit the effective species pool of colonists.

Keywords: benthic, MacArthur-Wilson equilibrium, succession, West Florida Shelf

Introduction

During summer of 2005, epibenthic hard-bottom communities in the Gulf of Mexico off west central Florida were negatively affected by a persistent red tide (harmful algal bloom) and subsequent hypoxic/anoxic conditions (CA Heil, pers. comm., 2006). Such catastrophic events have been documented since 1881, and observed for an even longer period of time. The organism responsible for the Florida red tide has been identified as *Karenia brevis* (Davis, 1948; Steidinger, 1975), but numerous questions still exist regarding the physical, chemical, and biological factors that lie behind the red tide blooms and subsequent mass mortalities of benthic animals and plants, fishes, and marine mammals. Given the near-annual regularity of minor and major bloom events (Walsh et al., 2006), surprisingly few studies have investigated the effects of red tides on benthic invertebrate and demersal fish communities on the west Florida shelf. One of the best-documented red tides occurred in the mid-eastern Gulf of Mexico during the summer of 1971. Observations before, during, and after the 1971 event provided insight into effects of a red tide bloom (Smith, 1975; Smith, 1979). After the red tide dissipated in September 1971, researchers assessed the impact on reef fish communities. They estimated that 80-90% of resident reef fish species perished in the event. On inshore reefs (13-18 m), an estimated 74% of the species were killed (Smith, 1975). Smith reported that invertebrate populations sustained even higher mortality than fish populations. Echinoderms, gastropod mollusks, decapod crustaceans, scleractinian corals, polychaetes, and sponges all declined drastically (based on qualitative observations).

Some biotic groups appeared to recolonize relatively rapidly (e.g., benthic algae and certain fishes), while others (*e.g.*, scleractinian corals and echinoderms) took several years to recruit. The post-impact recolonization study by Smith (1975) was instrumental in overturning previously held notions that the effects of red tides are negligible and short-lived, with only temporary effects on inshore and nearshore fisheries. His study asserted that major red tides might result in the near-extirpation of shallow-water reef biotas, requiring up to a decade or more for benthic communities to reestablish to pre-red tide conditions.

Decadal scale recovery rates of benthic as predicted by Smith (1975) are cause for concern in the Gulf of Mexico, whose oligotrophic waters support diverse, and very lucrative, commercial and recreational fisheries. In 2000, the commercial fish and shellfish harvest from the five U.S. Gulf states was estimated to be 772 million kg (approximately 20% of the total domestic landings in the United States) and valued at over $900 million (GulfBase.org). The recreational fishery is also significant as 40% of domestic landings come from the Gulf states, excluding Texas (O'Bannon, 2001). The productivity of coastal waters is due to the benthic-pelagic coupling (Marcus and Boero, 1998) in the form of biogeochemical cycling (the turnover of nutrients in form of living matter or its decomposed constituents), and the importance of fit, functioning benthic communities to the overall health of Gulf of Mexico fisheries cannot be overstated. It is essential, therefore, that we understand the population and community dynamics in the region in terms of the species present along the inner west Florida shelf as well as the ecological impacts and subsequent recovery rates of benthic invertebrate and demersal fish communities after a catastrophic disturbance such as the 2005 red tide and associated anoxic event.

The goals of this paper are two-fold. First, we aim to qualitatively describe the epibenthic and demersal fish communities on two natural hardbottom/ledge communities on the inner west Florida shelf, defined by Hine et al. (2003) as those areas landward of the 30 m isobath. Though there have been numerous papers published on the geological origin and characteristics of areas along inner shelf areas (Harrison et al., 2003; Twichell et al., 2003; Hine et al., 2003) as well as the physical oceanographic features that dominate (Nummedal et al., 1977; He and Weisberg, 2002; Liu and Weisberg, 2005), there have been relatively few descriptions of species that inhabit these areas. The lack of community-scale benthic descriptions makes it very difficult for scientists and managers to understand the impacts that acute stresses, such as the 2005 red tide event, have on natural ledge/hardbottom areas. This paper lays the groundwork for future quantitative, comparative studies by presenting a baseline species list from natural hardbottom habitats during a two-year study period that commenced shortly after the dissipation of the red tide. Although the seasonal/annual variability and quantitative statistics are not discussed, our second goal in the paper is to begin to speculate on the role of *Karenia brevis* as a potential community-structuring force on the west Florida shelf. The regular occurrence of minor and major red tide blooms (and the development of anoxic waters at depth) may play an important role in limiting the diversity of inner shelf communities. Qualitative observations and speculative discussions are presented in this paper, with quantitative analyses to follow in future publications.

Methods

Study Site Descriptions

The west central coast of Florida shelf is an estuarine, barrier island, inner shelf system of marked contrasts and contradictions (Hine et al., 2003). The coastal/shelf system is both wave and tide-dominated, relatively sediment-starved yet receives large inputs of sand from the Tampa Bay estuary, and is generally considered to be a low wave-energy system with indications of intermittent high wave-energy such as overwash fans and tidal cut inlets (Hine et al., 2003). A strong topographic influence is conferred on the region east of the 30 m isobath (representing the past 8,000 years of coastal and shelf evolution as evidenced by sea-level curves). The area is underlain by the Neogene limestone of a formerly active carbonate platform, with sediments rich in carbonate components and exposed hardbottom areas that occupy approximately 50% of the inner shelf. Ledges or scarps up to 4 m in relief are superimposed on the platform and tend shore-parallel (Obrochta et al., 2003). Two sites, Mastedon Tabletop (MT) and Fish and Wildlife Research Institute #1 (FWRI1) were chosen for monitoring after the dissipation of the 2005 red tide, as they had been severely affected by the anoxic conditions that developed during the event (Fish and Wildlife Research Institute, St. Petersburg, pers. comm.). GPS coordinates and site characteristics are displayed in Table 1. The two sites have the typical limestone outcroppings set in sandy substrate areas as described by Obrochta et al. (2003).

Table 1. Site Locations and Characteristics

Site	GPS Coordinates	Depth (m)
MT	27º90.20´ 83º10.79´	19.8
FWRI 1	27º91.31´ 83º10.55´	19.5

Benthic Data Collection

The project commenced in February 2006, approximately three months after the dissipation of the *Karenia brevis* bloom, and sampling was conducted on a monthly basis (weather permitting) through the next 22 months. A team of 4-6 divers entered the water upon reaching the site GPS coordinates. The divers navigated to a sub-surface buoy attached to a cinderblock, with attached temperature profiler that marked the site location. Three 15-m transect lines were laid on the bottom in a random fashion using headings determined *a priori* on board the vessel emanating at the marker. Digital photographs were acquired every 0.5 m along the transect line, at a consistent distance of 0.5 m from the bottom. The sampling strategy afforded the single benthic data collector enough time to attain maximum spatial coverage while remaining well within dive limits.

Substrate and biological cover attributes of the benthic photostations were assessed using point-count analysis (Curtis, 1968; Bohnsack, 1979; Carlton and Done, 1995; Jaap and McField, 2001; Jaap et al., 2003;). Twenty random points were superimposed on each image in Coral Point Count v.3.4 (Kohler and Gill, 2006), and the benthic component under each point was identified to provide an estimate of benthic cover (Hackett, 2002). Species were identified where possible, especially among the scleractinian corals and other sessile macroinvertebrates. Algae were identified to genus and, if possible, to species levels. General classifications of the algae were used if genera or species could not be determined in the photographs. These classifications include Rhodophyta (e.g, *Eucheuma* and *Gracilaria spp.*), Chlorophyta (e.g., *Udotea sp.*) and Phaeophyta (e.g., *Sargassum sp.*); if identification proved impossible due to poor quality of photograph, excess sedimentation, etc., the algae were grouped into Macroalgae and Turf Algae categories. Qualitative species lists were generated with assistance from past publications (Dawes and Lawrence, 1990) and are presented in this paper. Future work will focus on quantitative assessment of benthic component cover. The lists presented here are not meant to be comprehensive, as the data were collected through photographic analyses, thereby excluding cryptic species from appearing in the analyses.

Fish Data Collection

Fish censuses were conducted using a modified Bohnsack Point Count Method (Bohnsack and Bannerot, 1986; Bohnsack et al., 1994), with observers' fish identification skills evaluated prior to the surveys. All divers were Florida Aquarium REEF fish data collectors and they adapted easily to the Bohnsack Point Count Method. Once in the water, the divers rotated and counted fish within a five m radius cylinder (visibility permitting) extending from the surface to bottom for five minutes. Divers recorded fish species, abundances, and approximate sizes on underwater data sheets. Between one and three surveys were conducted in different, non-overlapping locations by each diver during each dive, to provide maximum spatial coverage. The data from the two sites were pooled and a complete species list for the sites was generated and is presented in this paper.

Results

Tables 2 and 3 list the benthic and fish populations, respectively, observed during the 22-month study period. The species listed in Table 2 represent those members of the epibenthos that are conspicuous in digital photographs; cryptic species that do no appear in the photographs are not listed. A number of the components could only be identified to the genus level, indicating that digital photographic transects may not be the best method for researchers that need to identify flora and fauna to the species level.

Table 2. Benthic Fauna and Flora

Corals *Cladocora arbuscula* (LeSueur, 1821) *Oculina diffusa* (Lamarck, 1816) *Phyllangia americana* (Milne-Edwards & Haime, 1849) *Siderastrea radians* (Pallas, 1766) *Siderastrea sidereal* (Ellis and Solander, 1786) *Solenastrea hyades* (Dana, 1846) *Stephanocoenia intersepta* (Lamarck, 1816)	**Algae** <u>Chlorophyta</u> *Acetabularia sp.* *Caulerpa mexicana* Sonder ex Kützing *C. prolifera* (Forskål) Lamouroux *C. sertularioides* (S.G. Gmelin) Howe *Codium isthmocladum* Vickers *Halimeda discoidea* Decaisne *Penicillus sp.*
Porifera *Cliona sp.* *Chondrosia collectrix* (Schmidt, 1870) *Cinachyra alloclada* (Uliczka, 1929)	<u>Rhodophyta</u> *Eucheuma isiforme* (C. Agardh) *Gracilaria sp.* *Laurencia corallopsis* (Montagne) Howe
Echinodermata *Arbacia punctulata* (Lamarck, 1816) *Astrophyton muricatum* (Lamarck, 1816)	<u>Phaeophyta</u> *Dictyota sp.* *Sargassum filipendula* C. Agardh
Chordata *Aplidium sp.* *Eudistoma sp.*	

Table 3 lists the 47 fish species observed and enumerated during the course of the study period. These species represent members of both the pelagic and dermersal community, with certain tropical species appearing during the summer samplings, as the waters in the Gulf of Mexico warm.

Table 3. Fish Community Components

Common Name	**Scientific Name (species author)**
Banded Butterflyfish	*Chaetodon striatus* (Linnaeus, 1758)
Bandtail Puffer	*Sphoeroides splengeri* (Bloch, 1785)
Barjack	*Caranx rubber* (Bloch, 1793)
Beaugregory	*Stegastes leucostictus* (Muller &Troschel, 1848)
Belted Sandfish	*Serranus subligarius* (Cope, 1870)
Black Margate	*Anisotremus surinamensis* (Bloch, 1791)
Black Seabass	*Centropristis striata* (Linnaeus, 1758)
Blue Angelfish	*Holacanthus bemudensis* (Goode, 1876)
Blue Goby	*Ptereleotris calliura* (Jordan & Gilbert, 1882)
Blue Runner	*Caranx crysos* (Mitchill, 1815)
Cocoa Damsel	*Stegastes variabilis* (Castelnau, 1855)
Cotton wick	*Haemulon melanurum* (Linnaeus, 1758)
Cubbyu	*Pareques umbrosus* (Jordan & Eigenmann, 1889)
Emerald Parrotfish	*Pareques umbrosus* (Jordan & Eigenmann, 1889)
Filefish	*Sp.?*
Gag Grouper	*Mycteroperca microlepis* (Goode & Bean, 1879)
Great Barracuda	*Sphyraena barracuda* (Edwards, 1771)
Greater Amberjack	*Seriola dumerili* (Risso, 1810)
Grey Triggerfish	*Balistes capriscus* Gmelin, 1951
Harlequin Bass	*Serranus tigrinus* (Bloch, 17900
Hogfish	*Lachnolaimus maximus* (Walbaum, 1792)
Inshore Lizardfish	*Synodus foetens* (Linnaeus, 1766)
Leopard Toadfish	*Opsanus pardus* (Goode & Beane, 1880)
Mangrove Snapper	*Lutjanus griseus* (Linnaeus, 1758)
Pilotfish	*Naucrates ductor* (Linnaeus, 1758)
Porgy	*Calamus sp.*
Red Grouper	*Epinephelus morio* (Valenciennes, 1828)
Round Scad	*Decapterus punctatus* (Cuvier, 1829)
Sand Diver	*Synodus intermedius* (Spix & Agassiz, 1829)
Sand Perch	*Diplectrum formosum* (Linnaeus, 1766)
Scamp Grouper	*Mycteroperca phenax* (Jordan & Swain, 1884)
Schoolmaster	*Lutjanus apodus* (Walbaum, 1792)
Seaweed Blenny	*Parablennius marmoreus* (Poey, 1876)
Sheepshead	*Archosargus probatocephalus* (Walbaum, 1792)
Sheepshead Porgy	*Calamus penna* (Valenciennes, 1830)
Slippery Dick	*Halichoeres bivittatus* (Bloch, 1791)
Spanish Mackerel	*Scomberomorus maculates* (Mitchill, 1815)
Spotfin Butterflyfish	*Chaetodon ocellatus* Bloch, 1787
Spottail Pinfish	*Diplodus holbrookii* (Bean, 1878)
Spotted Drum	*Equetus punctatus* (Bloch & Schneider, 1801)
Tomtate	*Haemulon aurolineatum* Cuvier, 1830
White Grunt	*Haemulon plumierii* (Lacapede, 1801)
Whitespotted Soapfish	*Rypticus maculates* Holbrook, 1855
Yellowfin Mojarra	*Gerres cinereus* (Walbaum, 1792)
Yellow Goatfish	*Mulloidichthys martinicus* (Cuvier, 1829)
Yellowtail Snapper	*Ocyurus chrysurus* (Bloch, 1791)
Yellow Wrasse	*Halichoeres sp.*

Discussion

The species lists presented in this paper contribute to a limited qualitative database of benthic and fish communities that develop on the natural hardbottom/limestone ledge outcroppings in the eastern Gulf of Mexico. Dawes and Lawrence (1990) observed that the limestone outcroppings are the major sites that support a macroalgal flora at depths below 10 m in the Gulf of Mexico, and contribute greatly to both biomass and energy levels, although overall the energy levels in these communities are lower and more erratic than in shallow water seagrass beds. They also observed that at certain times during the year, algal biomass on the limestone outcroppings accounted for less than 20% of the benthos (primarily during the winter and spring). The fluctuation in available biomass suggests that secondary production must be dependent on other sources such as the shallower, but more stable seagrass, communities. These observations coincide with initial quantitative assessments of herbivorous fish populations (not presented here), which appear to fluctuate on a seasonal basis, as the fish move into the shallower seagrass communities to feed during the colder months.

The seasonal emigration of fish populations, combined with the regular occurrence of acute stresses imposed on demersal fish and benthic macroinvertebrate communities, makes it difficult to apply typical dynamic equilibrium ecological models to definitions of community succession and structure. Smith (1979) proposed that eastern Gulf of Mexico reef-fish communities develop according to predictable, rather than chance processes. In this view, ultimate stability in species richness and composition represents the attainment of a 'climax' community, as opposed to a dynamic species equilibrium proposed by MacArthur and Wilson (1963) which predicts that species richness of insular (or insular-like) areas depends upon a balance of species immigration and species extinction rates. Immigration and extinction rates are in dynamic equilibrium, resulting in continual biotic turnover in contrast to the climax-community hypothesis (stable state). Smith attributed the development of a climax community to the inhospitable nature of the Gulf of Mexico which reduces the effective species pool of colonists. Hardy species (or species that produce hardy planktotrophic larvae) recruit (or settle) during the early stages of colonization and are difficult to displace. These characteristics, combined with the observations that benthic communities in the Gulf of Mexico are not isolated 'islands,' may make it difficult to apply the MacArthur-Wilson species equilibrium model to either benthic or fish communities along the inner west Florida shelf.

Communities along the west Florida shelf are composed of species that are tolerant and resilient to regular environmental stresses imposed on them as residents of temperate, shallow-water ledge communities. Populations may be restricted to those that are most resilient and able to withstand the red tide stress, as well as the synergistic effects that result when other natural and anthropogenic stresses, i.e., hurricanes, cold-water events, overfishing, and pollution, are factored in. Future analyses will quantify seasonal variability/stability of these communities, whose productivity contribute greatly to the socioeconomics of the west-central Florida region.

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Diving with Captive Sharks

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Abstract

For more than half a century, elasmobranchs have been placed on exhibit at aquariums and held in captive conditions for purposes of education and research. Much is still unknown about many species of sharks, including feeding habits, reproduction, and behavior. Captive populations have taught us a great deal and the number of captive held sharks continues to grow, with over one hundred public aquariums and marine laboratories now holding these animals. Many large facilities use diving to support the health and study of captive sharks, yet there is little literature discussing how this is done safely for both the sharks and the divers. This paper explores the current thinking and safety practices of 23 aquaria worldwide that dive with captive sharks and provides recommendations for dive program risk managers and areas for further study.

Keywords: aquarium, captive animal care, dive safety, elasmobranchs, risk analysis, risk management

Introduction

Diving is used to facilitate the health and study of captive sharks, and many facilities with these animals have active programs, however, other than the Elasmobranch Husbandry Manual (Smith et al., 2004) there is little literature on how this is done safely for both the sharks and the divers involved. Professional aquarists are considered experts in the husbandry of these animals and often on the biology and behavior of their charges. Together with dive safety officers they have developed safety strategies for diving with sharks, and much can be learned by consolidating their knowledge and experience. Most of these facilities have a focus on education or entertainment of their visitors, and today there are a variety of activities being done during dives, including what appears to be a growth in the areas of providing interpretive dives, guest diving programs and using divers to feed aquatic animals.

By comparing safety methods currently being used by aquaria around the world, this paper provides a view of the current standard of care; explores the current thinking and practices of diving with captive sharks; provides an overview of risk analysis techniques; and recommends areas for further study. Methods included a survey instrument, a literature search, and ethograms of individual sharks in the Oregon Coast Aquarium's Open Sea Exhibit.

Methods

The survey instrument was administered via telephone interviews, and in the case of several international facilities, electronic mail, and was designed to collect information from animal care professionals and/or dive officers around the world regarding their shark diving protocol. Selection of participating facilities within the USA was based largely on Association of Zoos and Aquariums

(AZA) membership as well as availability and receptivity. The AZA is an accreditation organization which accredits only those institutions that have achieved rigorous standards for animal care, education, wildlife conservation and science. Selection of participating facilities outside the USA was based on availability and receptivity.

The survey information collected included general exhibit characteristics, species composition, frequency and number of dives, purpose of dives, safety methods for dives, reported incidents, and the perception and assessment of risk by species. The assessment of risk by species was a subjective measure of how animal care professionals and dive officers view the shark species with which they are diving. Each were asked to rate their shark species on a scale of one to ten (one being the least, ten being the greatest) in two categories. The first category was how aggressive they believed their species to be (aggressive toward other sharks or inhabitants or divers). The second category was an assessment of the potential risk of injury (severity) to a diver for each of their species. The ratings were used as a way to quantify perceptions of aggressiveness and potential risk of injury to divers.

The manner of all survey questions was identical for every interview to ensure consistency. Once the survey was complete the data was organized and analyzed for number of sharks by species, frequency of dives, purpose of dives, safety methods for dives, reported incidents, and ratings by species. The resulting data presented here provides a good understanding of the current status of captive shark diving programs, but should be considered broadly, and not relied on too specifically or taken out of context. It is a good starting point for evaluating the practices of safe diving with captive sharks, but should be applied generally.

Additional methods were used internally at the Oregon Coast Aquarium to observe and monitor the swimming behavior of sharks in the Open Sea exhibit. The authors closely observed all nine Broadnose Sevengill sharks (*N. cepedianus*) from the acrylic tunnel to quantify their behavior before and during common work dives. Ethogram maps (based on data collection points every 20 sec) were used to record the depth, direction, and position of each shark, both before and during dives. This data was collected by the authors at similar times each day for four weeks with various volunteer and staff divers, which was representative of an average month of diving for this exhibit. These methods were adapted from those used by Russo, who conducted ethograms in the same exhibit in 2005, when there were only four Broadnose Sevengill sharks in the Open Sea collection (Russo N, personal communication, 2005).

Results and Discussion

Species of Sharks

Of the 27 species exhibited by participating aquaria (Figure 1), the most common species are the Sandbar, the Sand Tiger, and the Nurse. Sixteen out of the 23 participating aquaria (or 69.5%) have Sand Tigers. Five of the six international facilities have Sand Tigers, and three of the six have both Blacktip and Whitetip Reef sharks. The striped bars in Figure 1 represent those species which were involved in reported incidents.

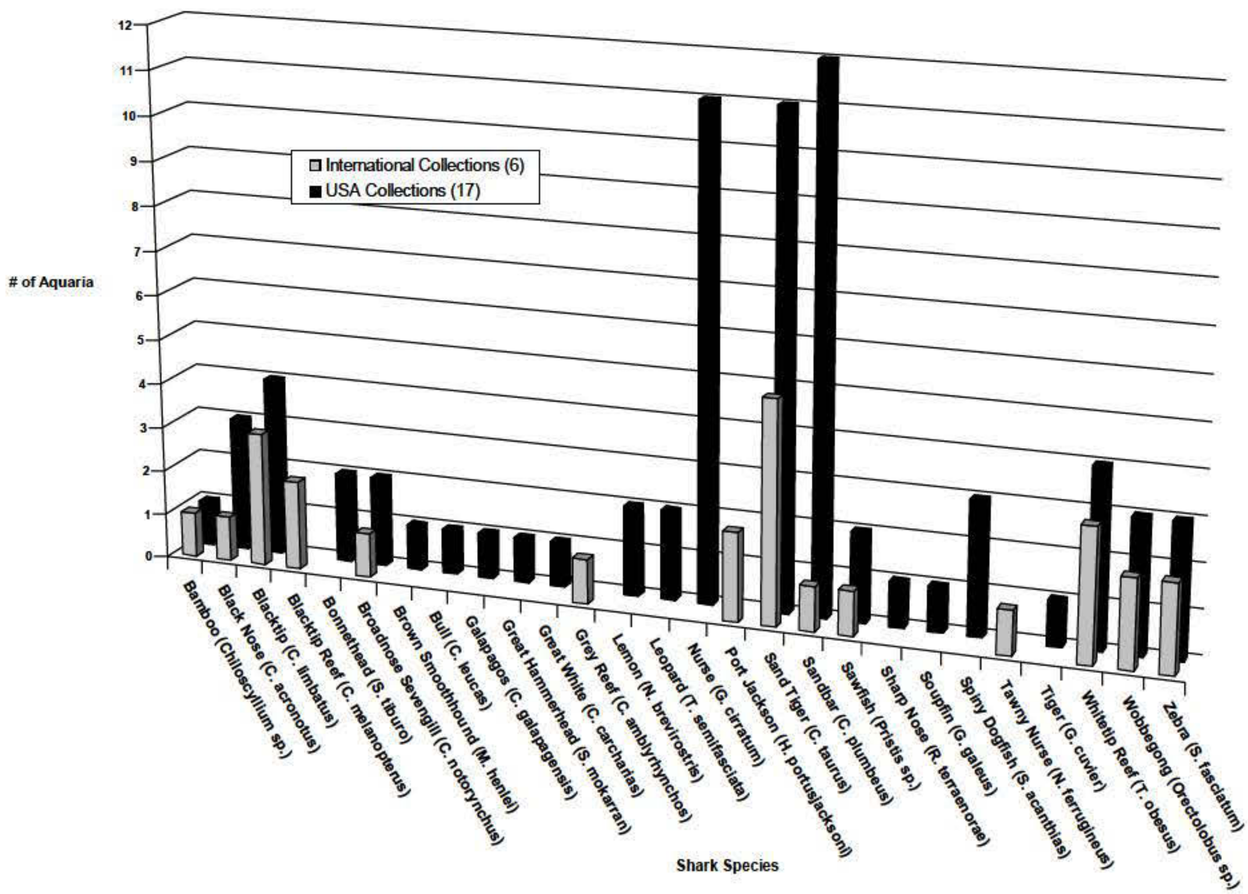

Figure 1. Reported shark species in participating aquaria among the 17 USA and 6 international collections. Note that all participating aquaria have multiple species.

Population density is also of interest, so we asked how many of each species these facilities have on exhibit (Figure 2). Spiny Dogfish were the most populous, with three facilities exhibiting a total of 224 individuals. Sand Tigers were also quite populous, with five international facilities exhibiting a total of 57 individuals, plus eleven US facilities exhibiting a total of 53 individuals.

The species exhibited is important for several reasons. It may be prudent to employ specific safety devices or strategies (e.g., using chain mail with a Great White) based purely on the elevated risk of injury severity associated with that species. There were several species that were held by only one respondent aquarium. This included one facility with a Great White, one with a Tiger shark, and one with Bull sharks. Each of these facilities reported that they use specialized devices (full body chain mail, and/or separation devices).

A number of survey participants suggested that population density and gender balance among the species are also issues that should be considered.

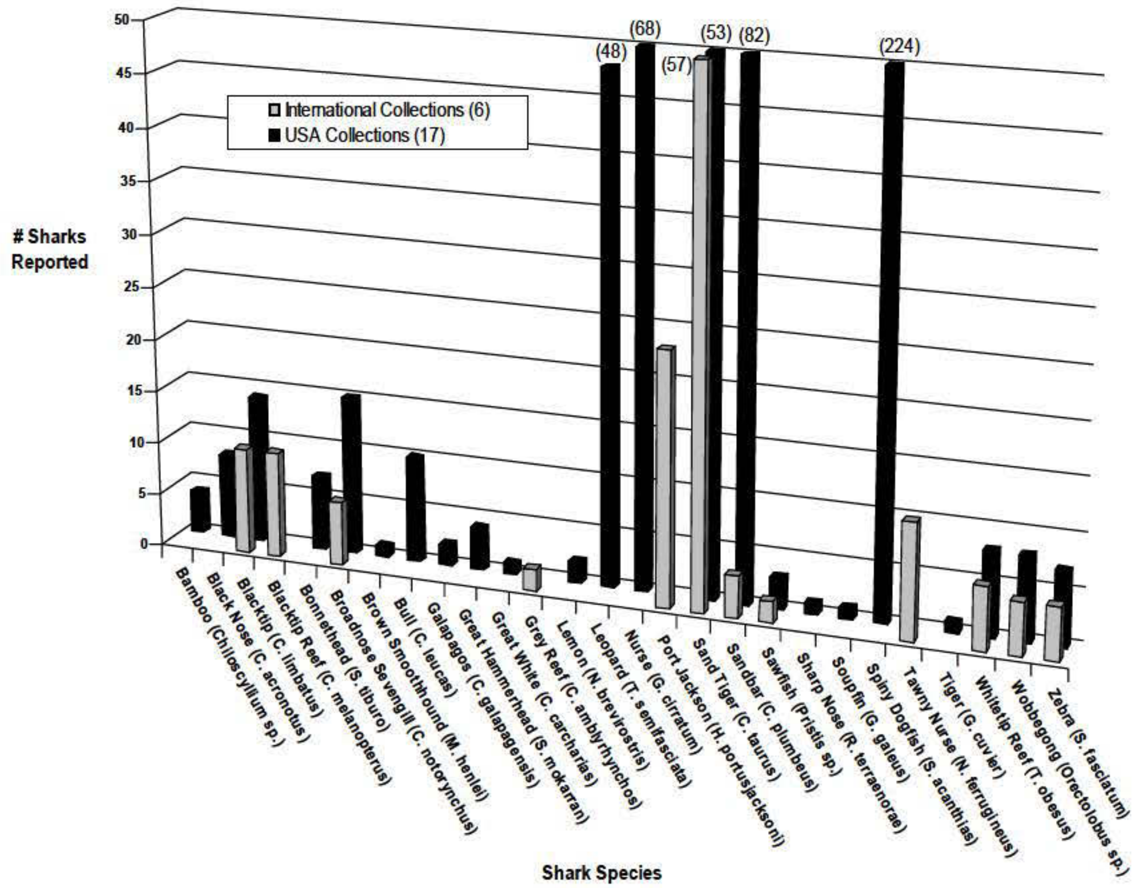

Figure 2. The total numbers of each shark species reported for participating aquaria in the 17 USA and 6 international collections.

Numbers of Dives

Determining the actual number of dives being done with captive sharks is fraught with difficulty. The data presented here provides a good understanding of the big picture, but should be considered broadly and should not be viewed as highly reliable or verifiable data. There is currently no central repository for such data – not in the commercial or science fields, nor through AZA. The dives reported here are averages based on the survey conversations with individuals at these organizations. Some of these organizations have excellent data management and are capable of providing exact numbers. Many simply averaged the number of dives they do weekly to provide estimates. There is also no clear date range for these reported dives. They are reported as representative of what each facility is currently doing, and do not necessarily represent past diving data. As a result, one cannot rely too specifically on this data; however it does provide a good picture of the basic volumes of dives being done, and is sufficient to draw some general conclusions.

There were 23 respondent facilities to the survey, and 26 shark exhibits in which dives are done.
Of these 26 exhibits, we were able to place them in four broad categories: those exhibits which had fewer than 100 dives per annum; those with between 101 and 500 dives per annum; those with between 501 and 1,000 dives per annum; and those with greater than 1,001 dives per annum (Table

1). The range was between 25 dives per year and 7,600 dives per year. It is interesting to note that four of the six exhibits that had more than 1,000 dives per year were international (non-US) facilities, and all of these offer guest diving programs. The average number of dives being done in US facilities was approximately 630 per year. The average number of dives being done in non-US (international facilities) were approximately 2,100 dives per year. The overall average total was approximately 1,027 dives per year.

Table 1. Total numbers of dives per annum and the number of participating aquaria for each grouping

# of Dives per Annum	# of Aquaria
1-100	4
101-500	9
501-1000	7
>1000	6

The number of dives can be an important part of risk analysis. If the dive data and incident data were reliable, this could form the first part of risk analysis. It is clear even from this limited data that a great number of dives are being done, with few incidents resulting. The safety procedures in use are obviously effective. As will be discussed below, this is only part of the risk picture, and should not be taken without regard to the consequences/severity of a potential incident, which is the second part of risk analysis.

Purpose of the Dives

The purpose of the dives could be broadly grouped into four categories; feeding dives, guest dives, interpretive dives, and those done for work or research (Table 2).

Feeding dives were described as those dives that were for the purpose of directly feeding either the sharks or other aquatic specimens in the shark exhibit. Three US-based and four international facilities use diving as a means to provide food directly to their sharks. Three US-based and one international facility use divers to feed the other specimens in the exhibit but not their sharks.

Guest dives are described as those programs that allow members of the general diving public to enter the exhibit and do a dive. These are typically paid guided dives with educational content and a recreational diving emphasis. Of the seven facilities that offer guest dives, four are international.

Interpretive dives are described as those programs that utilize divers to actively interpret the exhibit, usually with a focus on educational or conservation content. Divers are typically equipped with full face masks and underwater communications equipment and are able to present information directly to visitors, sometimes aided by an interpreter/docent on the visitor side. Of the ten facilities doing interpretive dive programs, two are international.

Work and research dives are those dives done to assist in the husbandry or study of the exhibit collection. All of the facilities participate in these types of dives.

Table 2. Purpose of dives for each participating aquarium

Facility	Purpose of Dives				
	Work	Interpretive	Guest	Feeding (non-shark)	Feeding (sharks)
US	X	X	X		X
US	X	X			X
US	X				X
US	X	X		X	
US	X			X	
US	X	X		X	
US	X	X	X		
US	X		X		
US	X	X			
US	X	X			
US	X	X			
US	X				
US	X				
US	X				
US	X				
US	X				
US	X				
International	X	X	X		X
International	X		X	X	X
International	X		X		X
International	X		X		X
International	X			X	
International	X	X			

The purpose of the dive is important because different tools, techniques and often divers are used. Work and research dives involve task loading, different tools depending on the task, and often will place divers in the swim patterns of the sharks. Guest dives include divers with a range of skills, which could impact safety. Interpretive dives introduce additional equipment issues, such as hardwired communications lines which could contribute to entanglement hazards (for both divers and sharks). Feeding dives also introduce potential hazards to both divers and sharks. Three US and four international facilities use divers to directly feed sharks, and an additional three US and two international facilities use divers to feed other (non-shark) species in their shark exhibits. It should also be noted that a majority of the reported incidents were related to feeding dives. Despite these incidents, a great number of feeding dives are being done safely, and many feel this is an acceptable activity and may be appropriate for some facilities, provided effective safety techniques are used. Some facilities choose to feed for animal care/husbandry reasons, to enhance the educational experience for their visitors, or both.

Safety Methods Used

Safety methods were broadly categorized into four basic strategies (Table 3), including separation (such as nets or physical barriers), chain mail, electronic (such as shark shield devices) and sticking. Five facilities used separation; three facilities used full body chain mail (with an additional two using chain mail gloves for feeding); and one facility has used electronic devices (though currently have it available only for contingency). The most common method was sticking, with 15 out of 23 of the facilities using this safety strategy. During this project we also learned of at least one facility that uses sticking in an open water environment during research dives with Sixgill sharks. The stick is used primarily as a visual and physical barrier between the divers and the sharks. It is typically used by a designated diver, whose role is to observe the swimming patterns of the sharks and intervene between the diver and shark where necessary. Most of the sticks are made of PVC and are four to five feet in length. Some are fitted with a T on one end which can be used to fend off a shark if necessary by placing the T under the lower jaw and gently redirecting the animal away from the divers. Several facilities described using the stick to condition their animals to avoid contact with the divers. Most view it as an effective low-cost tool with relatively simple training requirements.

Table 3. The physical safety method(s) used by each participating aquarium

	Safety Method Used				
Facility	None	Electronic	Sticking	Chain Mail	Separation
US	X				
US	X				
US			X		
US			X		
US			X		
US			X		
US			X		
US			X		
US			X		
US			X		
US			X		
US				body	
US	X			glove	
US		X	X	body	
US					X
US			X		X
US				body	X
International	X			glove	X
International	X				
International			X		
International			X		
International			X		
International			X		X

It is also notable that there were five facilities that used none of these physical strategies. Several of these facilities reflected that their strategy consisted of a strong culture of safety and an emphasis on training and preventive measures. All portrayed a high level of confidence in the safety of their programs.

From a historic perspective, safety protocol for diving with captive sharks has been an evolution. In the beginning, there was no previous experience to draw from, other than diving with wild populations, and little was understood about wild sharks.

One of the earliest reports of diving with captive sharks was provided by Harold (1968) of the Steinhart Aquarium, who summarized a number of incidents involving Sevengill sharks. Harold reported that in captivity Sevengills often refused to eat, and that the practice of capturing and force feeding them became normal procedure at the Steinhart Aquarium. He described an incident in 1962, in which a 16.5 lb Sevengill was captured by a diver in the aquarium and restrained, but escaped the diver's grasp. The shark circled back toward the diver who raised his right arm in front of his face. The shark bit, shook and twisted until it bit out a section of the forearm. Harold also described several incidents at San Francisco's 'Playland at the Beach' where large (up to 100 lb) Sevengills were often displayed in a 350,000 gallon exhibit. On one occasion a diver cleaning the windows accidentally kicked an eight foot Sevengill which spun around and grabbed the diver's fin, shaking it violently for about 30 sec, at which point the diver exited the water. On another occasion an employee had jumped into the water accidentally straddling a Sevengill, which turned and bit at him.

Reed (1987) also provided some early information on diving with sharks. He began diving with Sevengills at Marine World in Vallejo, California after they received a number of them from the Steinhart in 1978. Reed noted that initial fear of the animals had the divers actively trying to push the sharks away from the work area, but it was eventually recognized that the sharks were swimming in a specific pattern and had little choice in this pattern, given the shape and configuration of the exhibit. Once the divers realized this they were able to respond much more appropriately to the needs of the sharks and still get the required work done. Reed described becoming highly comfortable diving with these animals; however he was very surprised on his 2,000th dive when the large female Sevengill grabbed his head in her mouth and then suddenly released it without biting down. The routine nature of the dive and lack of provocation distressed Reed, who was unable to explain the behavior.

These early accounts highlight how little was understood about the management of captive sharks and how to dive safely with them, but since that time a large number of dives have been done, in a large variety of exhibits with many different species. The accreditation of a host of zoos and aquariums as AZA members has meant the establishment of industry standards for animal care, far superior communication between facilities, active sharing of animal behavior and husbandry techniques, and a culture of sharing the science, research, and anecdotal information between these facilities. What was a learn-as-you-go approach to keeping captive sharks has developed into a proactive professional approach, enhanced with the ability to draw upon the knowledge and experience of others through formal and informal networking. An aquarist at Dallas World Aquarium can call a colleague at Monterey Bay Aquarium and seek advice on how to introduce new species to their new exhibit, or the Oregon Coast Aquarium can enquire how the Melbourne Aquarium uses divers to feed their Sevengill sharks.

The diving chapter of the Elasmobranch Husbandry Manual recommends that the safety of both the animals and the divers should be considered even in the conceptual and design phases of exhibit development (Smith et al., 2004). Long before a diver straps fins on their feet, the exhibit design team should evaluate how safety can be built into the structure. This should be based on what species will be exhibited, how the exhibit will need to be cleaned, the expected swimming patterns of the sharks, obstructions and potential traffic conflict areas, the option of using physical barriers that can be lowered into the water, and the design and provision of holding and access areas (medical holding pools and side-entry pools). Engineers should consider design elements that minimize or nearly eliminate the need for cleaning dives, such as under-gravel filtration, currents and drains strategically placed to remove wastes, and surface access areas that enable cleaning to take place from the surface. Just the provision of a side entry pool can eliminate the need to physically remove animals from the water to separate them. This can be an important contribution to safety for both divers and animals, since there is an increased risk of injury while the animal is being handled. There is simply no excuse for exhibits being designed, built and populated before any thought is given to how divers will be required to enter, exit and carry out tasks in relative safety, but it still does occasionally occur. Post-construction changes to exhibits to accommodate the needs of divers can be quite costly.

Another element in the provision of an effective safety program is the experience and longevity of the Dive Safety Officer (DSO) and aquarist staff. Staff with well-rounded knowledge and experience with a variety of species and exhibits can avoid countless mistakes and contribute considerably to the well being of the animals as well as the safety of the divers. During this research it became clear that experienced skilled staff contributed substantially to the process of conducting thorough assessments of their safety needs. While some facilities employ specific safety measures primarily because that is the way it always has been done, a growing number are carefully and thoroughly evaluating their safety needs based on current conditions and the data they collect on their animals. At least one facility described that they chose the species of sharks to be exhibited based substantially on how easy they are to dive with. They made a management decision to remove their more aggressive and unpredictable Lemons and replace them with a milder species. Another facility related that they removed their Bull sharks for similar reasons.

Incidents

The definition of an incident was defined as any interaction between a shark and a diver that resulted in an injury to the diver. Collecting verifiable data on incidents and accidents proved difficult. As with diving data, no central repository exists. This meant that all information collected was based purely on the knowledge and memory of the person being interviewed. As a result, there is no clear date range for these incidents. Several respondents provided a fixed number of incidents, while others provided estimates. Nearly all were very candid and willing to openly discuss past incidents, recognizing the value of sharing such information. Since exact numbers cannot be determined, this information must be used with caution and considered anecdotal. Table 4 provides an overview of reported incidents by species, and some descriptive detail.

There were few reported incidents, particularly when taken in the context of the large numbers of reported dives, however it must be clear that this information is anecdotal and only broad observations should be made about it. As reported above, many of these incidents occurred during feeding dives, or while a shark was being handled or immobilized. The vast majority was minor, involving fingers, hands, and wrists, and many were considered diver error (such as feeding sharks out of the prescribed order or getting hands in the way of a shark's mouth). A number of incidents were followed by changes in safety protocol and/or equipment. These changes were sometimes quite minor, such as the addition of a chain mail glove to feed, while others were more sophisticated, such as the construction and use of a separation cage). It is also clear that even with diligent safety protocol diver error does occur and has resulted in incidents.

One point of interest is the species associated with these reported incidents (see the striped bars in Figure 3). It is not surprising that Sand Tigers were strongly associated with reported incidents, because they are the most common shark species (Figure 1) and are one of the most populous (Figure 2). Also notable is the Broadnose Sevengill, which represents a low percentage of the total population of captive sharks, but appears to be overrepresented in incidents. This may be partially explained by exposure, since a very large number of dives are being done with these animals.

Table 4. Incidents involving sharks reported by species

Species	Incident Descriptions
Bamboo	2 bites on exposure suit due to poor food placement.
Lemon	3 incidents reported.
Lemon	Some minor nicks/cuts.
Nurse	Fingers put in mouth of shark - sandpaper scrape - diver error.
Sand Tiger	9 bites on hands/fingers during feeds.
Sand Tiger	Some incidents reported during reproductive season.
Sand Tiger	2 incidents reported. Removing dead fish when shark bumped off divers mask, small cut on cheek.
Sand Tiger	1 bite on hand during a feed (puncture).
Sand Tiger	1 bite on shoulder during a public feed.
Sand Tiger	Incident(s) reported.
Sand Tiger	Presenter turned to point at another shark and stuck finger in mouth.
Sandbar	Many bites when provoked in target feeding.
Sandbar	3 incidents reported during feeding.
Sandbar	Some incidents reported before use of safety stick.
Sandbar	1 bite on wrist during feed - diver error - not following protocol.
Sandbar	Many incidents reported ignored stick and bumping divers.
Sevengill	Numerous "mouthing" incidents with head and regulator 1st stage. 1 finger bite during feed.
Sevengill	1 incident reported.
Spiny Dogfish	2 incidents reported.
Whitetip Reef	Many bites when captured and provoked.
Wobbegong	1 bite on hand.
Wobbegong	1 incident reported.

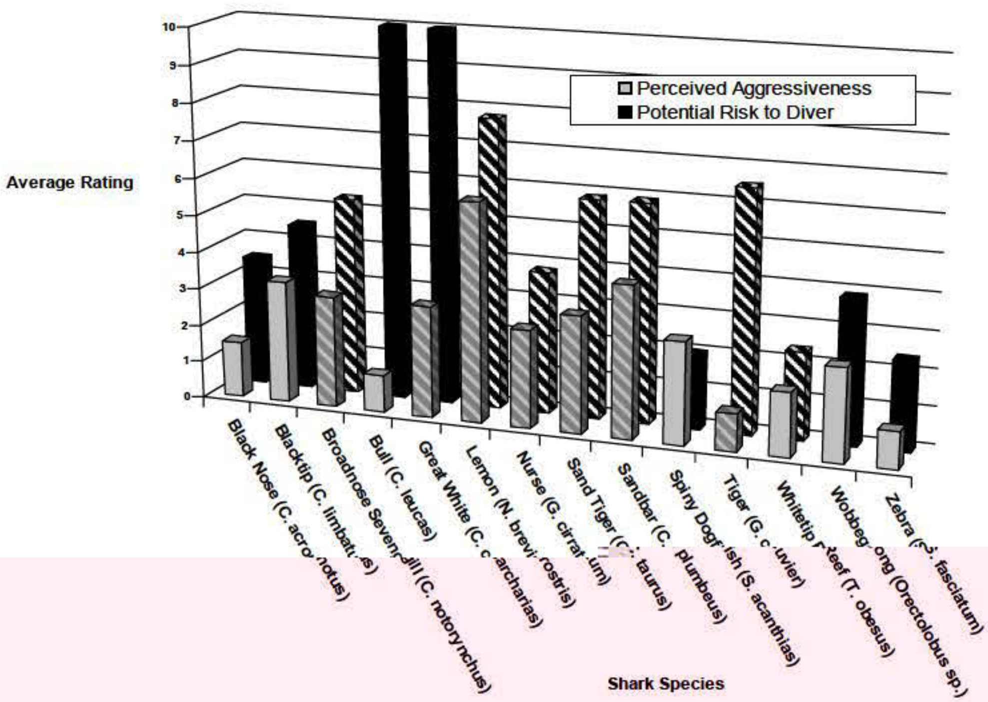

Figure 3. Average ratings for perceived aggressiveness and potential risk of injury (severity) to diver by species are from 1(least) to 10 (greatest). The striped bars represent species associated with incidents.

Perception of Risk

When developing a safety protocol for diving with sharks, one element that should be considered is the level of risk associated with any particular species (Hodges and Sabalones, 2005). The International Shark Attack File (Burgess and Buch, 2008) provides good data for the species of sharks which are implicated in attacks on divers in the wild, but it is not known how applicable this is to captive shark environments. During these surveys, one goal was to provide a model to quantify and assess risk. The survey assessment of risk by species was a subjective measure of how animal care professionals and dive officers view the shark species they are diving with. The development of safety protocol for diving with sharks is not prescriptive. There are no legal requirements or standards that govern diving with sharks. Each facility must develop safety protocol based on their local needs. This means that it is largely based on the perception of risk, which can be difficult to measure.

Each survey respondent was asked to rate their shark species on a scale of one to ten (one being the least, ten being the greatest) in two categories. The first category was how aggressive they believed their species to be (aggressive toward other sharks or inhabitants or divers). The second category was an assessment of the potential risk of injury (severity) to a diver for each of their species. The ratings were used as a way to quantify perceptions of aggressiveness and potential risk of injury to divers. To be clear, this is not a measure of how aggressive or injurious a species is, but rather how it is perceived.

Figure 3 shows the average perceived aggressiveness and the average perceived risk of injury. Figure 3 does not include those species reported in fewer than three aquariums, as they were considered insignificant for the average ratings. The exceptions to this include Bull, Great White, and Tiger species. These were included in the average ratings based on an assumption by the authors that these sharks are generally accepted as higher risk species.

The Lemon and the Sandbar received the highest averaged ratings for aggressiveness, while the Bull received very low ratings. The two species perceived as the highest risk of injury were the Great White and the Bull. This demonstrates an important concept of risk analysis. While the Bull shark is not perceived as aggressive, its capacity for inflicting serious injury is perceived to be very high. The severity of even a low probability event must be part of the consideration for mitigating risk. It is noted that the facilities that exhibit Bull sharks have taken additional safety measures. Another notable species is the Lemon, which was rated relatively high on both measures. It is noted that facilities exhibiting this species also have taken additional safety measures. It is also interesting to the authors that it appears the Lemon and Bull species are being selected less frequently as exhibit animals. Several survey respondents indicated they have moved away from these animals specifically to reduce risk for their divers. Perhaps the highest profile species, the Great White (which was only reported by one facility) was not perceived as particularly aggressive; yet again it was perceived as capable of inflicting serious injury and therefore warranted additional safety measures.

Risk Analysis

Risk analysis may be defined as the process for identifying potential risks, evaluating the probability and severity of those risks, and responding to these risks with a management plan.

Occupational health and safety regulations require all organizations to provide a safe environment for employees. We found several examples of risk management models used in the scientific diving community that may be applied to diving operations. The University of Hawaii uses a risk management form as part of each emergency plan for diving operations (University of Hawaii Dive Safety Program, 2008). The flowchart requires a description of the risk event, the probability of occurrence, the severity of an occurrence, the nature of the occurrence and mitigation or mediation of risk.

The University of Tasmania in Australia uses a workplace risk assessment and control guide as part of their dive operations planning (University of Tasmania, 2008). This guide provides a severity scale and a likelihood scale that are plotted on a risk matrix which provides a risk rating for the operation. This step by step guide walks the user through the risk management process, from systematically listing the activities; identifying potential incidents, accidents, hazards or consequences that may arise from the activity; allocating a severity rating and probability rating; to identifying control and safety measures.

An example of a generic guide for managing risk is provided by the Australian/New Zealand Standard for Risk Management, AS/NZS 4360 (Standards Australia, Standards New Zealand, 2004). This standard provides a logical and systematic method for risk management and highlights the importance of developing a culture of safety that is imbedded in the organization's procedures.

These are excellent tools and provide a good framework for how organizations should conduct risk analysis in general terms. More specific to diving with sharks, we also found several examples of risk response models used in the aquarium diving community. Figure 4 demonstrates how increasing levels of risk might be mitigated by moving from having no physical safety methods to using a safety diver, chain mail, separation or even to cease diving operations.

Figure 4. An example of a risk response model (adapted from Thomas 1991).

What conditions might suggest either extreme? Common sense would suggest that diving with small, non-aggressive species that have little or no capability of inflicting injury should require no additional safety measures beyond those used in other aquatic exhibits. Similarly, there may be occasions when diving operations are stopped for such things as a predation event resulting in highly excited feeding behaviors, the addition of new exhibit animals, or an escalating level of contact between divers and sharks.

Several facilities we surveyed had written protocol for response to shark behavior. One example is provided in Figure 5 (Broadhurst 2008).

SHARK-CON 1 =	Swimming slowly and predictably. Easy to guide with Safe-T-Sticks No contact necessary.
SHARK-CON 2 =	Swimming speed increased or odd behavior. More difficult to guide with Safe-T-Sticks. Occasional contact may be necessary.
SHARK-CON 3 =	Swimming speed greatly increased. More difficult to guide with Safe-T-Sticks. Routine contact may be necessary. Swimming patterns erratic, tight turns. Seem agitated and/or aggressive towards fish.
	If SHARK-CON level reaches level 3 an audible signal of 3 pings will be sounded. Divers will exercise caution while completing dive tasks expeditiously and exit the water as soon as practical.
SHARK-CON 4 =	Swimming speed greatly increased. Extremely difficult to guide with Safe-T-Sticks. Swimming patterns erratic with tight turns, head-bobs, arched back, and pectoral fins down. Aggressive and/or feeding behavior evident towards other fish. Sharks aggressive towards divers. Sharks may or may not strike Safe-T-Stick.
	If SHARK-CON level reaches level 4 an audible signal of 4 or more pings (in-water diver recall) will be sounded. Divers will exercise extreme caution and exit the water as soon as possible.

Figure 5. An example of written protocol for response to shark behavior (Broadhurst 2008)

Conducting a risk analysis for diving with sharks should include a broad range of factors, but we can group them in three categories: shark focused, diver focused, and exhibit focused. Shark focused factors include the aggressiveness of the species and individual animals; capacity for inflicting injury, such as the size of the animals, the size of the mouth, and tooth morphology; and issues of population density and gender balance. Diver focused factors include the tasks undertaken, such as feeding or handling, which may increase the exposure to risk; the experience, training and skill level of the divers present and their anxiety levels; the equipment used; the degree to which divers are task loaded; and the overall level of exposure. The higher the number of dives, the more statistical probability there is for an event. An alternative view offered by one animal care professional is that the more dives that are done with these animals, the more conditioned they become to the presence of divers, which may reduce risk. Exhibit focused factors include design and layout issues, such as traffic flow and bottlenecking. Some are designed to reduce the incidence of shark-diver interactions with consideration for swimming patterns, traffic constrictions, barriers, entry exit facilities and other environmental features. Others provide challenges that increase the risk for divers.

Operational Implications

During our surveys it became clear to us that all of the facilities we spoke to had engaged in some level of risk analysis, and described a variety of operational implications of diving with sharks. This included the need to develop training procedures for those diving with sharks; decisions on the use of volunteer divers vs. staff divers; the need to develop policy specific to diving with their sharks; and the need to be proactive and responsive to changes. One of the challenges of diving with captive sharks is the dynamic nature of their behavior, but proactively monitoring changes in shark behavior can be difficult. Several facilities described the need to make direct observations of their shark's behavior in an ongoing manner.

At the Oregon Coast Aquarium the authors used an ethogram model to track swimming patterns of each of the nine individual Sevengill sharks in the Open Sea exhibit, both before and during common work dives. This technique was used previously (Russo N, personal communication, 2005) with the original Open Sea exhibit population of 4 Sevengill sharks, however an additional five animals were added to this exhibit in mid 2007. This tool enabled us to begin to see differences in how each of the sharks behaved both with and without divers in the exhibit. Prior to any diving for the day, a shark was tracked and plotted on a map of the exhibit every 20 s, noting the approximate depth, location and direction of travel (Figures 6 and 7).

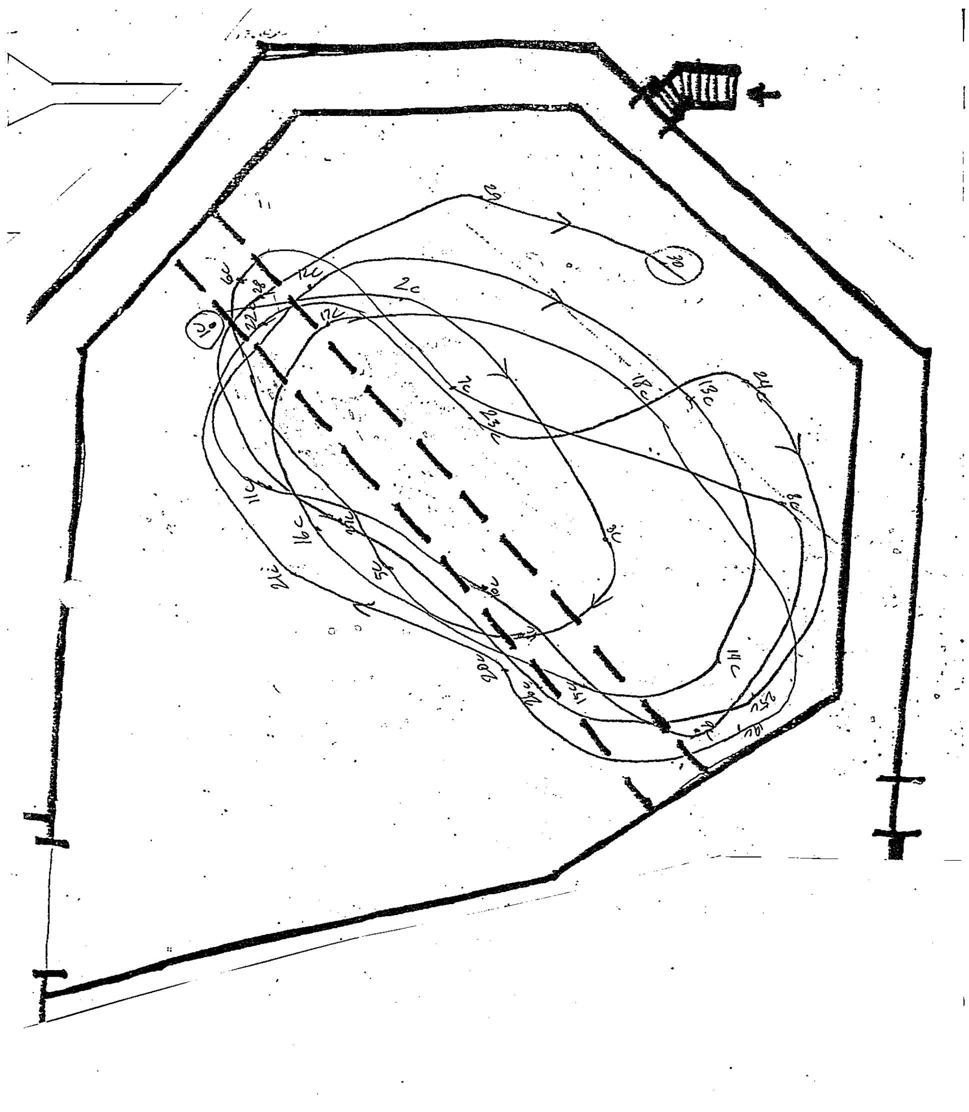

Figure 6. An example of an ethogram showing a relatively predictable swimming pattern

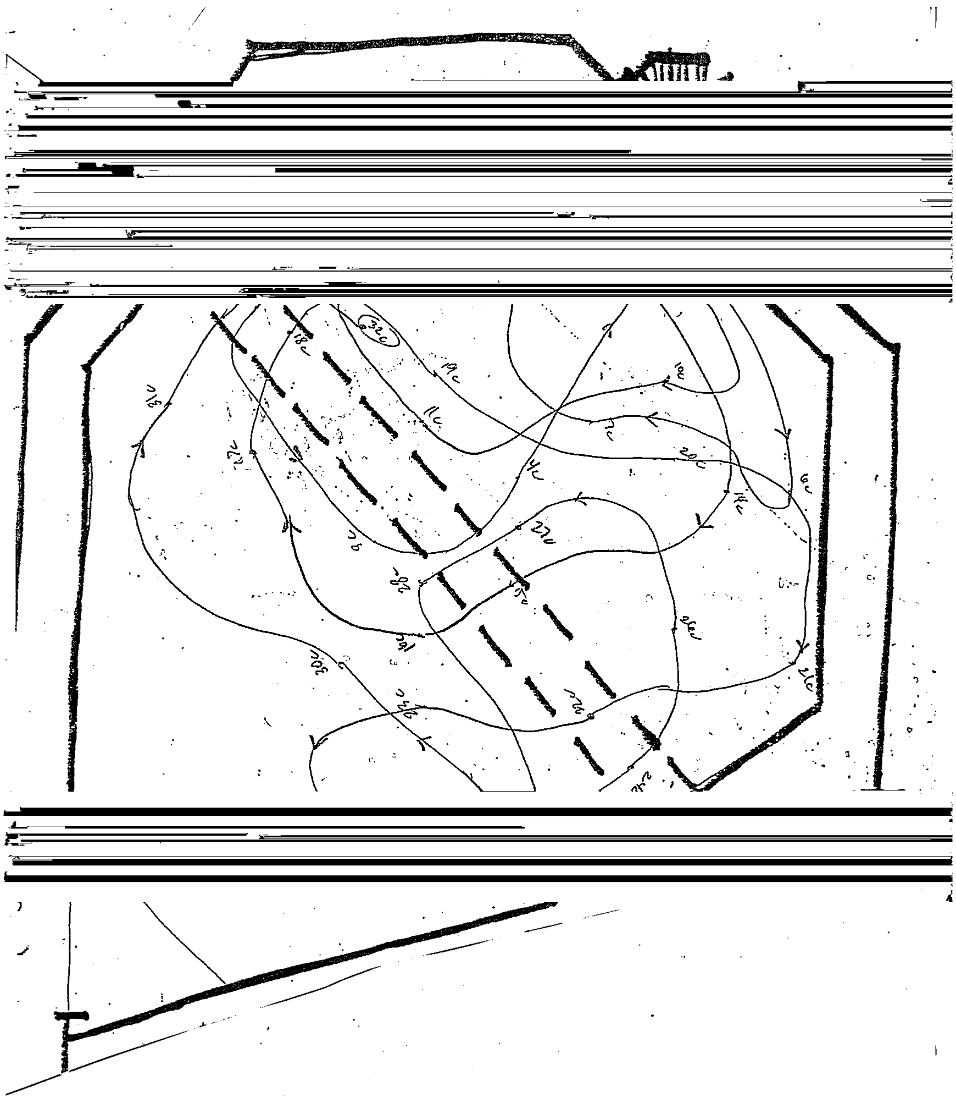

Figure 7. An example of an ethogram showing a relatively unpredictable swimming pattern

Tracking continued when divers entered o199 girm86(e)
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Recommendations

It is recommended that all facilities that dive with captive sharks conduct a thorough risk analysis and continue to monitor the behavior of their sharks to respond to changing safety needs. Changes to exhibits should be effectively planned and involve dive safety personnel. Likewise, facilities that are planning to build a shark exhibit should incorporate the safety needs of divers in the exhibit design stages.

A considerable number of dives are currently being done in captive shark environments, though no central database exists to evaluate these dives. Similarly, there is no central database to track incidents. The development of such a database is recommended and could assist those responsible for risk management decisions. One suggestion received from a survey participant that has merit is the notion of collecting the number of dives and the length of dives with each particular species, as well as the exhibit volume compared to the number of sharks and shark sizes (e.g., two million gallon exhibit with five 80 lb Sand Tigers and 10,000 dives or 6,500 h). A partnership between AZA and AAUS could provide an avenue for the collection and distribution of such statistics.

Anecdotally, we have identified three trends that bear watching. It appears that the use of divers to feed sharks (and/or other shark exhibit inhabitants) is increasing. Targeted feeding can provide excellent husbandry results, as well as a stimulating viewing and educational experience for visitors. Guest diving programs and interpretive dives also appear to be increasing. Tracking this increase in diving activity, along with the policies, techniques and challenges being encountered could provide valuable risk management information.

The ability to rapidly and effectively share information between facilities was described by numerous survey participants as an important contribution to safety. Formal and informal networking should be encouraged and supported by all organizations that dive with these animals. The AZA, AAUS, and the Association of Dive Program Administrators all provide networking tools, but these are each exclusive to their members, and only the AZA specifically targets elasmobranchs with their Shark Taxon Advisory Group (TAG). This group examines the conservation needs of the entire taxa and provides a forum for discussing husbandry, veterinary, ethical and other issues that apply to the entire taxa.

The experience and knowledge of dive safety and animal care staff is perhaps the greatest tool in developing effective dive safety protocol for diving with sharks. Personnel skilled in risk management can save an organization from financial, legal and operational losses, and should be viewed as part of the necessary costs associated with the risk management process. It should also be observed that risk is not just about preventing and managing negative consequences, but also opportunity cost (or the risk of failing to achieve intended outcomes).

The number of captive sharks held (and the accompanying dives) has continued to grow. These programs have provided considerable benefit for the missions of their organizations, including many positive research, educational, and visitor outcomes. The costs as well as the benefits should be factored into any dive program tasked with supporting such outcomes. As pointed out by the Australian/New Zealand Risk Management Standard, "organizations that manage risk effectively and efficiently are more likely to achieve their objectives and do so at lower overall cost" (Standards Australia, Standards New Zealand, 2004).

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Scuba Techniques for the Alaska Monitoring and Assessment Program (AKMAP) of the Aleutian Islands, Alaska

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Abstract

The Aleutian Islands extend over 2,000 km westward from Unimak Island to Attu Island, covering some of the most biologically diverse marine ecosystems in the world. The Aleutian Islands consists of volcanic oceanic islands delineating the North Pacific Ocean from the Bering Sea. Sub-littoral zones mainly consist of rocky substrates that are covered by encrusting coralline alga and a variety of kelps. Fine-grain sediments occur sporadically. Alaska Monitoring and Assessment Program coastal surveys are based on the Environmental Protection Agency, National Coastal Assessment (NCA) procedures using a probabilistic sampling design featuring monitoring of select environmental indicators. Data are integrated from multiple media, including data on water quality, sediment, biological, physical and chemical parameters to assess ecosystem conditions. Traditional NCA uses a multi-tiered sediment triad approach focused on fine-grain sediments, but cannot characterize the status of this region's rocky benthic ecological resource. As a result dive transect and quadrat methods were developed to collect benthic data for rocky and sedimentary benthic habitats of the nearshore Aleutian Islands in 2006 and 2007. This paper focuses on new sampling methodology implemented by scuba divers on hard substrates around the Aleutian Islands.

Keywords: assessment, benthic, contaminants, hard substrate, EMAP, nearshore

Introduction

The Environmental Monitoring and Assessment Program (EMAP) is a national research program led by EPA's Office of Research and Development (EPA-ORD). It is intended to develop the scientific tools and agency partnerships needed to broadly assess the status and trends of significant ecological systems. The goal of EMAP is "to monitor the condition of the Nation's ecological resources to evaluate the cumulative success of current policies and programs and to identify emerging problems before they become widespread or irreversible" (USEPA, 1997; 2004).

The Western States Coastal EMAP is a component of the National Coastal Assessment (NCA), led by EPA to survey the condition of the Nation's coastal resources. As a part of the overall EMAP, the NCA established a five-year effort (1999-2004) to monitor and assess the status and trends of significant estuarine and coastal resources to create an integrated and comprehensive coastal monitoring program among the coastal states (USEPA, 2004). Initially, this five-year effort did not include Alaska.

In 2001, the Alaska Department of Environmental Conservation (ADEC) developed a Cooperative Agreement with the University of Alaska Fairbanks (UAF) and the EPA to join collaboratively in the Western States Coastal EMAP. EPA divided Alaska's coastal regions into five provinces (Southeast, Southcentral, Aleutians, Bering Sea and Arctic). Surveys of two provinces, Southcentral and Southeast, were completed respectively in 2002 and 2004 (USEPA, 2004). EMAPs for Alaska were renamed Alaska Monitoring and Assessment Program (AKMAP). This paper addresses the methodology for the third AKMAP Coastal Survey, the Aleutian Island province. Whereas the first two AKMAP surveys utilized remote sampling gear (grabs and trawls) targeting soft sediments, the Aleutians required different methods due to the predominance of hard substrates. This paper builds upon the Aleutians AKMAP Scope of Work (Dasher et al., 2006).

Setting

From the Alaska Peninsula, the Aleutian Islands extend westward from Unimak Island to Attu Island over a distance of more than 2,000 km (Figure 1). Over 200 Aleutian Islands totaling about 1.1 million hectares form an arc that separates the North Pacific Ocean from the Bering Sea (Banks et al., 2000). Four main island groups comprise the Aleutian Islands: Fox Islands, closest to the Alaska Peninsula, consist of Unimak, Unalaska, and Akutan; Andreanof Islands includes Amlia, Atka, Adak, Kanaga and Tanaga; Rat Islands includes Amchitka, Semisopochnoi, and Kiska; and the western-most Near Islands includes Agattu and Attu. A smaller island group of the Semichi Islands, which includes Shemya, is close to the Near Island group. Almost the entire Aleutian Islands are part of the U.S. Fish and Wildlife Service Alaska Maritime Wildlife Refuge. Further to the west of the Near Islands, the Commander Islands, Russia, continue the island arc and are biologically linked to the Kamchatka shelf and coast. On the southern edge of this submerged mountain range, of which the Aleutian Islands are the exposed peaks, is a curving submarine trench as deep as 7,600 m extending across the North Pacific for 3,200 km from the Gulf of Alaska to Kamchatka Peninsula (Merritt et al., 1977).

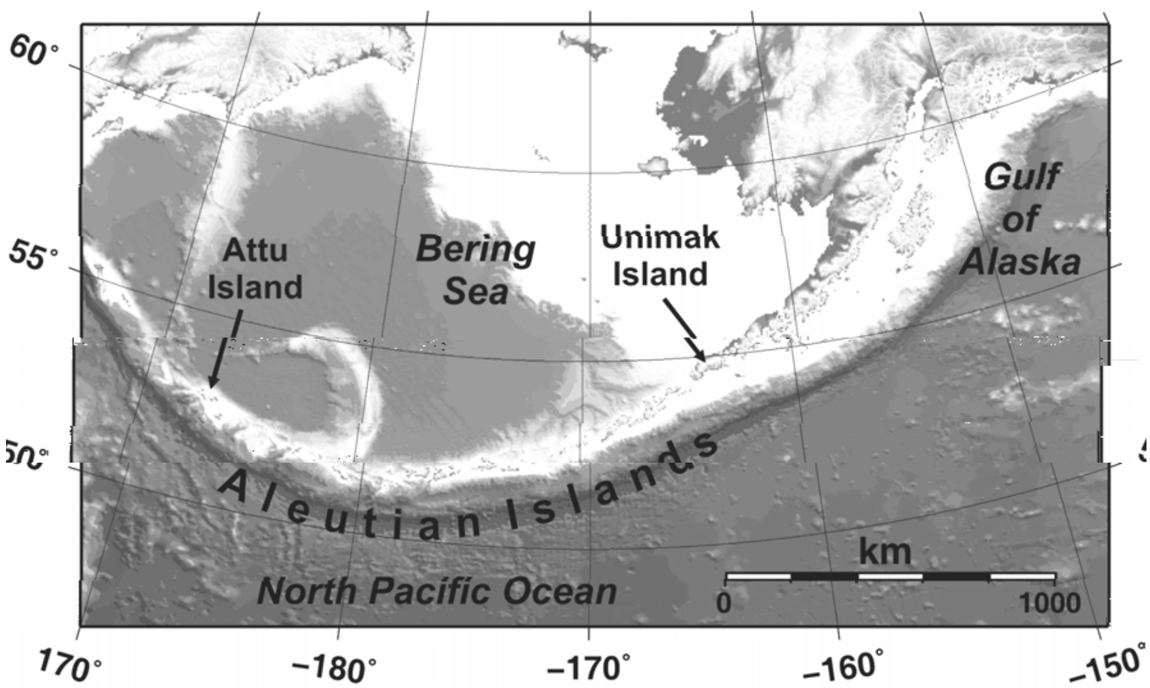

Figure 1. The Aleutian Islands, site of AKMAP investigations in 2006-07, spans nearly 2,000 km from Unimak Island to Attu Island

The Aleutian Islands rose from the volcanic activity resulting from the convergence of the Pacific and North American plates. Today the Aleutian Islands remain one of the most seismically and volcanically active regions in the world. Topographically the region consists of steep hillsides, shoreside cliffs, glacially carved basins, and volcanic peaks as high as 2,080 m. Islands are still being born out in the Aleutians, with Perry Island rising above sea level just over a hundred years ago (Johnson, 2003). The Pacific plate thrusts under the North American plate at a rate of 6-8 $cm \cdot y^{-1}$ and sections of the Aleutian Islands are moving in a westerly direction at 2 to 3 $cm \cdot y^{-1}$ (Consortium for Risk Evaluation with Stakeholder Participation, 2002).

Climatically the Aleutian Islands are located in a maritime zone, which exhibits heavy precipitation, extreme winds, frequent storms, cool summers, and warm winters (Johnson, 2003). The Aleutian low is a name given by meteorologists to the atmospheric pressure system that exerts the most influence on storms in this region during the winter time. During the winter season, when the Aleutian low is strong, an average of three to five storms move eastward along the Aleutian Chain (Johnson, 2003). In the summer, with periods of long daylight and high solar insulation, the Aleutian low is generally weak and the weather relatively benign for this region.

The southern edge the Aleutian Islands is bounded by the strong Alaska Current flowing in a westerly direction, with the easterly flowing Aleutian North Slope Current to the north of the islands. Significant flow from the Alaska Current occurs through 14 passes, providing relatively fresh surface waters and warm subsurface waters to the Bering Sea (Stabeno et al., 1999). Some of the most productive and biologically diverse marine ecosystems occur within the marine zones around the Aleutian Islands. Significant upwelling occurs in this region bringing nutrients to the surface creating a 'green belt' region of high levels of primary and secondary production along the Aleutian Arc (Springer et al., 1996). Numerous species of marine birds and mammals, fish, sea stars, crustaceans, mollusks, sea anemones, sponges and algae live in this region (Johnson, 2003). A small volcanic island, Bogoslof, barely 70 hectares and 100 m in elevation, west of Unalaska, Alaska, provides nesting habitat for nearly a 100,000 seabirds, including the red-legged kittiwakes, which breed at only 4 sites in the world, all in the Bering Sea region (Byrd, 1978).

Today the Aleutian Islands are sparsely populated by humans. Communities occur on only three islands, Unalaska Island (Unalaska), Atka Island (Atka) and Adak Island (Adak). Small US military installations occur on Shemya Island (U.S. Air Force) and Attu Island (U.S. Coast Guard Loran Site). Unalaska is the largest community with a population of about 4,000 residents and is the largest commercial fishing port in Alaska.

Fisheries harvests in this region, Aleutian Islands and Bering Sea, provide over 50% of the U.S. and around 10% of the global marine harvest of fish and shellfish. The Bering Sea harvest of walleye pollock, Pacific cod and other groundfish is worth an estimated $1 billion per year (Bering Ecosystem Study – Bering Sea Integrated Ecosystem Research Program, 2008). The fisheries and seafood processing industries employ thousands of Alaskans and many small communities are completely dependent upon the income from commercial fishing. The Aleutian Islands are within major migratory pathways of many subsistence food species used by the Aleut Natives. Aleut subsistence users harvest fish, marine mammals and other subsistence foods from around the Aleutian Islands. Gathering of subsistence foods is an important part of Aleut culture. Subsistence users in this region are concerned about their subsistence foods, with recent reports of global and local pollution sources and climate change impacts in this region (Johnson, 2003).

Near the end of the 20th Century, large scale ecosystem impacts were documented in the Aleutian Island and Bering Sea region, which have been attributed to climate change and human impacts on the system (Estes et al., 1997; Anthony et al., 1999; Bacon et al., 1999; Barron et al., 2003; Johnson, 2003). Effects of numerous anthropogenic stressors, ranging from commercial fisheries to invasive species, need to be understood if resource managers are to develop and practice adaptive management.

Numerous contaminated areas, consisting principally of petroleum products with some PCBs and heavy metals, exist in this region, with many sites related to World War II and Cold War activities (Stout, 2001). Aleutian Islands involved in World War II were Unalaska, Umnak, Adak, Amchitka, Kiska, Agattu and Attu. In addition to the local contaminant inputs, both marine and atmospherically coupled routes have been identified as pathways for the transport of contaminants from Asian and other Pacific Rim countries (Nilsson et al., 2002). Many are concerned that these contaminants pose potential threats to the marine ecosystem in the Aleutians and Bering Sea regions (Bering Ecosystem Study – Bering Sea Integrated Ecosystem Research Program, 2008).

Amchitka Island, mid-way in the Aleutian Arc, stands as a relic of the Cold War. The United States conducted its largest underground nuclear test (Project Cannikin) in 1971, with a yield of about 5 megatons at the Amchitka Island underground nuclear test area. Preceding that test were Projects Long Shot and Milrow, tests of approximately 80 kilotons and 1 megaton respectively. An estimated 15-16% of the total effective yield of all the U.S. underground nuclear tests occurred at Amchitka (Dasher et al., 2002). Leakage of radionuclides from these tests into the marine environment surrounding Amchitka remains a long-term concern (Burger et al., 2005, 2006, 2007a, b; Jewett et al., 2006). Tritium (^{3}H) is the principal radionuclide that will be present during the first 100 to 150 years after the tests in concentrations that could easily be detected above natural background in sea water near to any seepage points (Watanabe et al., 1991; Landa et al., 1999; Dasher et al., 2002).

A major pacific shipping route transits hundreds of ships a year between the U.S. West Coast and Asia through the Aleutian Islands. Shipwrecks in the past 15 years in this region have spilled over 1.6 million L of oil (ADEC, 2004). The most recent spill in 2004, the M/V *Selendang Ayu*, was carrying 1.7 million L of heavy oil and lost an estimated 1.3 million L of oil, in addition to 66,000 tons of soy beans (Morris, 2006). As the Arctic ice pack recedes due to climate change a major increase in shipping through this region is expected to occur as the Northern Sea routes open up (Johnson, 2003).

Implementation of the AKMAP Coastal Survey in the Aleutian Islands, and future Bering Sea and Arctic provinces is critical to providing ADEC a better understanding of the status and, as future assessments are done, trends in contaminant levels and ecosystem changes in the Alaska region.

Goals and Objective

Two overarching goals of the 2006-07 Aleutian AKMAP were 1) to assess the spatial extent of ecological conditions based on several measured indicators of marine environmental quality, and 2) establish baseline measurements to evaluate future changes in environmental quality or condition. In doing so, specific questions can be further evaluated and potentially answered. For example:

What proportions of the Aleutian Islands' coastal marine measured indicators have contaminant levels that indicate potential ecotoxicological impacts?

What is the prevalence of chemical contaminant loads in fish tissues that indicate exposure to contaminant sources?

What proportions of the Aleutian Islands' marine coastal waters have levels of nutrients, dissolved oxygen, or other tested water quality parameters that indicate poor water quality for resident benthic fish and invertebrates?

The objective of this paper is to present the methodology for sampling flora and fauna associated with the predominantly hard substrates of the nearshore Aleutian Islands.

Program Framework

The Aleutian EMAP survey was based on the principles used for national EMAP programs with a monitoring design that featured multi-tiered, integrated monitoring of selected environmental indicators. Data were integrated from multiple media, including water quality data, sediment data, biological, physical and chemical parameters. This provided a more complete evaluation and assessment of ecosystem 'health' or condition than more traditional monitoring, which typically emphasized single media and a stand-alone approach. Critical to the EMAP assessment process was the sampling design utilizing a probabilistic, stratified-random approach. This approach enabled interpretation of the general ecological health of large areas, such as the Aleutian Islands, to be assessed with a relatively small number of sampling sites.

The Aleutian AKMAP survey took a two-tiered approach. First, the EPA's national protocol, parameters or indicators sampled, analytical laboratory requirements, data QA/QC, data storage, data analysis and reporting methods were followed, whenever possible. Secondly, the rocky benthic habitat required modification in the EMAP methodology that are traditionally used, specifically by dropping benthic trawls and deemphasizing the sediment sampling because soft substrate was thought to be less prevalent. Aleutian AKMAP indicators were adapted and modified from those used in Hawaii EMAP (Nelson et al., 2007) and Guam EMAP (Guam EPA, 2004) to fit the unique environmental conditions and species found in the Aleutian Islands. Although many of the sampling parameters/indicators for the Aleutian AKMAP were carried out by UA divers, some sampling occurred from the research platform by ADEC scientists, i.e., water column and fish. Specific parameters for water column, sediments, fish tissue and benthic community habitat are listed in Table 1. Finally, the transect/quadrat protocol for sampling rocky regions in the Natural Geography in Shore Areas (NaGISA) investigations (Konar and Iken, 2003; Rigby et al., 2007) was modified for the Aleutians. The following methods describe alternate techniques used by divers to assess the predominantly rocky benthic habitat of the Aleutians.

Table 1. Sample parameters for the Aleutian AKMAP survey 2006-2007

Water Column

Measurements with Seabird 25 CTD continuous from surface to bottom on site

- Temperature
- pH
- Conductivity
- Salinity
- Dissolved Oxygen
- Fluorometry
- Photosyntheticly Available Radiation

Measurements 0.5 m below surface, mid-depth, 1 m off bottom

- Chlorophyll *a*
- Salinity
- Ammonia-nitrogen

Nitrate + Nitrite Nitrogen

Total Dissolved Nitrogen

Orthophoshpate

Total Dissolved Phosphorus

Silicate

Total Suspended Solids

Tritium

Sediments (chemical and physical measurements)

Grain Size

Total Organic Carbon

Trace metals: Aluminum, Antimony, Arsenic, Cadmium, Chromium, Copper, Iron, Lead, Lithium, Manganese, Mercury, Nickel, Selenium, Silver, Tin, Zinc

Chlorinated Pesticides: Aldrin, Alpha-chlorodane, Dieldrin, Endosulfan I, Endosulfan II, Endosulfan sulfate, Endrin, Heptachlor, Heptachlor epoxide, Hexachlorobenzene, Lindane (gamma-BHC), Mirex, Toxaphene, Trans-Nonachlor

DDT: 2,4' -DDD; 4,4' -DDD; 2,4' -DDE; 4,4' -DDE; 2,4' -DDT; 4,4' –DDT

Polynuclear Aromatic Hydrocarbons (PAHs): Acenaphthene, Acenaphthylene, Anthracene, Benz(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthene, Biphenyl, Benzo(k)fluoranthene, Benzo(g,h,i)perylene, Chrysene, Dibenz(a,h)anthracene, Dibenzothiophene, Fluoranthene, Fluorene, 2,6-dimethylnaphthalene, Indeno(1,2,3-c,d)pyrene, Naphthalene, Pyrene, 1-methylnaphthalene, 1-methyphenanthrene, 2-methylnaphthalene, 2,3,5-trimethylnaphthalene, 2,6-dimethylnaphthalene

PCB Congeners: PCB Numbers 8, 18, 28, 44, 52, 66, 101, 105, 110/77, 118, 126, 128, 138, 153, 170, 180, 187, 195, 206, 209

Tissues for Contaminants

(whole ground fish, mussel tissue, and macroalgae for organics, trace metals and lipids)

Taxonomic Identification

Total Length (fish)

Weight

Sex (fish)

Otoliths for aging (fish)

Tissue % Lipids

Chlorinated Pesticides: Aldrin, Alpha-chlorodane, Dieldrin, Endosulfan I, Endosulfan II, Endosulfan sulfate, Endrin, Heptachlor, Heptachlor epoxide, Hexachlorobenzene, Lindane (gamma-BHC), Mirex, Toxaphene, Trans-Nonachlor

DDT: 2,4' -DDD; 4,4' -DDD; 2,4' -DDE 4,4' -DDE; 2,4' -DDT; 4,4' –DDT

Trace metals: Aluminum, Antimony, Arsenic, Cadmium, Chromium, Copper, Iron, Lead, Lithium, Manganese, Mercury, Nickel, Selenium, Silver, Tin, Zinc

PCB Congeners: PCB Numbers 8, 18, 28, 44, 52, 66, 101, 105, 110/77, 118, 126, 128, 138, 153, 170, 180, 187, 195, 206, 209

Benthic Community Habitat (hard and soft substrates)

Epi-Infaunal composition/abundance

Substrate type

Aquatic Vegetation

Macroalgal abundance (% cover)

Anthropogenic debris identification

Threatened/Endangered Species

Methods

Sampling Sites

Sample locations were established by the United States Geological Survey National Wetlands Research Center, Gulf Breeze Project Office on a probabilistic sampling scheme based on a target population of waters ≤20 m deep around the Aleutian Islands. The target population was divided into two strata: 1) estuary waters ≤20 m, and 2) open marine waters ≤20 m. Estuary sites comprised 60% of the total selected sites. Hexagon grids of equal-sized cells were used. Fifty base or primary sites were selected with an additional (100% over sample) 50 alternate sites. For this study estuary was defined as any water body that was tidally influenced, saline, and had less than 50% of its perimeter adjacent to the ocean (Heitmuller, 2001). Open marine waters, the second target population stratum, were waters that did not meet the estuary definition. Wetlands and littoral zones were not part of the targeted population. A review of the sites selected indicated the preponderance of sites were likely rocky habitat common to the Aleutian Islands.

Weather conditions in the Aleutians often limit the ability to safely conduct monitoring operations, especially those near shore, during the period from September through May. Therefore, field operations took place within a late-June through July timeframe. The field survey of all 50 sites was scheduled for 2006 and 2007, with about half of the sites planned for each year. Research in both years was carried out aboard the 33-m R/V Norseman. Each site was located from a skiff with the aid of portable global positioning system (GPS) units (Garmin™ GPSmap 60 CS and 76CSx [accuracy <±15 m] with BlueChart electronic nautical charts) and a portable depth sounder (Speedtech Instruments™ Model SM-5) and marked with an anchor and surface float. Sample sites were to remain fixed, but if a sample site was in freshwater, extremely dense kelp beds, located on land or in the intertidal zone due to mapping inaccuracies, below safe diving depths (≤20 m), or otherwise presented a hazard to the dive team, program protocol allowed the site to be moved up to 500 m from the original location in a random direction. If, with the move the sample site still remained in an inappropriate location, the site was dropped and an alternate site was selected.

Alternate Sample Sites

An additional 50 alternate sites were randomly selected from the target population to provide back-up locations in the event that a primary site had to be dropped. The NCA Quality Assurance Project Plan (QAPP) 2001-2004 (Heitmuller, 2001) required that each of the remaining alternate sites be used sequentially, thus, when, say site 1 was dropped then alternate site 51 was added. Only sites that were originally selected from the target population were sampled. A tight sampling budget and the length of the sampling area, over 2,000 km, limited our ability to realistically utilize the next sequential alternate site once a base site had been dropped. While at times, selection of a sequential alternate site may have been located near-by, it was just as likely to be hundreds of miles away. Therefore, within a minimum radius of 7.4 km (4 nautical miles) around the unsampleable site, all alternate sites were randomly grouped and the first randomly selected alternate site was sampled.

Benthic Sampling

The Aleutian Islands AKMAP study was designed to sample and assess both soft and hard substrate habitats. The decision was made to use dive teams to sample both habitats to maximize efficiency of sampling techniques and project logistics. All diving adhered to no-decompression protocol and dive depths were limited to ≤20 m because of the long transit to the nearest hyperbaric medical facility in Anchorage. The dive team consisted of 6-7 divers from the University of Alaska Scientific Diving Program. Divers conducted the following tasks at each site:

- Lay sampling transect, record video, and place quadrats
- Benthic habitat description (dominant macroflora and macrofauna, marine debris, wave impact, threatened/endangered species).
- Soft substrate sampling (benthic infaunal community, sediment for physical characteristics, contaminant analyses, and toxicity).
- Hard substrate sampling (attached macroflora and macrofauna).
- Organisms for contaminants.

All sampling of biota by divers was covered under the Fish Resource Permits (CF-06-066 and CF-07-06) issued by the Alaska Department of Fish and Game.

Site Transect, video and 1-m^2 quadrats

A diver descended the site buoy line, attached a 25-m transect tape with a 5-m rope to the anchor (proximal end of transect) and swam into the current if present, otherwise swam right or left (randomly selected) laying out the tape parallel to shore along the depth contour (±2 m). The first 5 m of the rope nearest the anchor was not surveyed due to potential disturbance by the anchor. Once the tape was laid down, the diver slowly swam above the transect toward the anchor while video recording the transect (see Benthic Habitat Description for more information on the video activity).

Following video recording, quadrats were placed by a second diver at three random points along the transect tape as shown in Figure 2. Each 1 x 1 m (1 m^2) quadrat was placed on the offshore side of the transect regardless of substrate composition. This first sampling point along the transect was a random point within 0-15 m. Points two and three were each placed 5 and 10 m apart from the first randomly selected point. For example, if the random start was at 11 m, the second and third points were 16 and 21, respectfully. Quadrats were placed so that when facing inshore the frame leg closest to the anchor was at the random sampling point along the transect.

Benthic Habitat Description

Once the transect tape was laid out a diver swam from the end of tape to the anchor, videoing approximately a 1 m swath, or 1 x 25 m, recording the habitat, vegetation, macroinvertebrates (large epifauna), fish, marine debris, and threatened/endangered species. Video footage from the transects was intended to provide quantitative information on habitat characteristics and distribution of major taxa. Another diver either recorded on a slate or collected representatives of the dominant mature kelp and macrofauna from 1 m either side of the transect (2 x 25 m). Care was taken to ensure these collections did not occur within the three random 1-m^2 quadrats. All dominant kelp and invertebrate samples were recorded or placed in a mesh bag at depth. At the surface the samples were held in a cooler until returned to the main vessel, where they are refrigerated at 4°C or frozen at -20°C. Before the day ended a brief, qualitative Site Characterization was composed, incorporating information from the video records and dominant organisms recorded or collected. All divers reviewed the Site Characterization to ensure that it had been adequately characterized. An example of a Site Characterization is shown in Table 2.

Table 2. Example of a Site Characterization based on observations made by divers along a 2 x 25-m transect across the substrate

Sampling Location: Yunaska Island

AKMAP Site: AKALE06-0037

Sampling Date: July 17, 2006

Divers: R. Brewer, R. Clark, H. Chenelot, S. Harper, M. Hoberg, S. Jewett

This site was on the south side of Yunaska Island, adjacent to lava bluffs and caves. The target site was actually in about 33 m of water, so we moved it toward shore and set the site in 20 m. The visibility was about 15 m and the temperature was 5°C. The substrate was mixed, with large boulders to coarse black gravel. The boulders were covered with thin coralline red algae, *Lithothamnion* sp., but the gravel had no obvious biota. In fact, the gravel appeared to be quite sterile, presumably reflecting turbulent conditions across the substrate. The dominant algae, besides *Lithothamnion*, were two reds, *Schizymenia* sp. and an unidentified one with a large leathery blade. The dominant macrofauna was the sea urchin *(Strongylocentrotus polyacanthus)*. Dominant fish were the hexagrammids Atka mackerel *(Pleurogrammos monopterigius)*, rock greenling *(Hexagrammos lagocephalus)*, and kelp greenling *(H. decagrammus)*. One small school of about 30 Atka mackerel were seen near bottom.

Fish Community

Hawaii and Guam EMAP studies conducted quantitative fish censuses by divers along a 4 x 25-m strip transect. However, after several dives to rehearse this fish sampling protocol, the Aleutian AKMAP team determined fish densities were too low and kelp forests were often too dense to justify the standard EMAP fish census along a strip transect. As an alternative, fish were recorded by video along a 1 x 25-m transect when conducting the Benthic Habitat Description (see above).

Soft Substrates

Whenever soft sediments (sand) were encountered within the 1-m^2 quadrats, sediment and infauna were sampled. Adjacent to the first 1-m^2 quadrat that contained sand, another 1-m^2 quadrat was placed inshore of the transect to sample soft sediment (Figure 2) for the parameters listed in Tables 1 and 2. Any transect point on soft substrate had to be sufficiently large so that one 1-m^2 quadrat frame could be placed on the inshore as well as the offshore sides of the transect. Thus, for soft substrates, a transect had one 1-m^2 quadrat frame for sediment on the inside of the transect and up to three 1-m^2 quadrat frames for infauna on the outside of the transect. Sediment samples were always collected before the infauna samples to ensure no disturbance to the fine sediments.

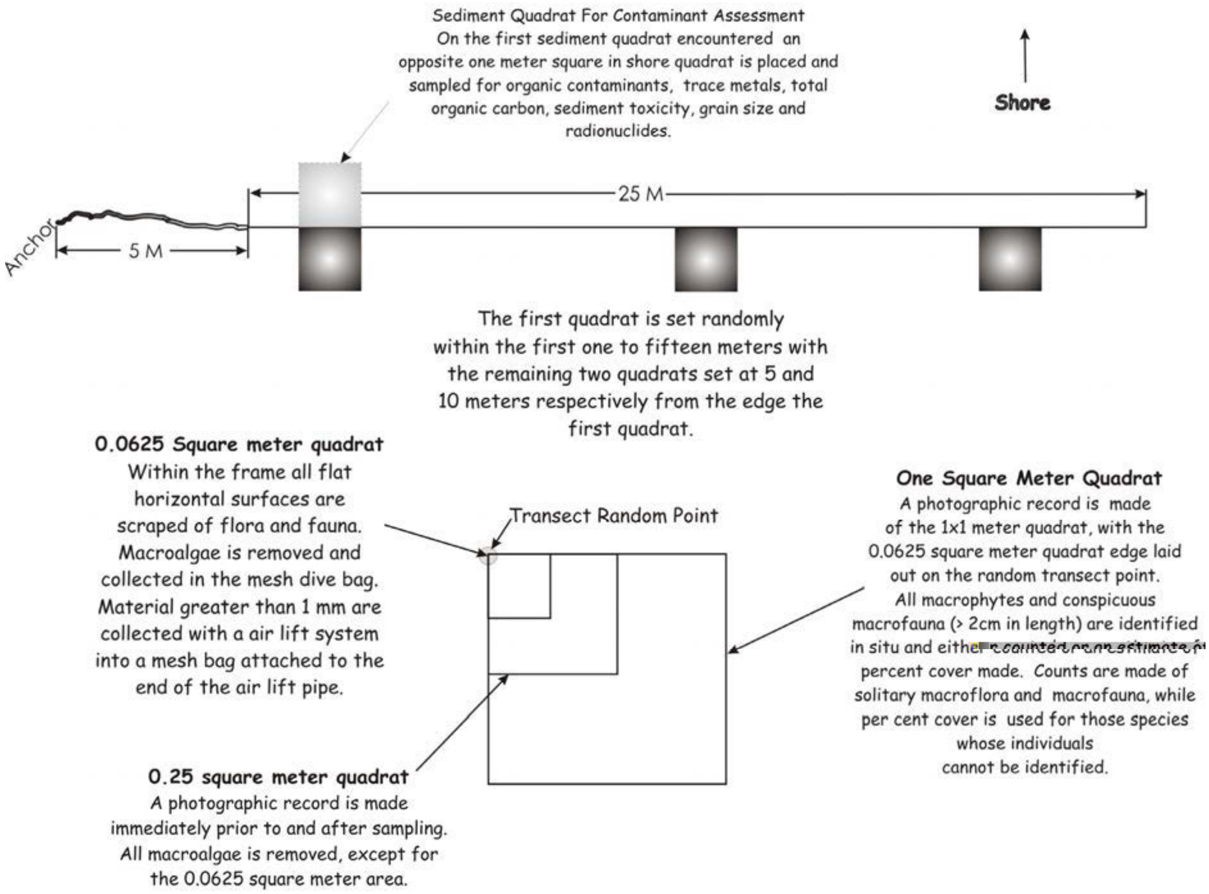

Figure 2. Hypothetical site sampling layout for scuba divers in the Aleutian AKMAP 2006-07

Using premarked jars sediment samples were collected in the 1-m^2 quadrat for inorganic (INO) and organic contaminants (ORG), grain size (GRN), radionuclides (RAD), total organic carbon (TOC), and sediment bioassays (BIO). The following collection gear was used by two divers:

- 1-500 mL ICHEM™ jar (ORG)
- 1-125 mL HDPE jars (INO)
- 3-125 mL glass jars (GRN + RAD + TOC)
- 2-2 L plastic containers (BIO)
- 2 collection bags for samples

All sediment samples were taken to a sediment depth of 5 cm and from an undisturbed portion of the 1 m^2 quadrat. All samples were placed in a mesh bag at depth. At the sea surface the samples were held in a cooler until returned to the main vessel, where they are refrigerated at 4°C or frozen at -20°C.

Soft Substrate Benthic Sampling

Within each 1-m^2 offshore quadrat a 25 x 25 cm (0.06 m^2) quadrat was placed for infauna sampling. The smaller quadrat was placed so that when facing inshore the frame leg closest to the anchor was at the transect sampling point (Figure 2). The entire 0.06 m^2 was removed to a substrate depth of 10 cm by a diver-operated airlift with a 0.5-mm mesh bag. The retained portion of the airlift, mainly live biological material, was preserved in 10% buffered formalin and labeled. Three benthic samples were collected on each site transect.

Hard Substrate Benthic Sampling

Whenever hard substrate (gravel, cobble, boulder, bedrock) were encountered within the transect, divers collect biota from three quadrat sizes at up to three random transect points along the offshore side of the transect (see Figure 2 and methods in *Site Transect, video and 1-m^2 quadrats*). At each random point along the transect three different quadrat sizes (1 x 1 m [1 m^2], 50 x 50 cm [0.25 m^2], and 25 x 25 cm [0.06 m^2]) were nested (see Figure 2). Quadrats were placed so that when facing inshore the frame leg closest to the anchor was at the transect random sampling point.

Still photographs (Canon PowerShot S80 digital camera) of the 1 x 1 m, 50 x 50 cm, and 25 x 25 cm quadrats were taken to enable a more detailed analysis of the habitat-diversity associations. If conditions did not permit such a photographic record to be made (e.g., poor visibility) then a hand-drawn map was constructed as an alternative.

Within each 1 x 1 m quadrat, a photographic image record was made immediately prior to sampling. All macrophytes and conspicuous macrofauna (>2 cm length) within the 1 x 1 m quadrat were identified in situ, and either counted or an estimate of percent cover was made using a standard technique. Counts were made of solitary macroflora and macrofauna, while percent cover was used for species whose individual could not be differentiated (e.g., colonial organisms).

Within each 50 x 50 cm quadrat, a 25 x 25 cm quadrat was placed (always the same position within the larger sample). In each 50 x 50 cm quadrat, a photographic image record (digital) was made immediately prior to and after sampling. Within the 50 x 50 cm quadrat all macroalgae were completely removed, except for the 25 x 25 cm area. The algae collected from within the 25 x 25 cm quadrat was added to 50 x 50 cm quadrat after all invertebrates were removed from the algal thally. This 50 x 50 cm sample was taken in order to ensure sufficient algal reference material to support the *in situ* observation.

All macrophytes and fauna within the 25 x 25 cm quadrat were carefully removed using knife or hand scraper and an airlift system with 1 mm mesh bags. In each 25 x 25 cm quadrat, a photographic image record was made immediately prior to and after sampling. All portions of the sample were separately fixed and preserved 10% buffered formalin. A wet weight was determined to the nearest gram for red, green, and brown algae within each sample. A detailed algal species list was recorded for each site.

Organism Sampling for Contaminants

The organisms collected for contaminants by divers included macroalgae (*Druehlia [Alaria] fistulosa*), horse mussels (*Modiolus modiolus*) and blue mussels (*Mytilus trossulus*). Macroalgae was collected at the dive site, if available within the sampling transect, rinsed in site water, weighed, placed in plastic, ziploc™ bags and frozen at -20°C. Horse mussels (*Modiolus modiolus*) and blue mussels (*Mytilus trossulus*) also, if present, were collected from the dive site, rinsed in site water and stored at -20°C. Fish were collected for contaminants by hook and line from the research vessel. The dominant fish mainly included greenlings (*Hexagrammos lagocephalus* and *H. decagrammus*), Irish lords (*Hemilepidotus jordani* and *H. hemilepidotus*), and rock sole (*Lepidopsetta polyxystra*)

Results and Discussion

The 50 sites that were sampled in 2006-2007 ranged from Tigalda Island in the east to Attu Island in the west (Figure 3; Table 3). A total of 35 primary sites and 15 alternate sites were sampled over a period of 63 field days. Bad weather forced no diving on only three days. Most of the sites sampled consisted of hard substrates (35 sites), i.e., gravel, cobble, boulder, or bedrock. There were 11 sites with sand only and three sites with a mixture of sand and hard substrates. Seven divers made 440 dives for 308 hours of bottom time, with no diving incidences. Samples analyses are underway at various laboratories and a report is anticipated in late 2009.

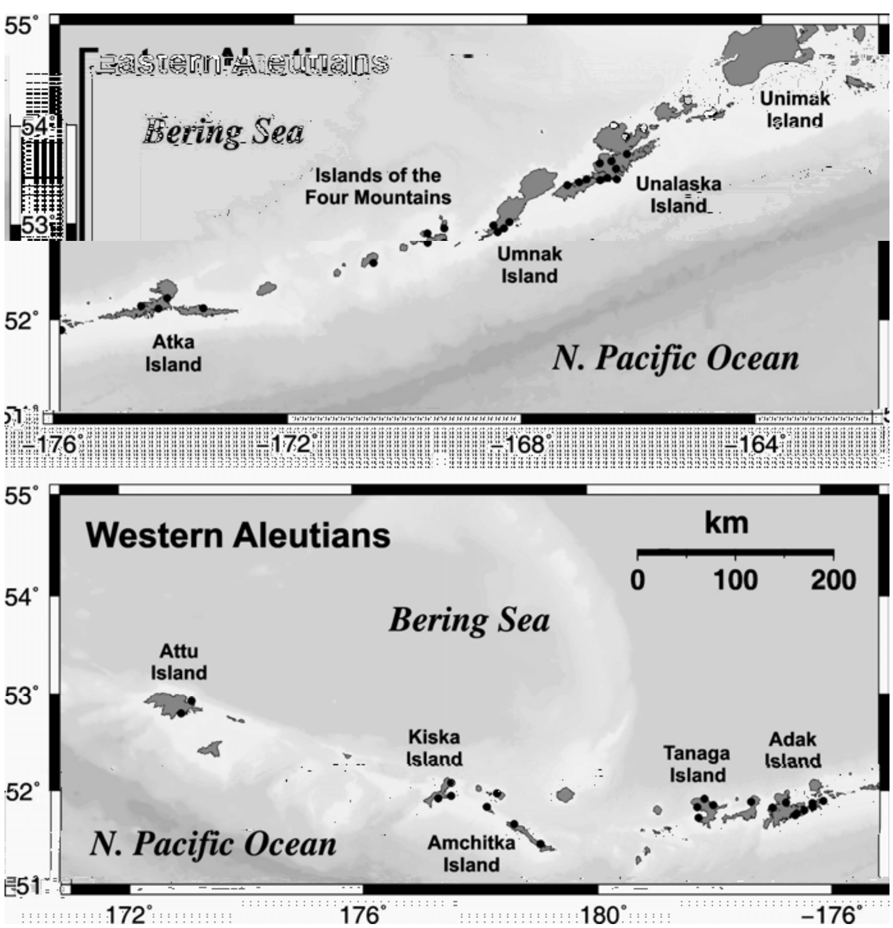

Figure 3. Fifty random sites sampled by scuba divers in the Aleutian AKMAP 2006-07

Table 3. Sampling site information for Aleutian Islands AKMAP, 2006-07

Sequence#	Date	Site ID	Island, Bay	Depth (m)	Substrate***	Lat and Long (NAD 83)
1	07/03/06	AKALE06-0039	Tigalda Is., Tigalda Bay	6	BO	N54 07.510 W165 06.633
2	07/04/06	AKALE06-0026	Tigalda Is., Avantanak Strait	15	BO	N54 06.400 W165 12.675
3	07/05/06	AKALE06-0002	Akun Is., Heliathus Cove	15	C/BO	N54 14.400 W165 32.590
4	07/07/06	AKALE06-0021	Unalaska Is., Unalaska Bay, Nateekin	17	S	N53 53.490 W166 36.709
5	07/08/06	AKALE06-0044	Unalaska Is., Driftwood Bay	13	BE	N53 59.689 W166 49.116
6	07/09/06	AKALE06-0007	Unalaska Is., Makushin Bay, Naginak	15	S	N53 38.292 W166 51.133
7	07/10/06	AKALE06-0020	Unalaska Is., West Arm Scan Bay	16	S	N53 36.978 W167 03.339
8	07/12/06	AKALE06-A0018	Unalaska Is., Aspid Bay	12	S/G/C	N53 25.560 W167 24.490
9	07/13/06	AKALE06-0012	Unalaska Is., Kismaliuk Bay	11	S/G/C	N53 27.311 W167 16.688
10	07/14/06	AKALE06-0027	Unalaska Is., Peacock Pt.	14	BO/BE	N53 23.703 W167 36.599
11	07/15/06	AKALE06-0011	Carlisle Is., Carlisle Pass	7	BO	N52 53.973 W169 59.730
12	07/15/06	AKALE06-0024	Kagamil Is., Kagamil Pass	17	S	N52 57.124 W169 42.804
13	07/16/06	AKALE06-0010	Chuginadak Is., Samalga Pass	17	BO	N52 48.289 W169 42.526
14	07/17/06	AKALE06-0037	Yunaska Is.	18	G/BO	N52 35.604 W170 38.245
15	07/18/06	AKALE06-A0034	Umnak Is., Cape Udak	14	BO	N52 54.715 W168 47.852
16	07/18/06	AKALE06-0025	Umnak Is., Traders Cove	11	BO/BE	N52 57.091 W168 41.546
17	07/19/06	AKALE06-0043	Umnak Is., Lookout Pt.	14	BE	N53 01.034 W168 35.947
18	07/20/06	AKALE06-A0012	Unalaska Is., Kuliliak Bay	10	BE	N53 26.897 W167 03.042
19	07/20/06	AKALE06-A0006	Unalaska Is., Eagle Bay	10	G/C/BO	N53 27.980 W166 55.419
20	07/21/06	AKALE06-0023	Unalaska Is., Usof Bay	15	BO	N53 27.247 W166 45.426
21	07/21/06	AKALE06-0028	Unalaska Is., Usof Bay	17	S	N53 33.812 W166 46.505
22	07/22/06	AKALE06-0030	Unalaska Is., Beaver Inlet, Kisselen B	17	G/C/BO	N53 42.734 W166 35.131
23	07/22/06	AKALE06-A0002	Unalaska Is., Kalekta Bay	10	S	N53 58.234 W166 18.053
24	06/25/07	AKALE07-0019	Attu Is., Chichagof Harbor	8	G/BO	N52 55.861 E173 15.295
25	06/26/07	AKALE07-0004	Attu Is., Temnac Bay	11	S	N52 48.164 E173 04.487
26	06/29/07	AKALE07-0017	Kiska Is., Vega Bay	14	BE	N51 54.869 E177 26.865
27	06/29/07	AKALE07-0046	Little Kiska Is., E of Yug Pt.	16	BE	N51 56.422 E177 40.022
28	06/30/07	AKALE07-A0048	Kiska Is., Haycock Rock	11	BO	N52 04.640 E177 40.253
29	07/01/07	AKALE07-0045	Little Sitkin Is., near Finger Pt.	11	BO	N51 58.201 E178 27.184
30	07/01/07	AKALE07-A0031	Rat Is.	14	BO	N51 49.594 E178 16.676
31	07/02/07	AKALE07-0016	Amchitka Is., Kirilof Bay	7	C/BO	N51 25.850 E179 13.403
32	07/03/07	AKALE07-DD0002	Amchitka Is.	15	BE/BO	N51 38.560 E178 44.928
33	07/05/07	AKALE07-A0016	Tanaga Is.	8	BE/BO	N51 49.288 W178 07.256
34	07/06/07	AKALE07-0042	Tanaga Is.	16	BO/S	N51 54.750 W178 00.187
35	07/06/07	AKALE07-0050	Tanaga Is.	13	BE/BO	N51 50.697 W177 51.210
36	07/07/07	AKALE07-DD0003	Tanaga Is.	12	BE/BO	N51 42.615 W178 06.201

37	07/08/07 AKALE07-A0028	Kanaga Is.	15	BO	N51 52.872 W177 12.279
38	07/09/07 AKALE07-0005	Adak Is., Bay of Islands	16	BO	N51 49.023 W176 50.385
39	07/10/07 AKALE07-A0021	Adak Is., S. Kagalaska Strait	11	C/BO	N51 45.358 W176 25.437
40	07/10/07 AKALE07-0047	Adak Is., SE	10	C/BO	N51 44.106 W176 27.229
41	07/12/07 AKALE07-A0014	Adak Is., Kuluk Bay, Gannet Rocks	16	BO/BE	N51 52.215 W176 36.383
42	07/12/07 AKALE07-A0019**	Kagalaska Is.	13	S/BO	N51 47.549 W176 17.405
43	07/13/07 AKALE07-A0005	Little Tanaga Is., Chisak Bay	10	S	N51 49.603 W176 09.001
44	07/14/07 AKALE07-0018	Little Tanaga Is., Umak Pass	14	C/BO	N51 52.005 W176 09.232
45	07/14/07 AKALE07-0013	Umak Is., Umak Bight	8	C/BO	N51 53.425 W175 58.282
46	07/15/07 AKALE07-0031	Atka Is., Deep Bay	9	BO/BE	N52 08.454 W174 36.409
47	07/16/07 AKALE07-0032	Atka Is., Vasilief Bay	19	S	N52 06.909 W174 18.951
48	07/17/07 AKALE07-0008	Atka Is., Nazan Bay	9	S	N52 13.417 W174 09.718
49	07/18/07 AKALE07-0035	Amlia Is., S. of Cape Idalug	14	S	N52 07.179 W173 32.484
50	07/21/07 AKALE07-0029	Umnak Is., Nikolski Bay	13	C/BO/BE	N52 58.969 W168 52.419

* Alternate site for AKALE07-0006

** Original coordinates for AKALE07-A0019 placed it on land.

*** Substrate categories (subjective, not based on Phi size): S=sand; C=cobble; G=gravel; BO=boulder; BE=bedrock

The shallow, mixed-substrate, subtidal region of Aleutians Islands required sampling techniques much different from the Hawaii and Guam EMAP studies (Table 4). The protocol implemented in this study was based, in part, on our knowledge of the shallow subtidal communities in the Rat Islands [Amchitka and Kiska] (Jewett et al., 2006). Because we knew that these communities were dominated by encrusting coralline red algae, kelps and diverse sessile epifauna, the standard EMAP 4 x 25-m area was anticipated to be too time-consuming to assess kelp, epifauna, and fish. Furthermore, fish were seldom seen within a 4 x 25-m area when the transect tape was being laid out. Ordinarily fish are assessed on the first swim because they may be subsequently frightened from the area. However, because fish density was so low we determined that it was appropriate to conduct the video after the transect tape had been laid down, so as to use the tape as an area guide. Furthermore, some sites had dense macroalgae canopy and understory, making quantification of biota very difficult. So, rather than take time to assess fish along the 4 x 25-m area, we assessed fish, along with kelps and epifauna, in the 1 x 25-m video. Organisms on or over hard substrates often included brown algae (*Agarum* spp., *Druehlia (Alaria) fistulosa*, *Cymathere triplicata*, *Desmarestia* spp., *Laminaria yezoensis*, *Nereocystis lutkeana*, *Saccharina* spp., and *Thalassiophylum clathrum*), green algae (*Codium ritteri*, *Ulva* spp.), red algae (*Constantinea* spp., *Fauchea* sp., and encrusting corallines), invertebrates such sponges, sea anemones, giant Pacific chiton (*Cryptochiton stelleri*), horse mussel (*Modiolus modiolus*), blue mussel (*Mytilus trossulus*), rock jingle (*Pododesmus macrochisma*), Oregon triton (*Fusitriton oregonensis*), giant octopus (*Enteroctopus dofleini*), sea urchin (*Strongylocentrotus droebachiensis* and *S. polyacanthus*), and various sea stars. Although fish were less common, they included the territorial demersal greenlings (*Hexagrammos lagocephalus* and *H. decagrammus*), Irish lords (*Hemilepidotus jordani* and *H. hemilepidotus*) and dusky rockfish (*Sebastes ciliatus*).

The transect and quadrats methods ultimately used were modified from the procedure used in the international NaGISA investigations (e.g., Konar and Iken, 2003). The NaGISA approach was designed for seagrass and macroalgal communities on mixed substrates in intertidal and shallow subtidal to depths of 20 m. Whereas NaGISA methods called for five replicates of nested quadrats along a 30-m transect per depth stratum, we decided on three replicates along a 25-m transect. In view of all the diving tasks required at each site, three replicates were more appropriate for our dive time. Using the NaGISA methods was deemed most appropriate for sampling in the nearshore Aleutians where kelp canopy and understory and sessile organisms predominates.

Table 4. Comparison of EMAP techniques used in studies on mixed substrates

Technique	*Aleutian Islands*	*Hawaii**	*Guam***
Site placement adjustment	≤500 m	Not available	~93 m
Site accuracy	<± 15 m with GPS	± 3 m with DGPS	± 3 m with DGPS
Water column sampler	Seabird 25 CTD	Hydrolab H_2O Datasonde	YSI Datasonde
Sediment depth	5 cm	15 cm	5 cm
Infaunal sampler area	3-0.06 m^2 quads to 10 cm	1-0.0098 m^2 core (11.2 cm dia) to 5 cm	1-0.0045 m^2 core (7.6 cm dia) to 10 cm; 0.02 m^2 grab
Macroalgae/macrofaunal cens	1 x 25 m video; 3-1 m^2 quads on 25-m transect for counts and % cover of sessile forms	4 x 25 m - count macrofauna >2 cm; 6-1 m^2 quads on 25-transect for % cover of sessile forms	4 x 25 m - count macrofauna >2 cm; 6-1 m^2 quads on 25-m transect for % cover of sessile forms
Fish census	1 x 25 m video	4 x 25 m	4 x 25 m
Tissues for contaminants	Fish by hook and lin macroalgae, horse mussels, blue mussels by divers	Sea cucumbers by divers	Sea cucumbers and crabs by divers

* Nelson et al., 2007

**Guam EPA, 2004

The depth of penetration for sediment sampling, 5 cm, was the same as used in the Guam EMAP study (Guam EPA, 2004) on mixed substrates, but not as deep (15 cm) as in the Hawaii EMAP study (Nelson et al., 2007). Traditional sediment sampling for granulometry and contaminants target the surficial sediments to 5 cm deep, because these are the depths associated with most infauna, epifauna and demersal fish (Jewett et al., 1999; 2006).

Whenever a site had soft sediments infaunal sampling was conducted within up to 3-0.0625 m^2 quadrats or a total of 0.18 m^2 per site, to a sediment depth of 10 cm. This is considerably greater site coverage than in other EMAP studies. Hawaii and Guam sampled infauna at each site using a single core. Hawaii used a 0.0098 m^2 core to a sediment depth of 5 cm and Guam used a 0.0045 m^2 core to a depth of 10 cm (Table 4). So, infaunal sampling in this study covered 18 times more area per site than Hawaii or 40 times more area per site than Guam. Guam also used a 0.02 m^2 grab if conditions were unsafe for sampling by divers. We rationalized that the additional infaunal sampling coverage was warranted because few soft sediments sites were anticipated over the vast Aleutian Islands that spans nearly 2,000 km. Furthermore, a single core, whether 0.0098 m^2 or 0.0045 m^2, seemed too small to characterize the infaunal community.

As noted above, macroalgae and macrofauna were assessed in the 1 x 25-m video transect, however, more detailed quantification (counts and % cover) was conducted from 3-1 m^2 quadrats per site. Nevertheless, this is still less site coverage than occurred in the Hawaii and Guam EMAP investigations. In both of those studies, counts of large (>2 cm) macrofauna were made along 4 x 25-m transects, in addition to % cover of sessile forms within 6-1 m^2 quadrats per site (Table 4). Once again, the reduced site sampling area in the current study, in comparison to Hawaii and Guam studies, stems from the dense coverage of diverse macroalgae and macrofauna in the Aleutians.

Other EMAP monitoring studies of soft-substrate environments targeted trawl-caught fish for contaminant analyses. For hard-substrate environments Guam EMAP used a sea cucumber (*Holothuria atra*) and crabs (*Scylla serrata* and *Uca* sp.) and Hawaii EMAP used sea cucumbers (*Holothuria atra* and *H. whitmaeri*) for tissue contaminants (Table 4). In the Aleutian Islands both the Sea Otter (*Enhydra lutris*) and Stellar Sea Lion (*Eumetopias jubatus*) are considered threatened and there is a concern with anthropogenic contaminants in the food web accumulating in these apex animals (Estes et al., 1997; Bacon et al., 1999; Barron et al., 2003). Use of sea anemones, albeit a different genera than the sea cucumber (*Holothuria* sp.*),* may have maintained some biological comparability to Hawaii and Guam studies, but it is not a key species in the nearshore food web we were assessing and there is a lack of information on the usefulness of sea anemones as bioindicators for trace metals and organic contaminants. This guided our selection of organisms to be sampled, resulting in the Aleutian AKMAP monitoring using fish collected by hook and line, and dominant macroalgae and mussels collected by divers for tissue contaminant analyses.

Ideally, EMAP methods designed for hard substrates should be similar for comparative purposes, just like the methods designed for soft substrates. Complete similarity cannot be maintained, such as uniformity of type of organisms sampled, when attempting to compare such different systems as tropical coral reefs to arctic cold water hard-substrate near-shore kelp beds. This investigation in the Aleutian Islands highlighted the need for adaptability in sampling methods. It is quite probable that the next EMAP study done elsewhere on hard substrates will also require adaptive methodology specific to the particular region.

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Dive Logistics of the Turner to Wakulla Cave Traverse

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Abstract

Since its official inception in 1990, the mission of Global Underwater Explorers' Woodville Karst Plain Project (WKPP) has been to explore, survey, and protect the underwater cave systems of North Florida's Woodville Karst Plain. On December 15 2007, the WKPP exploration team of Jarrod Jablonski and Casey McKinlay conducted a seven mile underwater traverse between Turner Sink (Leon Sinks Cave System) and Wakulla Springs to verify the connection the team made between the two systems earlier in the year. With no possible exit to the surface along the way, the journey took 6.5 hours at a depth of 300 ffw (91 mfw) followed by approximately 14 hours of decompression. An estimated 50 international team members were present to support this dive, with tasks including equipment staging, decompression monitoring, and surface management. Following established WKPP protocol, two-person support teams worked in three hour shifts over a 24 hour period. All WKPP equipment, gasses and procedures are standardized to maximize efficiency and flexibility. Roles and experience levels vary, but support teams are broadly interoperable because project members share common equipment, gasses, and training. Continuous support was present in the water and on the surface to handle potential injuries or problems, food, drink and additional gas supplies. Using this standardized approach, the WKPP successfully completed the traverse and set two world records, including the longest cave dive between two entrances and the longest traverse in a deep cave. The connection of the two cave systems yields the Wakulla-Leon Sinks Cave System, currently the longest underwater cave in the United States and the fourth largest in the world at a total of 28 miles of surveyed passages.

Keywords: cave diving, exploration, rebreather, trimix

Introduction

Project History

The Woodville Karst Plain (WKP) is a 270 mi^2 (700 km^2) region stretching from the southern edges of Tallahassee, Florida to the Gulf Coast (Figure 1). Characterized by a layer of unconsolidated sands overlying a thick sequence of carbonate deposits (Hendry and Sproul, 1966), the WKP is distinguished by the presence of sinkholes, karst windows, sinking streams and large springs (Werner, 1997). The WKP contains seven of Florida's 27 first magnitude springs, defined as those that discharge at least 100 cubic feet of water per second (cfs), or about 64.6 million gallons per day (mgd). These first magnitude springs include Wakulla Springs, Spring Creek, Indian Springs, and Shepard Springs (Rupert and Spencer, 1988) (Figure 2).

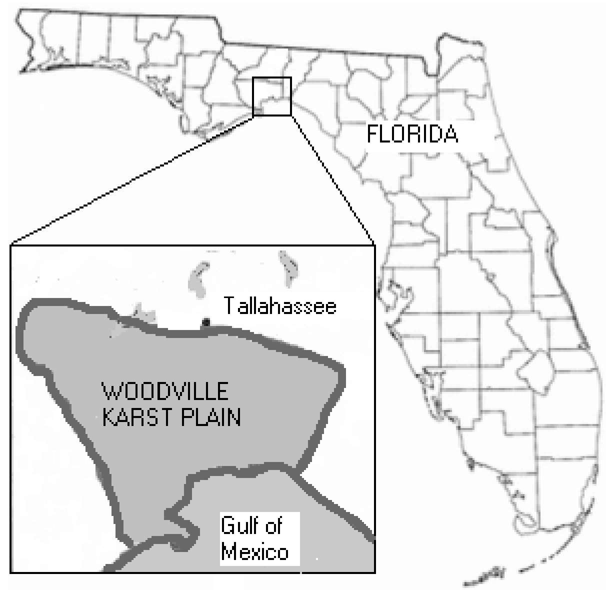

Figure 1. Woodville Karst Plain Geological Area

Underwater cave exploration has taken place within the WKP since the 1950s; however, it was not until the mid 1980s that substantial progress was made. Technological advances, improved team organization, and a better understanding of decompression physiology had to be reached in order to overcome the impedances experienced by past exploration teams given the depth and horizontal extent of the caves. In 1985, a team of divers undertaking exploration at Sullivan Sink cave started what is currently known as the WKPP. Implementing a standardized equipment configuration and team-oriented system, these divers were able to explore new cave at an increased rate.

The WKPP received official project status recognition from the National Speleological Society in 1990. Project members have since continued to expand on the initial efforts of the Sullivan Sink group through the development of new technology (i.e., extended range diver propulsion vehicles), decompression procedures, and exploration protocol. Between 1990 and 2007, approximately 300 volunteers have participated as members of the WKPP. International participants from Germany, Singapore, Japan, Italy, Sweden, France, Australia and the United Kingdom have frequently attended project events. At any given time the team roster has included exploration and support divers, surface management personnel, medical consultants, videographers, photographers, and scientific collaborators. A standardized approach to equipment, training, and on-site procedures has been established within the team in order to maximize efficiency and safety.

The average WKPP exploration dive from 1990 through the end of 2007 required a total of 15 hours (bottom time plus decompression), with the longest dive exposure running 26.5 hours. With these exposures, a substantial time, financial, and physical commitment was necessary for everyone involved. Team personnel have invested a total of 300,000 person-hours on a volunteer basis over the course of 300 scheduled events. Financially, an estimated total of US$4,500,000 has been personally contributed by project members stemming from direct costs incurred and equipment purchases. In addition to diving labor, an average of 15,000 lbs (6,818 kg) of equipment has been moved in and out of the water during a single team event (McKinlay, 2007).

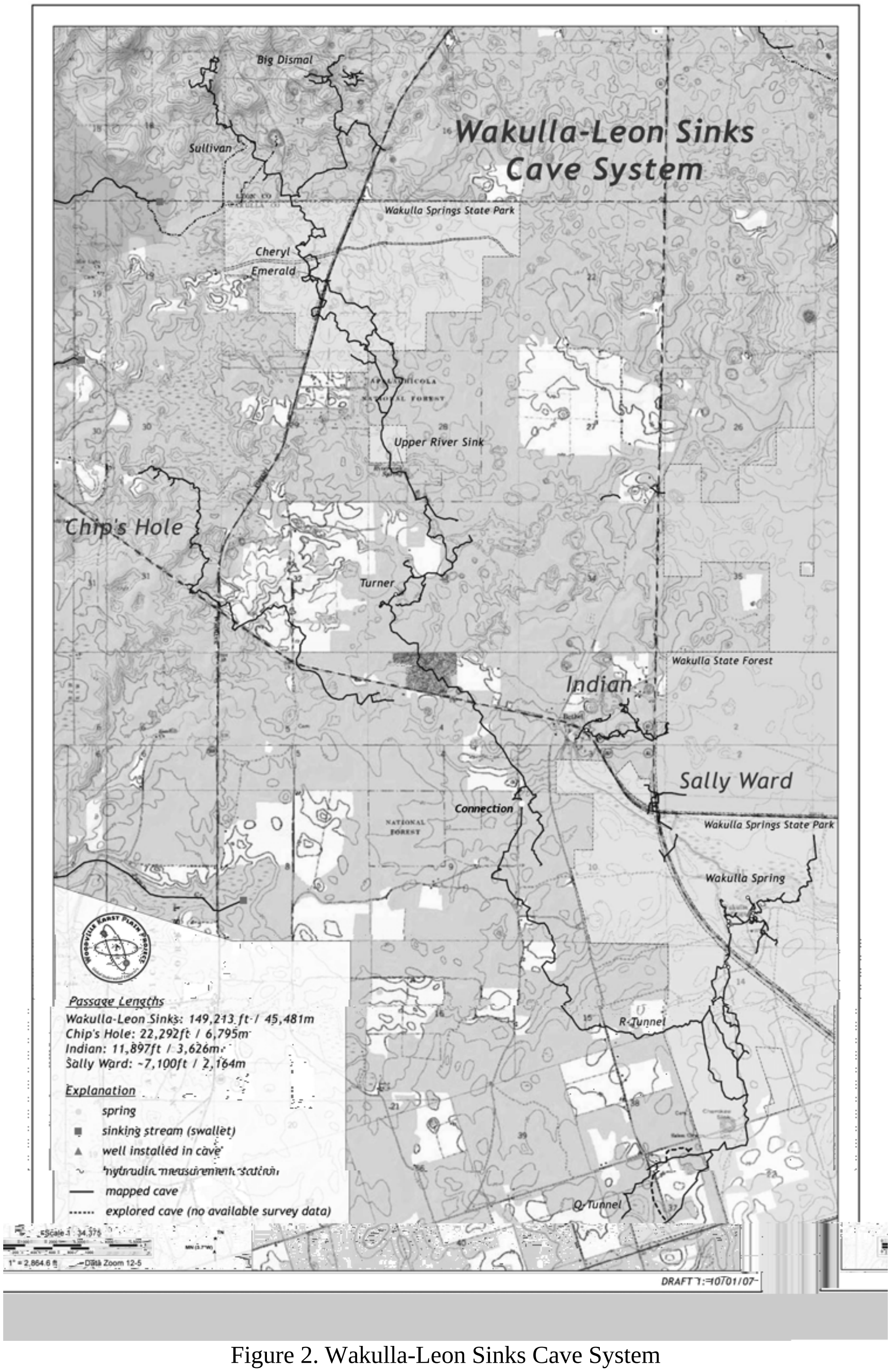

Figure 2. Wakulla-Leon Sinks Cave System

A non-profit affiliate of Global Underwater Explorers (GUE), the WKPP established an ongoing mission to explore, survey, connect, and protect the flooded cave systems located within the WKP. Through partnerships with state, federal, and private landowners, the project has been positioned to promote public awareness, education, and scientific discovery through research support. Using information collected by the WKPP, resource managers, policy makers, landowners, and researchers have been able to formulate necessary and responsible land use decisions to protect these invaluable water resources for future generations (McKinlay, 2007).

The Turner-Wakulla Connection

Nearly two decades of exploration led up to the final connection of the Wakulla Springs and Leon Sinks cave systems. Following a number of successful exploration dives at Turner Sink (one of the entrances to the Leon Sinks cave system) during 2006, the team returned there in May 2007 to attempt a Wakulla-Turner connection. Unexpectedly, the cave passage ended in every direction that the divers attempted to explore. Saturday June 23, 2007, the team of Jarrod Jablonski and Casey McKinlay pushed beyond the end of the "R Tunnel" line of Wakulla Springs cave (starting 16,500 ft (5,029 m) from the entrance). Their effort resulted in the exploration of 7,120 ft (2,170 m) of new cave passage. In addition to significantly lengthening the amount of surveyed cave passage at Wakulla Springs, project hydrogeologist Dr. Todd Kincaid also saw that the new passage was trending towards the Leon Sinks cave system and close to a connection.

Saturday July 28, 2007, McKinlay and Jablonski entered the Leon Sinks cave system through Turner Sink. According to the map, the plotted intersection between Wakulla Springs and Leon Sinks was located approximately one mile from the end of the surveyed passage of downstream Turner. If the survey data was correct, the exploration team would encounter the connection tunnel 11,000 ft (3,353 m) away from the entrance of Turner.

Exploration diver Casey McKinlay described the dive leading up the actual connection to Wakulla Springs (McKinlay 2007):

"Approximately 11,200 ft (3,413 m) downstream of Turner Sink Jarrod and I entered a massive room with floor to ceiling relief of 120 ft (37 m) + and wall to wall distance in excess of 100 ft (30 m) . The floor was 300 ft (91 m) + and the line was positioned on the left side of the room. I held the line while Jarrod scouted a large opening on the right wall behind a large silt bank. A few minutes later he returned and with a calm sort of confidence indicated it looked reasonable for perhaps the first of several attempts to locate the incoming R-Tunnel from Wakulla. The map indicated it was in this general vicinity somewhere but what would it look like? Would it be passable? All good questions with nothing taken for granted after the May 19th, 450 minute trip to the end of the line where the cave shut down. I switched on the video camera and 50 watt light as Jarrod tied in and headed for the dark spot on the wall. The tunnel looked reasonable and quickly turned left around a corner and into another massive room with openings right and left. Too many options in my opinion as both anxiety and anticipation began to build. With a fresh stage (bottle) plugged in and two extras clipped off, we were committed to spending as much time as necessary to find the way. We made another left into a medium sized room that did not look good as I held back to look at the large, dark opening to the right. Too many left turns already. I was concerned we would end up intersecting the Turner line in some sort of loop. Jarrod backed up and went right into another massive room and the tunnel began to take shape. We took another right as Jarrod wrapped on a huge rock center conduit and continued on. This was good, keep making right turns. It was difficult to check the compass on my left arm because holding the camera steady with the scooter took both hands. I started thinking out loud that the line was here somewhere. Perhaps over the next rise or around the next corner? Where was it, the anticipation was killing me. It would be tied off on the right wall at a corner with a large rock outcropping and it could not be much farther unless the survey was totally off. As we rounded the next corner and looked at the right wall I saw something that did not look natural hanging down from a rock outcropping. It was a loop of line. If there was a blue arrow on the other side of that rock with "Wakulla Springs" on it we had done it. The arrow was right where we had left it a month earlier on the 580 min, 23,810 ft (7,257 m) ride from the Wakulla entrance. The long awaited physical connection between the Wakulla Springs and Leon Sinks cave systems was established Saturday, July 28 at 12:20 pm. After 17 years the WKPP had finally connected Wakulla Springs to Leon Sinks. The team would be pleased. I could feel the pressure that had been building over the years begin to release. It was a good day for the WKPP."

Methods

Pre-Dive Planning

Following connection of the Wakulla and Leon Sinks cave systems, team personnel started making plans to verify the connection by traversing from Turner Sink to Wakulla Springs. Based on decompression logistics, the exploration team of Jablonski and McKinlay decided to start the dive at Turner and exit at Wakulla. The conditions at Wakulla Springs were significantly more favorable for running decompression due to in-water and surface conditions. Approximately four weeks prior to the scheduled day of the traverse (Saturday December 15, 2007), team members were notified of the plan through the WKPP's official email list. At that time, all personnel started making the necessary preparations depending on their position within the project (Table 1).

Table 1. Pre-Dive Task List

Pre-Plan	**Individuals Responsible (total number)**	**Comments**
Draft exploration plan	Casey McKinlay (1)	Review with Jablonski and coordinators
Request for team availability	Todd Leonard, Mark Messersmith (2)	Build roster for event
Submit dive plan to Florida DEP	Casey McKinlay (1)	
Make travel arrangements	Team Individuals (50)	Friday through Sunday
Place media on notice	Casey McKinlay, Jarrod Jablonski (2)	TV, WEB, Newspapers, Partners
Gas fills	Team Individuals (50)	Arrange for tank filling and gas supplies
Equipment	Team Individuals (50)	Inventory equipment, burn test batteries
Video and Photos	Jarrod Jablonski (1)	Coordinate video equipment, draft video and photo plan
Research Support	Dr. Todd Kincaid, Dr. Chris Werner (2)	Coordinate with researchers

Gas and Equipment

Exploration and support personnel individually completed equipment preparation and gas fills appropriate for their diving assignments during the week leading up to the traverse. All WKPP divers followed the team's protocol for bottle marking and breathing gasses, which designates specific mixes according to depth and purpose (Table 2). The standardized gasses represented the maximum allowed oxygen and minimum allowed helium percentages. Each bottle was marked by maximum allowable depth measured in feet, horizontally on opposing sides of the bottle, in three inch high numbers. Since a "20" and "70" foot bottle can look similar, the oxygen bottles were horizontally marked

"OXYGEN." Gases were analyzed immediately after filling and again on-site. Bottles were also marked with a label at the neck of the tank, logging oxygen and helium percentage, the analyzer's initials and date of analysis.

Table 2. WKPP Standard Gas for the Turner-Wakulla Traverse

Depth Range (ft (m))	**Maximum O_2 %**	**Minimum He %**
Exploration and Setup Diver Bottom Gas		
190 (58) + (Exploration)	10	85
190 (58) + (Setup)	12	70
Exploration Diver Decompression Gas		
130-190 (40-58)	21	70
80-120 (24-37)	35	60
30-70 (9-24)	50	0
Support Diver Bottom Gas		
190 (58) + 0-190 (0-58)	12 18	70 45
Support and Setup Diver Decompression Gas		
200-240 (61-73) 130-190 (40-58) 80-120 (24-37) 30-70 (9-24)	16 21 35 50	45 35 25 0
100% oxygen at 30 ft ONLY When dry, out of the water and in the habitat		
0-20 (0-6)	100	0
Back Gas Breaks during Decompression		
Setup Diver	16	45
Exploration Diver	12	25
Breaks done at stop prior to gas switch (e.g., 130, 80, 30 ft (40, 24, 9 m)) Cycle during 100% oxygen decompression: 12 min O_2/ 6 min break gas Extended bottom times: Break gas at 50 ft for 20 min		
Drysuit Inflation Gas: 100% Argon		

Support and exploration divers adhered to the team's equipment protocol during every team dive. Standard gear configuration included a drysuit, undergarments, argon inflation system, backplate and harness, primary regulator on a seven foot hose, backup regulator attached to a bungee cord worn around the neck, primary light, two backup lights, and double back-mounted tanks. Divers used 80 ft^3 (11 L) stage bottles (tanks configured with a regulator and a pressure gauge) which were clipped off to the harness, keeping the back gas in reserve. The WKPP's standardized approach to equipment choice and configuration allows maximum flexibility and interoperability among team members.

Additional equipment used over the course of the weekend included Gavin scooters and Halcyon RB80 semiclosed-circuit rebreathers. Gavin scooters were used by both WKPP support and exploration divers to maximize in-water efficiency. The Halcyon RB80 was used by the primary exploration team, the long-range setup team, and the Turner support team.

Accident Management Plan

Prior to the traverse weekend, the local hyperbaric treatment facility and emergency response units were notified of the team's scheduled event so the appropriate staff could be placed on call. The

WKPP's medical coordinator, Mr. Gene Hobbs, was also on call to provide medical histories to treatment facilities in the case of an emergency.

Medical oxygen and first aid kits were set up at both sites before divers entered the water. The surface management teams verified that emergency contact numbers were entered into multiple mobile phones at both Turner and Wakulla.

Support Logistics

The project weekend started with placement of decompression equipment the Friday before the traverse, including decompression bottles and accessories (Table 3). At Turner and Wakulla, setup divers staged the equipment by clipping it off to established lines or habitats at their appropriate depths (Table 6). Although the exploration team planned to exit and decompress at Wakulla, decompression bottles had to be available at Turner in case the exploration team had to abort the traverse and return to Turner. Equipment placement continued into Saturday morning, with delivery of the Halcyon decompression rebreathers, food, water and electric heat packs to their staging depths. Troughs and habitats were also filled with air and checked for proper placement.

The first support team in the water escorted the long range setup team (Team 2) to 190 ft (68 m). Meanwhile, other members of the team assisted with moving gear into the water and monitoring the initial descent of Team 2 from the observation tower. After Team 2 started their dive, the Turner support crew moved from Wakulla to start setting up for the dive.

The surface managers at Wakulla and Turner stayed in frequent communication by cell phone to update support schedules. The traverse team (Team 1) started their dive with the company of escort and video teams. After these support teams returned to the surface at Turner, a group of surface and in-water support remained at Turner. If the traverse team turned the dive early for any reason, the entire operation would have to move to Turner Sink. The in-water support crew at Turner made scheduled checks at 120 ft (37 m) for the possible return of the exploration team; this schedule continued until the Wakulla surface manager called with the news that the traverse was successful.

Support teams continued to rotate through three hour diving shifts at Wakulla Springs (Table 3). The first several shifts were given the task of supporting Team 2 through their decompression. After greeting the team at depth and taking superfluous gear away from the divers to make their decompression easier, support divers monitored the team for signs of oxygen toxicity or other issues which might arise. They also provided a mode of communication between the decompressing divers and the surface by passing waterproof notes between the two groups. At the end of each support shift, the divers brought used equipment (scooters, bottles, rebreathers which were no longer necessary) with them so the gear could be removed from the water by the surface team.

The weather provided an added complexity to the weekend. The day of the traverse was characterized by a misty rain. As night time approached, a line of severe storms loomed on the horizon to the west. Tornado warnings were announced as dangerous winds, frequent lightning, and heavy rain moved into the area. The surface support crews at both sites covered exposed equipment while maintaining the necessary personnel to continue monitoring diving activity.

The long-range setup team was preparing to exit the habitat and begin their ascent to the surface at 1930, so a three-person team was sent in so each decompressing diver had an individual monitor. At 2000, a deep support team also entered the water to begin checking for the appearance of the traverse team's lights at 190 ft (68 m). On their first check, there was no sign of the team. Approximately 45 minutes later, they returned for a second look but once again there was no sign of the traverse team.

They surfaced again and waited until 2140 for a third check. This time was a success, and the deep support team returned to the surface with the news that the team had arrived at Wakulla along with a cache of used bottles and scooters. After receiving verification that the team was decompressing at Wakulla, surface manager Todd Leonard called Turner surface manager Dawn Kernagis to pass along the information. The Turner crew proceeded to pull the remaining decompression equipment out of the cave, clean the site, and return to Wakulla Springs at 0100 on Sunday to continue support duties.

Table 3. Support Diver Plan and Schedule

Task	**Personnel**
Friday PM Setup at Wakulla: Setup team to place gear in advance of dive	
100% oxygen and accessories at 30 ft (9 m) on habitat	Shallow: John Kendall, Richard Walker Deep: Scott Cox, Doug Mudry
50% nitrox at 70 ft (21 m)	
35/25 trimix at 120 ft (37 m)	
21/35 trimix at 190 ft (68 m)	
Habitats filled with air and checked	
Accessories at 120 and 50 (37 and 15 m)	
Decompression Halcyon rebreathers at 120 ft (37 m)	
Electric heat packs at 120 ft (37 m)	
Saturday AM Setup	
Surface Manager at Wakulla	Todd Leonard, Shellie Foss
Surface Manager at Turner Sink	Dawn Kernagis, Kell Canty
Escort divers into Wakulla	Richard Lundgren, Doug Mudry
Escort divers into Turner Sink	Scott Cox, Marc Singer
Video team at Wakulla - surface, underwater	Anthony Rue
Video team at Turner Sink - surface, underwater	Anthony Rue, Richard Lundgren, Doug Mudry
Sherpa at Wakulla	Adam Gonzales, Curtis Baldwin
Sherpa at Turner Sink	Curtis Baldwin, Claudia Milz, Antonio Giorgetti
Tower Monitor at Wakulla	Adam Gonzales, Curtis Baldwin
Wakulla Support Schedule Saturday-Sunday	
Surface Managers	Todd Leonard, Shellie Foss, Sonya Tittle
1100-1440 Meet Team 2, pull gear	David Doolette, Gideon Liew
1450-1815 Team 2 into habitat	Dean Marshall, John Bailey
1930-2135 Escort Team 2 to surface	Claudia Milz, Bill Oigarden, Antonio Giorgetti
2000-2345 Meet Team 1	Doug Mudry, Chris Werner
2315-0232 (Sunday) Support Team 1	Gideon Liew, Mario Arena
0250-0602 Support Team 1	Antonio Giorgetti, John Kendall, Paul Gore
0754-0945 Escort Team 1 to surface	Richard Lundgren, John Bailey, Mario Arena
Turner Sink Support Schedule Saturday-Sunday	
Surface Managers	Dawn Kernagis, Kell Canty
Decompression equipment placement; all day until Team 1 arrival at Wakulla has been confirmed	Scott Cox, Marc Singer, Todd Kincaid

Support dives continued through the night at Wakulla until the traverse team entered the habitat at 30 ft (9 m). At this time, the divers worked on moving the used gear out of the cavern to the surface. Personnel pulled the equipment out of the water, and other team members helped load the pieces of gear into their respective vehicles in order to minimize post-dive exertion by the exploration team. At 0745, the final support team entered the water to escort the traverse divers through the final stages of their decompression out of the habitat. McKinlay and Jablonski surfaced at 0900 on Sunday December 16, 2007 after a total in-water time of 20.5 hours. The support team removed the last pieces of equipment from the water and cleaned the dive site as they concluded the weekend's events.

Setup Team Logistics

The first task of the day was to have the long-range setup team (Mark Garland, Mark Messersmith, David Rhea) place extra bottles and scooters 6,500 ft (1,981 m) from the entrance of Wakulla for the traversing divers to pick up on their way out. The traverse could not be completed until their delivery was confirmed. Their planned bottom time was estimated between 150-200 min at a depth of 300 ffw (91 mfw), with a total decompression time of 700 min (Table 4).

Richard Lundgren and Doug Mudry escorted the rebreather team through their initial descent to 190 ffw (58 mfw) before returning to the surface for additional support work. The setup team then successfully delivered the cache of equipment and replaced a malfunctioning flow meter in the cave, returning to Wakulla after a total bottom time of 180 min.

Table 4. Setup Team (Garland, Messersmith, Rhea) Dive Plan

Tasks	**Comments**
Transport equipment for exploration team into Wakulla Springs	Carry 1 scooter and 1 stage bottle for each exploration diver
Place equipment at 6,500 ft (1,981 m) depot	Total estimated time at depth: 150-200 min
Remove malfunctioning flow meter in D-Tunnel	Total estimated decompression: 700 min
Install new flow meter in D-Tunnel	Alternate diver: Marc Singer

Exploration Team Logistics

The exploration team (Casey McKinlay, Jarrod Jablonski) drove from Wakulla to Turner to continue preparing for their dive after the setup team left the surface. They waited to receive word that the setup team successfully delivered the bottles and scooters. Wakulla surface manager Todd Leonard contacted the Turner Sink crew to give them the news that the setup team was successful, and Jablonski and McKinlay entered the water to begin the traverse.

Table 5. Exploration Team (McKinlay, Jablonski) Dive Plan

Task	Comments
Traverse from Turner Sink to Wakulla Springs	Distance: 7 miles Estimated time at depth: 360-450 min Estimated decompression: 660-780 min Target surface time at Wakulla: Sunday, 0800 Required departure time from Turner: Saturday, 1400 Requires confirmation that setup team has delivered equipment on Wakulla side and visibility inside cave is acceptable
Use single stage and single scooter to 7,000 ft (2,134 m)	Carry scooters 2, 3, 4 and stages 2, 3, 4
Leave stage 1 and scooter 1 at 7,000 ft (2,134 m)	Retrieve on cleanup dive
Use scooter 2 for 1 hour	Switch estimate at 13,000 ft
Point of No Return to Turner Sink	Estimate at 13,000-15,000 ft (3,962-4,572 m)
Use scooter 3 for 1 hour, switch stages	Switch estimate at 20,000 ft (6,096 m)
Use scooter 4 for 1 hour	Switch estimate at 27,000 ft (8,230 m)
Pickup scooter 5 and stage 5 for exit	Switch estimate at 31,000 ft (9,449 m); 5,000 ft (1,524 m) to Wakulla basin
Exit at Wakulla Springs basin	Begin decompression
Exploration Logistics	
Time and Distance per stage tank at 300 ft (91 m)	2 hours, 2 miles each stage; 3 stages for the 7,000-31,000 ft (2,134 -9,449 m) section allows for the possibility of losing 1 stage per diver without abort
Safety tanks	Turner: 3 at 3,000 ft (914 m); 3 at 6,000 ft (1,828 m); 3 at 10,500 ft (3,200 m)
	Wakulla: 2 at 9,000 ft (2,743 m); 2 at 7,000 ft (2,134 m); 3 at 6,500 ft (1,981 m); 3 at 3,500 ft (1,067 m); 3 at 2,200 ft (671 m)
No-Turnaround Point	13,000-15,000 ft (3,962-4,572 m) downstream from Turner Sink
Scooters	155 min and 15,000 ft (4,572 m) range each
	3 scooters each for the 7,000-31,000 ft (2,134 -9,449 m) section allows for the possibility of losing 1 scooter per diver without abort
Speed	Estimate 100-150 ft (30-46 m) per min
Halcyon Rebreathers	Estimate 10-12 hours run time before breakthrough
Traverse Time Points	**Minutes**
Sink to 190 ft (68 m) drop and switch	35
190 ft (68 m) drop to Switch 1 at 7,000 ft (2,134 m)	60
7,000 ft (2,134 m) to Switch 2 at 13,000 ft (3,962 m)	50
13,000 ft (3,962 m) to Switch 3 at 19,000 ft (5,791 m)	50
19,000 ft (5,791 m) to Switch 4 at 25,000 ft (7,620 m)	60
25,000 ft (7,620 m) to Switch 5 at 30,000 ft (9,144 m) (or Wakulla 6,500 ft (1,981 m))	60
31,000 ft (9,449 m) to Wakulla Springs Basin	80
Total	**395**

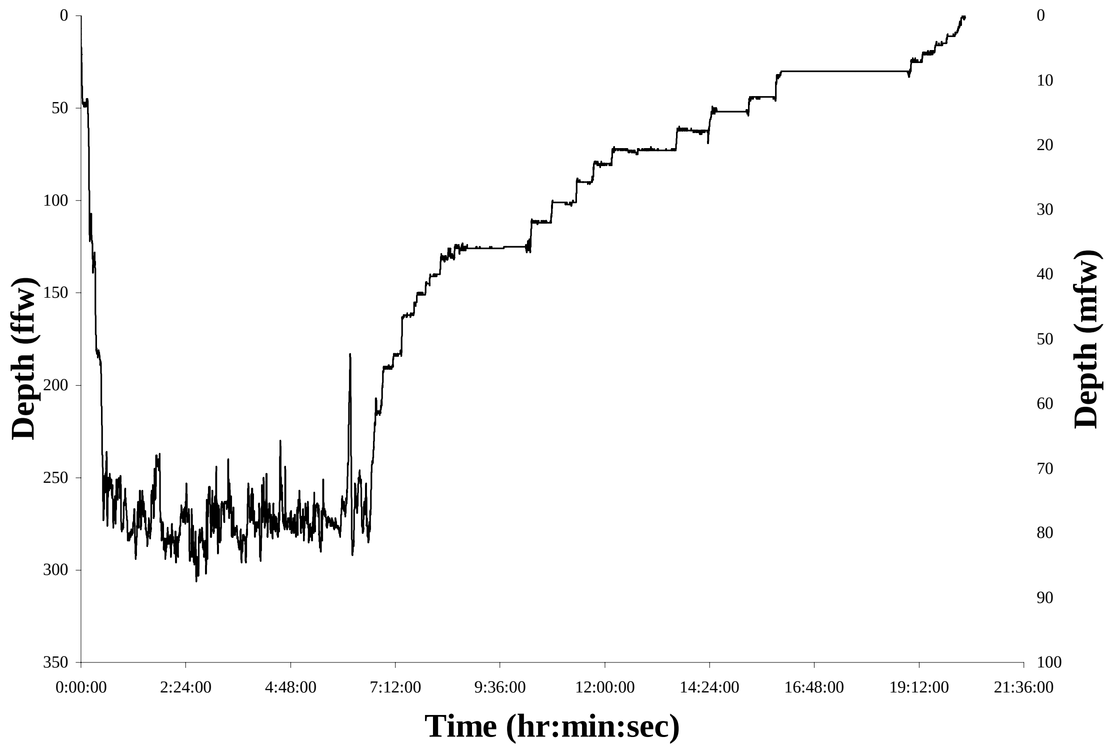

Figure 3. Turner to Wakulla traverse and decompression profile for McKinlay and Jablonski

On Saturday December 15, 2007 at 13:20, Jablonski and McKinlay started their dive from Turner Sink to Wakulla Springs. The journey covered approximately seven miles of underwater cave passage, at an average depth of 300 ffw (91 m). Initially carrying four stage bottles and four scooters each, the exploration team planned a timeline for when they would switch their use of each bottle and scooter (Table 5). The first bottle and scooter used by each diver was clipped off to the line 7,000 ft (2,134 m) from the Turner entrance; these bottles would be taken out of the cave on a future dive. As the team followed their switch timeline, they came upon the gear cache that was delivered earlier in the day by the setup team. An extra bottle and scooter was picked up by each diver, and they continued on their way to the basin of Wakulla Springs. The total bottom time for the traverse was 6.5 hours, with a total decompression time of approximately 14 hours (Figure 3).

Discussion

On the weekend of December 15-16, 2007, the WKPP successfully completed the traverse between Turner Sink and Wakulla Springs. The connection of the two cave systems yielded the Wakulla-Leon Sinks Cave System, the longest underwater cave in the United States and the fourth largest in the world at a total of 28 miles (45 km) of surveyed passages. Through the completion of this dive, the team also set two world records for the longest cave dive between two entrances and the longest traverse in a deep cave.

The WKPP immediately set its sights on future exploration possibilities after the Wakulla-Leon connection was made. The team's proposed plans included searching for a connection to the Gulf of Mexico, while continuing their research and conservation efforts within the WKP.

Acknowledgments

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Diver Eco-Tourism and the Behavior of Reef Sharks and Rays – an Overview

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Abstract

This paper addresses the issue of the expanding tourist dive industry that is focused on seeing sharks and other elasmobranchs. The two competing approaches involve baiting and feeding sharks and rays verses natural observations without feeding. In terms of shark feeding dive operations, there are two main questions: 1) How safe are divers on tourist operations that attract sharks and rays using bait? 2) How is the fish's behavior and ecology being affected by this interaction? The broader issue is: what is the best practice in terms of conservation and ecology? I offer some observations on shark behavior and suggestions for future scientific research.

Keywords: bait, diving, elasmobranchs, feeding, safety, scuba, tourist

Introduction

Dive destinations compete vigorously to attract recreational scuba divers to their resorts. One major attraction for any dive site is the reliability of seeing big fishes and especially sharks and rays. This raises interest in scientific questions about fish behavior, as natural resource managers need to know basic information concerning home range and habitat usage in order to best define the boundaries for a marine protected area. Other behavioral questions such as how the fish habituate (or not) to scuba divers depends both on the behavior as well as the movement patterns of the species. Such information is also important to safety considerations regarding tourist's interactions with wildlife underwater. This is also a question for scientific divers (and AAUS) who may undertake to study these sharks attracted by the tour.

This paper is an overview based upon my research on the movement patterns of the grey reef shark, *Carcharhinus amblyrhynchos*, in the Pacific Ocean. The purpose is to highlight the research questions and practical applications as well as to initiate discussion of the diver safety questions**.**

Diving with sharks, skates and rays is a main attraction for diving tourists worldwide. Consequently, the economic success of any local diving tourism industry can be dependent on the reliability of seeing sharks and rays up close. Some locations, such as Blue Corner, Palau, have natural aggregations of sharks that are not fed by divers. At other locations (e.g., Bahamas, Fiji, Tahiti) dive operations attract sharks and stingrays using bait operators (Brunnschweiler and Earle, 2006). These operations allow divers to watch underwater as the divemasters hand feed these fish (Figures 1, 2, 3). Baiting is a highly controversial practice, and there have been several instances of divers being bitten and even killed. Of course, every location and dive operation has its own unique set of circumstances and operating procedures. Successful shark feeding operations do best where they have individual sharks that are regular visitors and can be 'trained.' There is always concern by the dive guides when unfamiliar sharks come into a hand-feeding arena (Lobel, personal observation).

Figure 1. Shark feeding tourist dive in Fij, bull shark, *Carcharhinus leucas.* Photo credit: Phillip S. Lobel.

Figure 2. Shark feeding in Tahiti with tourists lined up along a rope. Photo credit: Phillip S. Lobel.

Figure 3. Tourists wading with large stingrays in Tahiti. Rays were attracted by bait.
Photo credit: Phillip S. Lobel.

As we all know, sharks are in danger due mainly to fishing for fins and as by-catch in other fisheries. The shark fin fishery is a global threat that has to be acted upon by most local governments to protect species in their legal territorial waters. The nation of Palau has lead Earth's nations by enacting pioneering legislation backed up by effective law enforcement to protect sharks in its territorial waters. This is great, but the scientific data are completely lacking to evaluate whether this will save sharks only locally. In other words, do reef sharks move offshore at some times, and thereby become vulnerable to open seas fisheries?

A grey reef shark with a tangled lure (figure 4) was swimming at Blue Corner, Palau (Lobel, personal communication). The fishing line was stainless steel and the stainless hook with lure attached was imbedded in her jaw. This female grey reef shark was very thin and gradually looked worse over time until she disappeared from Blue Corner. This is an obvious risk to the animal because she may well not survive; it is also a risk to divers if this shark becomes aggressive, and a risk to the local economy by losing 'watchable' wildlife.

Figure 4. *Carcharhinus amblyrhynchos*, Grey reef shark with a fishing lure at Blue Corner , Palau.
Photo credit: Phillip S. Lobel.

In terms of elasmobranch behavior, we need to know the home range of individual sharks and rays in order to better determine the size of marine protective areas. These same data allow for estimating the predictability of observing the animals for site-specific ecotourism. For these studies, tracking sharks using acoustic tags is the most feasible methodology. One of the key issues, however, is determination of the best method for tagging a shark that will cause the least harm to the animal (Skomal et al., 2007). This is an especially relevant concern given that research in the USA is governed by permits from the Institutional Animal Care and Use Committee (IACUC).

The unifying scientific question in terms of understanding how dive operations may or may not be impacting sharks and rays and the associated diver safety issues is whether the individual sharks and rays are local residents and thus able to learn and habituate to divers. Alternatively, if these sharks and rays rarely reside for long-term in the same areas, then they are much less likely to become accustomed to a site-specific feeding dive. I base this on my own experiences, especially with the grey reef shark *Carcharhinus amblyrhynchos*. I have observed that sharks unfamiliar with divers are more aggressive (Figure 5) and less predictable than those that have habituated to the presence of divers, such as we now see in numerous marine protected areas such as Blue Corner, Palau, and Shark Reef Marine Reserve, Fiji.

Figure 5. Grey reef sharks can be aggressive when first encountering scuba divers, but they habituate to the presence of divers to the point that they ignore flash photography and bubbles (photo Fanning Atoll, 1974). Photo credit: Phillip S. Lobel.

Methods and Results

My research has examined the movement patterns and reproduction of the grey reef shark, *Carcharhinus amblyrhynchos*, at two locations in the Pacific Ocean: Johnston Atoll and Palau. The objective was to determine whether this shark species exhibited any measurable degree of local site fidelity, or if they just wandered widely and haphazardly. At Johnston Atoll, the local question concerned the degree of exposure to individual sharks that were found in reef habitats contaminated with PCBs and dioxins. At Palau, the local question concerned if and how far individual sharks wander beyond the boundaries of the marine protected area at the Blue Corner dive site.

The overall aim of this research was to address both conservation and scientific issues. The basic issues are:

- Socio-economic issues
  - Marine Protected Area – how big?
  - Fisheries prohibitions – on what scale?
  - Making the case for conservation based on solid science and applied economics
    - How much is a shark worth to ecotourism?
    - Are individuals resident longtime?
    - Do scuba divers adversely affect a shark's behavior?
- Scientific questions
  - Behavioral ecology, natural history of a top predator
    - Home range: hunting and feeding habits- orientation to spawning fishes?
  - Trophic ecology: Bioaccumulation of contaminants and ciguatera

Observation of Reproduction

One aspect of shark biology that is easy for scuba divers to note is the relative abundance of males and females. It is also simple to note if females have been recently mated or appear pregnant. Male and female sharks are easily distinguished by the presence of claspers on the males (Figures 6, 7). A recent mating event can be recognized by scars on a female (Figure 8) that result from the male biting and holding her during copulation (Whitney et al., 2004). Fresh mating wounds are clearly recognizable on females of many species of elasmobranchs (Pratt, 1979; Kajiura et al., 1999; Jensen et al., 2002; Porcher, 2005). It is a little more difficult to assess pregnancy in sharks, but females in advance gestation are much 'fatter' than others (Figures 9, 10). Pregnant female grey reef sharks have been observed to gather in aggregations in shallow water away from their usual territory (Economakis and Lobel, 1998).

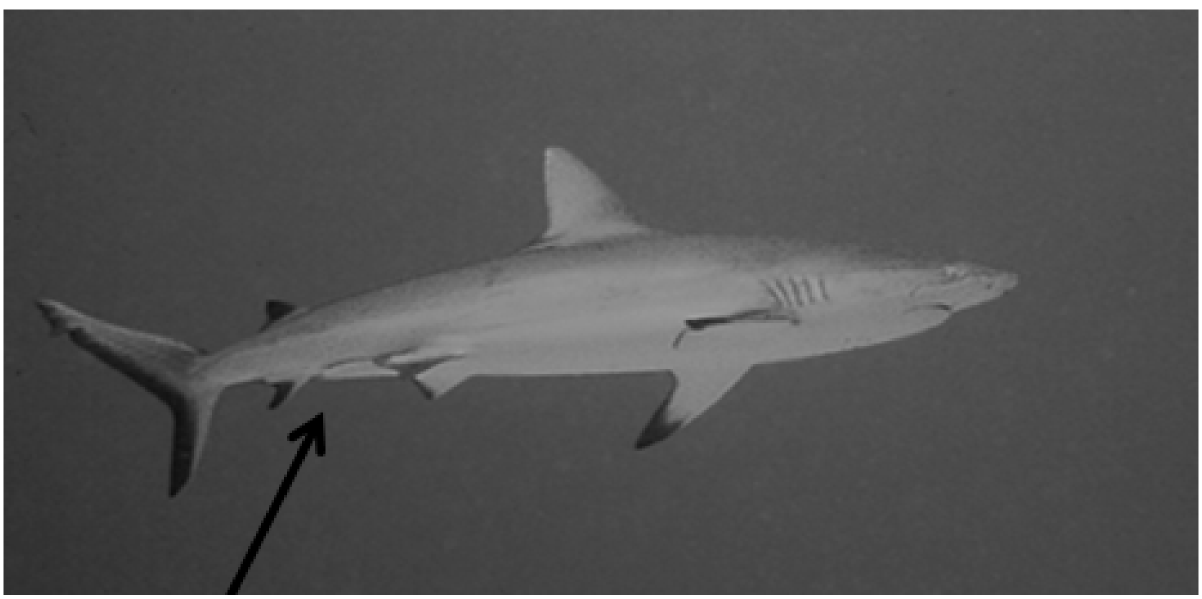

Figure 6. Male grey reef shark showing claspers. Photo credit: Phillip S. Lobel.

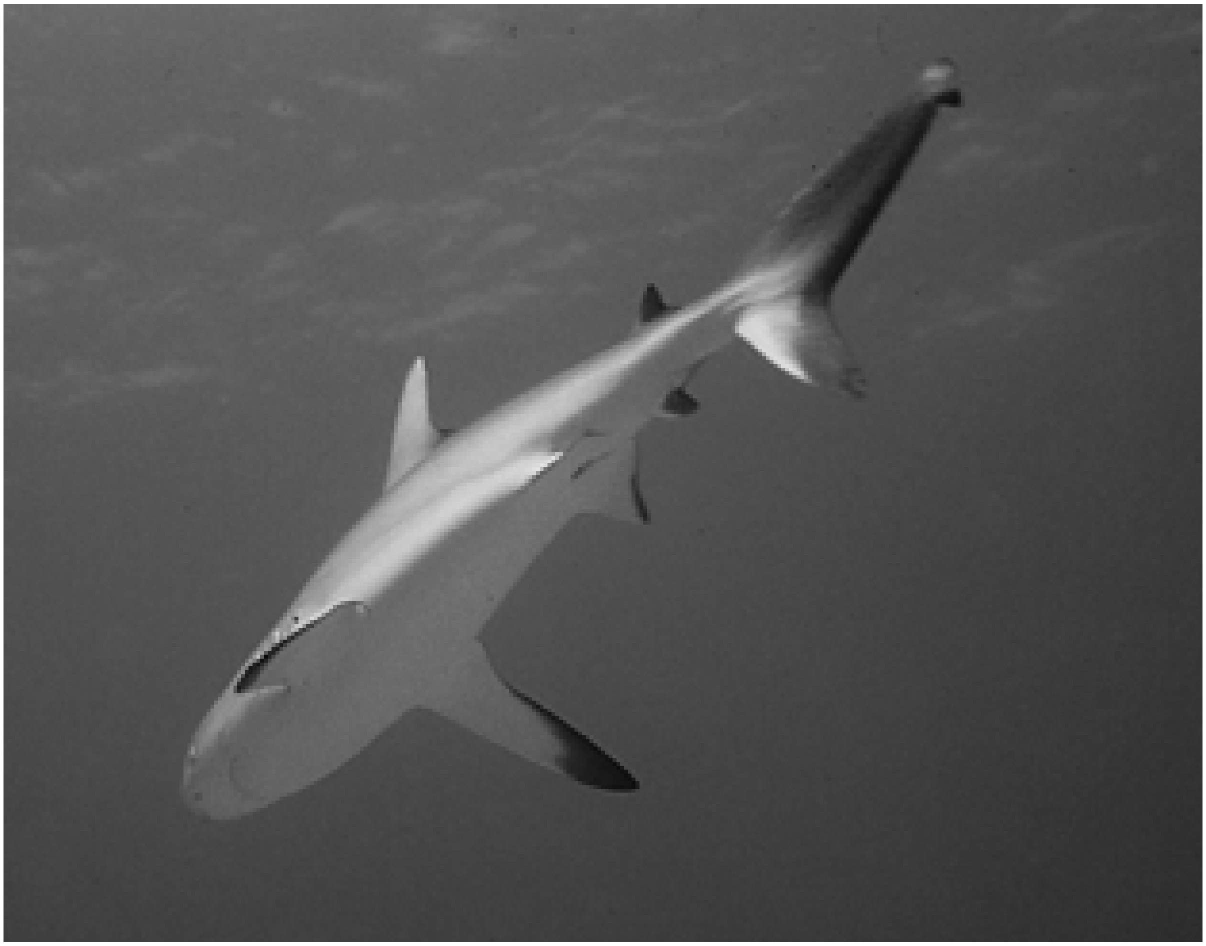

Figure 7. Female grey reef shark, no claspers. Photo credit: Phillip S. Lobel.

Figure 8. Female grey reef shark showing mating scar and torn pectoral fin. Photo credit: Phillip S. Lobel.

Figure 9. Female grey ref shark with a large belly that is typical when pregnant. Photo credit: Phillip S. Lobel.

Figure 10. A typically thin female shark. Photo credit: Phillip S. Lobel.

The Acoustic Bracelet Tag

We developed a new method of tagging sharks that was minimally physically intrusive. The design is to have the tag fall off (by corrosion) after the battery lifetime of the tag has ended. Sharks were captured by baited hook, promptly tagged and released. An acoustics bracelet tag was banded onto a shark's caudal peduncle (Figure 11) and tracked by underwater loggers (Figure 12) strategically deployed at up to 16 locations. The bracelets were made of stainless fishing lure braid and were crimped with non-stainless crimps. These last about 15 months in seawater jars in the laboratory. In addition to the acoustic bracelet tag, several of the sharks were also equipped with the National Geographic Cittercam camera system (Figure 13, Skomal et al., 2007).

Figure 11. The newly developed acoustic bracelet tag on the shark's caudal peduncle.
Photo credit: Phillip S. Lobel.

Figure 12. Diver deploying underwater acoustic loggers for recording passage of sharks that have been tagged in Palau. Photo credit: Phillip S. Lobel.

Figure 13. The National Geographic Crittercam on a grey reef shark at Blue Corner, Palau. The results from these deployments have been featured in the 'Wild Chronicles' NGS TV series on PBS. Photo credit: Phillip S. Lobel.

The study of grey reef sharks at Johnston Atoll began in 1983 with acoustic tracking during 1999 to 2003. The long-term study at Johnston Atoll included detailed census surveys of females (most being pregnant) in shallow water aggregations and acoustic tracking. Johnston Atoll was a military base with extensive chemical and nuclear operations since the 1930s (Lobel, 2003; Lobel and Lobel, 2008). Johnston Atoll had no tourists and relatively few divers. Palau, in contrast, is a sport diver Mecca with as many as 300 divers per day at Blue Corner. The Palau study was from 2003 to 2006.

The Study of Sharks at Johnston Atoll: Pollution and Ciguatera

The answer to the question about how far and wide a reef shark may roam also has major implications to pollution impact studies and remedial pollution cleanup actions. The consequence of the decisions to clean up a site or 'remediate in place' also have economic implications as the price tag for clean-up is always in millions of dollars. In our case study at Johnston Atoll, a central Pacific Ocean U.S. Department of Defense installation, cleanup requirements are based upon an evaluation of potential ecological impact, particularly to top-level carnivores and other threatened or endangered species. Our studies addressed this issue by studying sharks as well as contaminants in potential prey fishes and in the marine sediments that prey fauna consume while feeding (Lobel and Lobel, 2008).

On another tangent that is interesting scientifically, one of the long time questions in animal behavior can be paraphrased as 'what makes a good fish go bad?' It is well known that certain contaminants that accumulate in the tissues of an animal can affect that animal's behavior. Lead and mercury can cause learning abnormalities and could lead to abnormal behavior, perhaps even increased aggression ('mad as a hatter'). The impact of the mix of other chemicals in the ocean, both pollutants and natural, is really unknown. We are particularly interested in the effects of the natural phenomenon of ciguatera on the survivorship and behavior of fishes. Ciguatera is a natural toxic phenomenon that is pervasive on tropical marine reefs. Sharks are well known to be top-level carnivores and have been shown to have high levels of ciguatera in some areas at some times. We have been evaluating the impacts from military contamination on reefs from these several perspectives.

The objective of the study at Johnston Atoll was to determine where sharks ranged relative to underwater sites contaminated with Agent Orange, dioxins, PCBs, and plutonium from nuclear weapons detonations. At Johnston Atoll, we found concentrations of contaminants in shark tissue samples that were much higher than expected. The levels were of concern because if the sharks were feeding randomly throughout the entire atoll, it would imply a greater environmental impact from the contaminants than if, on the other hand, the sharks were homebodies. If the sharks were individually very site specific and were staying on a particular local reef area, then their feeding perhaps could be focused on where there were contaminated reef fish (such as on adjacent reef to the Navy pier, the old Loran station and the former Herbicide Orange storage site.. If that was the case, individuals may be impacted but not necessarily a significant portion of the overall population of the atoll.

The Study of Sharks in Palau

Scuba divers worldwide come to Palau to see and photograph sharks. The abundance and diversity of shark species that can be seen on any given day guarantee the economic survivorship of Palau's ecotourism diving industry. But more than that, Palau provides an educational product that introduces sharks as wondrous animals to those watching films in theaters, in schools and on TV. It is fair to state that the reliability of filming sharks in Palauan waters attracts film crews from all international venues. These films show sharks in their natural habitat, and help audiences appreciate their positive role in the coral reef ecosystem.

Specifically, we do not yet know if the common reef sharks seen by divers every day at such famous dive locations such as Palau's Blue Corner, are resident or migratory. We do not know if these same sharks may at some time in their life cycle venture far out into international waters. If they do, they could become easy prey to high seas fisheries legally operating in international waters. More complicated, is the legal prosecution of captured fishing boats found, for example, within Palau's sovereign waters with shark fins aboard. These boats can claim that the fins were taken legally in international waters and not within Palau's protected economic zone. The law enforcement case can only be made if the boats were actually witnessed fishing in restricted waters *OR* if science can demonstrate beyond a reasonable doubt that the species of sharks in the boat could only be found and caught inside of the protected exclusive economic zone.

Palau's Blue Corner is arguably one of the best diving sites in the world, where divers come to specifically see sharks. Are these the same sharks they are seeing on a daily basis? How do the sharks react to the people? And, as a dive master for tourists, are there things you can do that would make it easier for people to see the sharks and be less intrusive to them? The scientist needs to answer the question about where different shark species spend their time. Are the so-called 'reef sharks' really restricted to only coastal coral reefs? Can these 'reef sharks' migrate between Pacific Islands such as from Palau to Yap to Guam? These questions remain to be answered.

Discussion

Data obtained, so far, indicate that the grey reef sharks are highly site resident for extended periods of time from weeks to months, but that they also range elsewhere (at least outside of the range of the acoustic logger array) for some time periods and then later return. Analysis of these data is in progress, and will be presented in forthcoming research papers.

Acknowledgments

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Subtidal Application of Copper in the Study of Gastropod-Algal Interactions

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Abstract

Copper metal and copper-based anti-fouling paints are commonly used in intertidal ecology to restrict movement of molluscan grazers. As a chemical barrier, copper provides a method for examining grazer-algal interactions without the confounding factors associated with caging effects. The study of these interactions subtidally has mainly incorporated methods of manual removal or the use of cages with high artifactual effects. This study introduces the use of copper as a chemical barrier to a subtidal herbivorous gastropod, *Chlorostoma brunnea*, that grazes directly on the giant kelp, *Macrocystis pyrifera*, in central California. Cages constructed of copper pipe were placed at the base of each of ten *M. pyrifera* individuals, effectively restricting the immigration and emigration of *C. brunnea* on *M. pyrifera.* This paper discusses the methods for making, deploying and maintaining these subtidal copper cages.

Keywords: algae, cage, *Chlorostoma brunnea*, exclusion, experimental design, gastropod, *Macrocystis pyrifera*

Introduction

Experimental research is essential to the study of species interactions and community dynamics (Connell, 1974; Paine, 1977). More specifically, examining grazer effects through experimental manipulation is a powerful way to discover the influence a species has in its community (Hall et al., 1990). Manipulations, such as these, are usually created through the implementation of exclusion or inclusion cages or by manual removals or additions of the species being studied. Much of this type of research has been performed to examine the relationship between herbivorous mollusks and algae (Lubchenco and Gaines, 1981). Caging methods have proven effective in describing many grazer-algae interactions but often artifacts that can alter the environment of the experimental areas (i.e., shading, temperature, increased sedimentation inside cages, and abnormal predator behavior) and must be incorporated into the experimental design, thus increasing the total number of treatments and replication (Hall et al., 1990; Hayworth and Quinn, 1990).

To counteract caging artifacts, many researchers choose to implement techniques using chemical barriers to allow for superior investigation of grazer-algal interactions (Johnson 1992). The repulsion by certain molluscan species to copper (either the metal or as an additive to paint) is not understood (Cubit, 1984), but is quite effective and has replaced the use of cages for many intertidal field experiments. The use of copper paint has become an important experimental method for manipulating grazers such as limpets and chitons in rocky shore environments (Johnson 1992).

Subtidally, exclusion cages have primarily been used to examine interspecific interactions between fish species (Anderson, 2001). Few subtidal studies have experimentally examined the effects of fish grazers on algae (e.g., Hatcher and Rimmer, 1985; Kennelly, 1991). Studies that have examined non-fish grazers have done so using manual removals and implementation of caging techniques (e.g., Choat and Andrew, 1986; Fletcher, 1987; Leonard, 1994). No present data exists on the use of chemical barriers to study subtidal grazer-algal interactions. This paper introduces copper metal as a barrier to study the dynamics of subtidal gastropods and their preferred algal food source.

Project Description

The giant kelp, *Macrocystis pyrifera*, is a large subtidal alga that forms extensive beds along the coastlines of New Zealand, southern Australia, North and South America, and South Africa (Steneck et al., 2002). Kelp forests such as these form complex structure that hosts numerous associated species of fish, arthropods, echinoderms, molluscs, mammals, and other algae (Mann, 1973). Many specific and interspecific interactions have been well studied in these giant kelp systems (North, 1971; Dayton, 1985a; Dayton, 1985b; Foster and Schiel, 1985; North, 1994; Steneck et al., 2002). However, a more thorough understanding of the strength of trophic interactions is essential to determine the overall dynamics of the kelp forest community (Paine, 1969; North, 1971; Dayton, 1985; Foster and Schiel, 1985; Estes and Duggins, 1995).

Three species of the gastropod genus *Chlorostoma* (formally *Tegula*) graze directly on attached *Macrocystis pyrifera* and are highly abundant in central California (Watanabe, 1984a), with density estimates ranging from 150 to 200 *Chlorostoma* per *M. pyrifera* sporophyte (Watanabe, 1984a; S. McMillan unpublished data). These herbivores, *Chlorostoma montereyi, C. pulligo,* and *C. brunnea*, use *M. pyrifera* as their preferred food source and shelter from benthic predators such as *Pisaster giganteus* and *Pycnopodia helianthoides* (Watanabe, 1984b). Although removal of an adult *M. pyrifera* sporophyte by gastropod grazers has never been recorded, their grazing may indirectly affect the structure of the kelp forest (Graham et al., 2007). Species that graze on *M. pyrifera* adults that do not directly remove individuals may indirectly remove all or parts of the sporophyte through the weakening of tissues leaving the sporophyte vulnerable to surge, epiphytes, and bacterial infections (Foster and Schiel, 1985). Grazing may also lead to a reduction in reproductive potential by removing reproductive blades or causing stress to the sporophyte initiating reduction in production of sori in favor of allocation of materials for new growth (Foster and Schiel, 1985). The role of these intermediate grazers may be important but has not been investigated experimentally.

Methods

The purpose of this study was to evaluate how the most abundant herbivorous subtidal gastropod genus in central California, *Chlorostoma*, affects the productivity, reproductive potential, and survivorship of *Macrocystis pyrifera* within giant kelp forests. The effects of the most abundant of this genus, *Chlorostoma brunnea*, were investigated using experimental field manipulations of *M. pyrifera* sporophytes at a central California location, Stillwater Cove, Carmel (36°34'N, 121°56'W).

In order to examine the relationship between *Macrocystis pyrifera* and *Chlorostoma brunnea*, I developed a caging technique to exclude and include *C. brunnae* from individual *M. pyrifera* sporophytes. Each exclusion/inclusion cage was constructed of a ½" (schedule M) copper square frame with four legs. Rebar was inserted into the frame for increased durability and weight. Frame dimensions were 1 m square with legs measuring 23 cm. All corners of the frame were soldered together using street 90 'L's and 'T's to attach the legs. All excess solder was then sanded away to reduce possible movement of snails over the copper barrier via the solder. On each frame a ¼" mesh nylon netting 'Macrocystis skirt' was sewn through holes drilled on the inside of the frame and a nylon drawstring was sewn into the inner edge of the skirt. This skirt was then synched by the drawstring midway around the holdfast to prevent snails from climbing on or off the sporophyte via the holdfast. Therefore, these cages created a moat around the base of the plant (Figure 1).

Figure 1: *Macrocystis pyrifera* with copper cage with netting attached to the base of the sporophyte. Photo credit: Serena McMillan (used with permission).

Holes were drilled into the granite substrate using a Chicago Pneumatic CP-09 Handril. Concrete anchors wrapped in a layer of z-spar epoxy were then hammered into the substrate. Eyebolts were screwed into the concrete anchors and the cage legs were secured to the eyebolts via cable ties.

Twenty *Macrocystis pyrifera* sporophytes with similar stipe numbers were selected at approximately 10 m depth. Five sporophytes were randomly chosen as controls (i.e., no manipulation). Ten individuals were randomly chosen to be stocked with varying densities of *Chlorostoma brunnea* (0, 50, 100, 150, 200, 250, 300, 350, 400 and 450 snails per sporophyte). All conspicuous mobile invertebrates were removed from fronds prior to the addition of snails. The *C. brunnea* used to stock the kelp sporophytes were collected from kelp forests near the experimental site. Five individuals were randomly chosen as artifact controls. Cages without nylon netting were placed around these sporophytes to control for any effect of the copper (Figure 2).

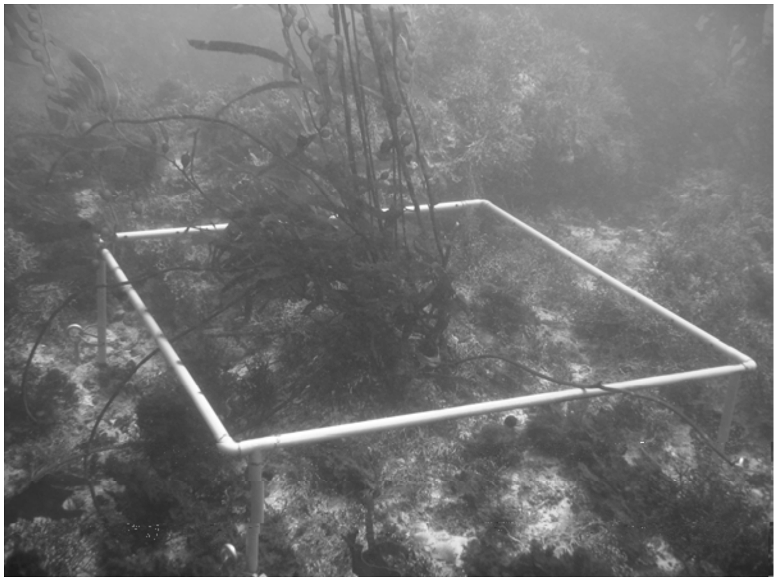

Figure 2: *Macrocystis pyrifera* with a copper cage without netting (artifact control).
Photo credit: Serena McMillan (used with permission).

All *Macrocystis pyrifera* individuals were surveyed every two weeks for stipe number, growth rate, and reproductive potential. Visual surveys of all treatment sporophytes were made to insure densities of snails remained consistent with stocked values and maintenance to all cages were performed weekly. Initiation of the experiment occurred October 31, 2007, and the experiment was to run for one year. However, due to the most intense storm to hit the northwest coast in over 25 years (Lewitsky et al., 2008), 80% of all experimental cages were destroyed on the week of December 4, 2007.

Results

The copper cages were successful as chemical barriers to *Chlorostoma* species and restricted the movement of gastropods to and from the *Macrocystis pyrifera* individuals. The densities remained consistent with the original stocked values. After three weeks, snails were removed from the treatment sporophytes stocked with 0 and 50 snails to estimate any migration of snails. Eight snails were found on the individual stocked with 0 snails and 62 snails were found on the treatment stocked with 50.

Discussion

The change in snail densities from the original stocking number found after three weeks suggests that some snails may be able to cross over the cage and skirt barrier. However, during the time that the experiment was running, many winter storms did create high surge at the site. This surge caused the *Macrocystis pyrifera* individuals to lean over and come in contact with other *M. pyrifera* individuals, and fronds and sporophylls periodically touched bottom. These conditions allowed for migration of snails on and off of the treatments. These events were observed and anticipated prior to the initiation of the experiment, and plans were made to remove all snails from treatments and replace with new snails at stock values monthly. However, this plan was never implemented due to the large storm occurrence that prematurely ended the experiment after one month.

This study introduces the use of copper metal as an effective way to investigate grazer-algal interactions without the use of vigorous manual removals or cages that enclose algae and may cause shading and deposition of sediments, among other effects. However, as cautioned by Johnson (1992), any experimental design that includes any type of caging or chemical barriers requires the application of artifact controls. This study did include such controls, and that data will be presented in a subsequent paper.

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Developing a Training Program for Scientific Diving Environmental Health and Safety Professionals

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Abstract

Many individuals become involved in scientific diving to support their research activity during academic training. Diving is typically a tool and not the focus of the educational program. Many diving safety officers are drawn from this pool with only modest familiarization with the more formal elements involved in traditional environmental health and safety programming. The integration of diving safety into such programs makes clear the value of developing opportunities for education directed specifically at careers in health and safety based on diving expertise. This paper will discuss the history of scientific diving safety officer qualifications and training and present an outline for a scholarship program to prepare student candidates as environmental health and safety diving professionals.

Keywords: diving safety officer, education, professional development, students

Introduction

Scientific Diving

Many individuals become involved in scientific diving to support research activity during academic training. Diving is typically a tool and not focal to the educational program. Many diving safety officers (DSO) are drawn from this pool with only modest familiarization with the more formal elements involved in traditional occupational health and safety programming. The objectives of this paper are to review the evolution of DSO requirements and describe opportunities to develop an educational pipeline to build the supply of scientific diving professionals.

The History of Scientific Diving Regulation

The first scientific diving safety program was developed at Scripps Institution of Oceanography, La Jolla, California in 1954. Programs followed at other facilities, often fostered by personal contacts between interested individuals taking the lead. The person who would become the diving officer or DSO was typically a strong diver, usually with instructor credentials and teaching experience. Educational backgrounds varied widely, from practical to academic. Ancillary skills were similarly variable, often reflecting the focus of the institution. There was little oversight in these early programs. Regulations were generally based on resident expert experience or, when available, expert consensus. The evaluation of individuals and operations relied heavily on evaluator experience and interest. Many approved procedures were consistent with or developed from those of other diving groups, primarily the recreational and military communities, but also the commercial community for certain operations. The standard of the scientific community was marked by independence and flexibility to meet shifting targets.

The autonomy of the scientific diving community in the United States was seriously threatened in 1975. The United Brotherhood of Carpenters and Joiners of America, supported by the American Federation of Labor and Congress of Industrial Organizations (AFL-CIO), petitioned the Federal Government to issue an emergency temporary standard (ETS) applicable to all professional diving operations. The ETS was issued on June 15, 1976 (effective July 15, 1976). While the ETS was successfully challenged in the U.S. Court of Appeals and withdrawn (November, 1976), a permanent standard with a very similar structure was made effective October 20, 1977 against minimal serious opposition. This rule exempted recreational diving instructors but not diving scientists.

The approval of the new permanent standard finally caught the attention of the scientific diving community. A coalition was organized in 1977 with the express intent of fighting the inclusion of scientific diving under what was perceived to be a commercial diving standard. Arguments were submitted to the federal Occupational Safety and Health Administration (OSHA) in October, 1979. The coalition requested an exemption for scientific diving, citing the established history of self-regulation and a lower incident rate than that demonstrated by the commercial diving industry. After extensive public discussion and deliberation, an exemption for scientific diving was granted, effective November 28, 1982.

The American Academy of Underwater Sciences (AAUS) was formed out of the coalition group that won the exemption. The organization was incorporated in California in 1983 to support the ongoing autonomy of the scientific diving community.

The situation was not dissimilar in Canada, where the Canadian Association for Underwater Science (CAUS) was founded in 1983 to improve communication within the scientific diving community and address regulatory issues. The issues arose following the 1982 release of the Canadian Standards Association Z275.2 "Occupational Safety Code for Diving Operations" that arbitrarily encompassed much of scientific diving through broad definitions (Sparks, 1985; 1986). The strong historical record of scientific diving safety (Sparks, 1984) was not sufficient to protect autonomy. While text was put in a later revision of the Canadian Standards Association standard stating the equivalency of the CAUS standards, the regulatory authorities did not embrace the concept of autonomy for scientific diving to the extent seen in the United States.

The increased involvement with regulatory authorities did benefit the scientific community. Procedures and practices were reviewed, some modified and some more carefully defined to reflect general health and safety sensitivities. For some, this meant learning a substantial amount of arcane language from another field. The advantage of bringing OSHA culture to scientific diving was the opportunity to make the needs and nuances of scientific diving community more understandable to the regulatory authority.

Diving Safety Officers Credentials

DSOs were once hired almost exclusively based on their diving credentials. These would generally include diving skills (from air diving through advanced technologies), support skills (e.g., gear service, vessel operations), and teaching qualifications. This skill set was unusual enough that it was sometimes practical for other non-diving-related qualifications to be waived or accommodated as the case required. Sufficiently large scientific diving programs would be the sole responsibility of the DSO. Small diving programs could find DSOs with a range of non-DSO responsibilities, from lab technician to terminal degree academic.

Since the 1980s, the administration of scientific diving programs has increasingly been incorporated into broader occupational health and safety programs. This tends to increase the pressure for cross-qualification and a mix of responsibilities. This is reflected in job postings in which the desired qualifications seem to conform more to those of a traditional safety officer than a DSO. The following text was extracted (and anonymized) from a university job posting released in January 2008:

> *DIVING AND BOATING SAFETY OFFICER / OCCUPATIONAL HEALTH AND SAFETY SPECIALIST*
>
> *Position Summary: Diving and Boating Safety Officer/OHS Specialist position opening to manage all diving and boating operations for all campuses and leased spaces. Selectee will serve as the University Diving and Boating Safety Officer plus assist Safety Coordinators, Assistant Director, Risk Manager and or the Director in routine safety and health duties. Some of these other duties may include training, safety inspections, and waste disposal operations, developing manuals, policies and emergency response.*
> *Minimum Qualification: High school diploma, five years appropriate experience, computer skills to manage diver databases, excellent mechanical aptitude, organizational skills, able to communicate effectively both verbally and in writing, able to meet the medical requirements to be a diver in good standing, certified scuba diving instructor and meet the criteria to be a scientific diver. Prefer additional OSHA safety training history or basic safety courses.*

While the most qualified candidate would undoubtedly be selected, it is unclear if diving qualifications would be the most important factor. The threshold for diving experience in the example above is extremely modest. A certified scuba instructor able to meet the criteria to be a scientific diver may not have any direct experience in scientific diving. Such a person may learn the regulations and guidelines, but may not be in the best position to evaluate and promote operational safety in a scientific diving program. The range of skills requested in the posting, however, could make it possible for other experience to outweigh diving experience. This should be a concern within the scientific diving community.

Discussion

Diving Safety Officer Responsibilities

DSOs have many areas of responsibility. Direct contact with program divers include teaching, skill evaluation, and risk assessment of individual divers and dive plans. Administrative responsibilities include diver enrollment, diver activity tracking, and budgeting. Communication responsibilities include internal interaction with program divers, participating departments, and the institutional administration. External communications will be required with professional organizations, equipment manufacturers, regulatory agencies, and sometimes other subject matter experts. Ancillary responsibilities may include equipment maintenance, vessel operation, or a range of non-diving health and safety obligations.

Mastering the various elements requires a broad base of experience and confidence. Ensuring the capability and readiness of individual divers and dive teams is clearly the most important goal. The path to this endpoint can vary. Experience in teaching recreational diving is not necessary to develop the critical skill set, but the experiences of working with students can effectively sharpen both rapid assessment and rapid intervention capabilities. Extensive, direct involvement in scientific diving is extremely desirable to understand both operations and risk assessment. Leadership experience is of further value for appreciating the range of capabilities expected in any group of divers and understanding how to strengthen team performance. Active involvement in organizations such as AAUS improves the understanding of issues faced within the scientific diving community. Experience across multiple levels of diving or multiple diving disciplines can improve capabilities of risk assessment and incident review and increase flexibility to deal with new situations. A strong understanding of health and safety rules and procedures will facilitate successful interactions with regulators and likely protect the institution from aggravation that could spill into other areas.

The critical factors of a successful DSO are experience, competence and initiative. A person with high scores on these scales as they regard diving will likely either be ready to take on the responsibilities of a DSO or will work effectively to overcome any shortcomings. The question is whether the community is doing as much as possible to facilitate the development of those drawn to the field. It is in our own best interest to nurture a strong pool of candidates able to become scientific diving leaders.

Diving Safety Officer Integration

A large number of DSOs come up from within scientific diving programs, gaining practical experience with the administration from the user side. Depending on the focus of the program and the leadership qualities of the existing DSO -- the primary source of leadership support within the institution -- the lessons learned can provide a variable foundation.

An important role of professional organizations like AAUS is to augment the support available at an institutional level. Communications provide the core pathway. Annual meetings offer a superb opportunity for professionals and fledgling professionals to meet colleagues and establish networks that can be accessed for future need. AAUS strives to make the annual meetings useful to the entire membership. Students can get presentation experience and established members can scout for new talent. Fledgling professionals get extensive support through direct training programs. These include the New DSO Orientation and the DSO Certification Workshop. Full papers from the individual presentations are included in the proceedings of each meeting for the future use of all, whether or not they were able to attend the meeting.

Annual meetings provide an excellent opportunity to bring groups together, but more frequent communications are necessary to disseminate timely updates and new information. The publication *The Slate* served this role as a quarterly release for AAUS through 2007. Recognizing the need for a more frequent (and cost-effective) vehicle for communication, the *E-Slate* was established in June 2006 as a monthly electronic newsletter for the membership. The frequent release dates and flexible structure provides a useful tool in updating the membership on business, developments and other items of interest. DSOs are also able to forward the *E-Slate* throughout their institutions, minimizing the demands on their time to maintain regular internal communications.

Areas for Continuing Development

There are several areas that require further development to best serve the scientific diving community. The virtual office capabilities of the AAUS website can play a more important role in bringing the community together both to learn and to reduce the administrative load on all.

Efforts to facilitate submission of dive records, both by divers and by DSOs, can reduce the burden on DSOs and improve the documentation of scientific diving safety. Similarly, establishing an accessible central record of scientific diver, DSO and institution status could be a valuable service to the community when transfer and reciprocity questions frequently arise. The provision of new and evolving opportunities for continuing education could also be helpful to both individual divers and member institutions. The documentation of continuing education is an increasingly popular requirement in many applications. Developing diving-related programs reduces the burden on member organizations and DSOs, increases outside awareness of the efforts and nature of the academy, and can aid in developing the pipeline for new scientific diving professionals. Web-based training programs represent a tremendous opportunity to combine training, education, communication and documentation. Once established, programs can be completed, scored, feedback provided and statistics collected with minimal additional effort. Topics addressed can range from general to specific interest and from basic to advanced study.

Developing and updating the kinds of materials and programs discussed above requires technical support and manpower. In many cases, the materials are less the problem than the manpower in adapting the material to the correct platform. It is here that the power of leveraging resources becomes attractive. Programs can be developed to provide a training opportunity for students that also serve the needs of the academy -- the long term need of expanding the pool of scientific diving professionals and the short term need of manpower.

Scholarship Training Programs for Students

AAUS currently provides scholarship support for both doctoral and master level students. The current programs offer modest stipends on a competitive basis for individuals employing scientific diving to conduct their academic research or studying diving relevant to the scientific diving community as their academic research. The projects being funded are independently generated and, while valuable, are not selected for the benefit of the academy. Expansion of the scholarship offerings could allow AAUS to address some of the internal needs in program development and increase the educational opportunities for students. An example of how this can work is provided by the AAUS *E-Slate*, a vehicle developed by capitalizing on the time and energies of a Divers Alert Network intern (Roxanne Robertson) posted to AAUS for a three month internship in the summer of 2007. The DAN-AAUS collaboration demonstrated how a relatively small investment could provide an educational opportunity in scientific diving for an interested student and manpower to complete projects of specific interest to the academy.

Program options could be designed for both undergraduate and graduate students (Table 1). The initial effort would best be directed at undergraduate or immediately post-graduate students. This would augment but also be completely distinct from the current scholarship programs funding master and doctoral level research projects.

The undergraduate research internship scholarship program would include four core elements - academic, communications, administrative and diving. The academic component would include diving medicine and physiology, diving safety techniques, and research techniques. The communications component would include oral, written, and public speaking elements. The administrative component would include orientation to DSO tools, institutional diving procedures, and health and safety regulations. The diving component would include diver safety training, underwater research techniques, and a transition to leadership involvement if competency and qualifications allow.

Table 1. Proposed scientific diving research scholarship programs

Element	Undergraduate Research Intern	Graduate Student Researcher
Level	undergraduate/immediate post-graduate	master
Duration	3-4 months	12 months
Location	assigned placement at AAUS central office or organization member site	home institution or placement
Mentorship	academic with scientific diving interest	academic with scientific diving interest
Effort	split between AAUS projects and host site projects	research project pertinent to scientific diving health and safety (major paper or thesis project/sub-project)
Application	competitive process	competitive process
Prerequisites	established scientific diver and clear interest in science and diving	established scientific diver with interest in relevant research topic
Academic status	academic studies with good fit for host institution	enrolled in or having applied to graduate program
Cost	$3,000-$5,000	$5,000-$7,500

The graduate student researcher scholarship program would be tailored to the skills and interests of the successful candidate as driven by the thesis topic. The research question could be independently generated but would more likely be adapted from a series of possible questions of interest to the academy provided in the announcement of the competition. The primary focus would be on the academic component, but, given the nature of the question, this might well include substantial elements classified as administrative or diving in the undergraduate program description. The recipient would also have to demonstrate communications skill through articles written for AAUS publications and presentations given within and without the scientific diving communities.

The proposed scholarship programs could be funded in part by user fees charged for certifications provided upon the successful completion of continuing education web-based training programs. It is expected that individual members and divers operating under the auspices of organizational member institutions could access the programs at no charge. A modest charge could be levied, however, for those without membership affiliation. Continuing education requirements are associated with an increasing number of certifications. AAUS offerings would be of educational value and increase the Academy profile in the larger community.

Conclusions

AAUS can demonstrate a substantial history of commitment to scientific diving. A current challenge for the academy is to ensure an adequate supply of new professionals to support the needs of institutional programs. Expanding the opportunities for professional development through new scholarship programs would create opportunities for individuals interested in learning about scientific and provide an important source of manpower to develop other initiatives necessary to serve the existing scientific diving community. It is expected that directed scholarship programs could benefit the students, the academy and the scientific diving community.

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Assessment of Fish Communities Associated with South Carolina Permitted Artificial Reef Sites with Comparable Natural Hard Bottom Sites by South Carolina Aquarium Volunteer Divers

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Abstract

The Marine Resources Division (MRD) of the South Carolina Department of Natural Resources currently manages a system of 45 permitted marine artificial reef (PAR) areas or sites off the South Carolina coast and within SC estuarine (internal) waters that generated an estimated economic impact of ~$83 million during 2006. Given the environmental and economic importance of this system, MRD is faced with formulating management policies regarding the PAR system despite limited information on these systems. In 2007, MRD in conjunction with South Carolina Sea Grant Consortium funded the South Carolina Aquarium (SCA) to implement and conduct field research to assist MRD in improving the understanding of fish assemblages associated with selected PAR sites and comparable hard bottom areas off of South Carolina. Moreover, the SCA needed to use a cooperative fisheries research approach that involved trained volunteer divers participating in the collection of underwater observational data. Based upon recommendations by the MRD Artificial Reef Program staff, three different sites were selected for surveying per dive trip: one offshore man-made structure, i.e., Y-73, within a PAR area, one comparable offshore natural HB site, i.e., locally called 'The Gardens', and one near-shore PAR site, i.e., 'The Charleston 60'. For each survey site selected, two fish assessment trips per month to each of the three selected sites per trip (i.e., a total of three assessment dives per trip) have been attempted starting in July 2007 with plans of continuing these trips through April 2008. During each fish assessment survey at selected sites, advance trained SCA volunteer divers using the Roving Diver Technique have been recording species observed during the dive as well as other biotic (e.g., notes on fish feeding behavior, etc.) and selected abiotic information related to the survey site on a standardized diver fish assessment survey form. Site information is recorded by the surveying divers after each dive also includes assigning recorded species to specific abundance categories (i.e., four $\log_{10}$ categories), survey depth ranges, temperatures and estimated visibility while surveying. By the end of February 2008, 110 dive surveys have been completed and 127 different fish species have been observed by SCA volunteer divers. In addition to enhancing the understanding of coastal temperate water fish assemblages, project results are also expected to assist MRD in evaluating the utility of cooperative fishery research projects involving trained volunteer recreational divers especially with regard to conducting future fish assessment surveys related to the SC artificial reef sites. Furthermore, the experience that the SCA staff has acquired conducting this study has increased the SCA capabilities to participate in applied cooperative research involving the recreational diving community, MRD and other stakeholder groups.

Keywords: fish communities, fish identification training, roving diver technique

Introduction

The Marine Resources Division (MRD) of the South Carolina Department of Natural Resources developed and currently manages a system of 45 permitted marine artificial reef (PAR) areas or sites off the South Carolina (SC) coast and within SC estuarine (internal) waters. This vast array or system of artificial reef sites enhances saltwater recreational fishing and diving opportunities while directly mitigating heavy utilization impacts on limited natural hard-bottom areas of SC (Robert Martore, MRD, personal communication). It is also apparent that the SC marine artificial reef system, as developed and managed by the MRD, is clearly a significant component of the entire SC coastal economy. For example, during 2006, aggregate expenditures by private boat anglers and charter divers making trips involving these AR sites generated an economic impact (i.e., economic importance) of approximately $83 million in total sales (output) that directly and indirectly supported approximately 1,000 jobs (Rhodes and Pan, 2007).

Given the environmental and economic importance of this system, MRD like other marine resource management agencies is often faced with formulating policies and/or regulatory decisions regarding natural systems and related habitats like their PAR system despite limited information on these systems. Additionally, MRD is interested in evaluating the utility of cooperative fishery research approaches involving fishery stakeholders working in conjunction with professional scientists. In general, cooperative fishery research involves stakeholders such as marine biologists, user groups (e.g., commercial fishermen, recreational divers, etc.) and non-profit organizations "…in the design, conduct and communication of biophysical, gear design and engineering and social science research…" with the nature and degree of involvement varying with the partnership (Hartley and Robertson, 2006).

Using a National Oceanic and Atmospheric Administration (NOAA) grant, MRD in conjunction with South Carolina Sea Grant Consortium funded the South Carolina Aquarium (SCA) starting in 2007 to implement and conduct field research to assist MRD in improving the understanding of fish assemblages associated with selected PAR sites and comparable hard bottom areas off of South Carolina using a core of volunteer divers trained by the SCA. Specifically, this cooperative fisheries research (MRD-SCSGC, 2007) needed to involve trained volunteer divers participating in the collection of observational data "…to examine the importance of variables such as: depth, location, construction materials, age, or productivity on an artificial reef versus comparable natural hard bottom areas." According to Van Dolah et al., 2008, natural 'hard bottom' (HB) areas off of South Carolina's coast are characterized as "…broad expanses of smooth sand bottom interspersed with areas of low relief hard ground and rocky outcrops. These habitats provide hard substrate for a diverse assemblage of sessile invertebrates which, in turn, attract a variety of motile species including many demersal fishes."

In this study, this need or objective was addressed by implementing a cooperative fisheries research project involving volunteer divers trained by the SCA with these divers routinely collecting field observations on fish species associated with selected man-made structures within PAR areas and a comparable HB area starting in 2007.

Since the South Carolina Aquarium (SCA) opened in 2000, the community of SC recreational scuba divers have routinely volunteered to perform various diving tasks essential to the cost-effective operation of the SCA as a private not-for-profit organization. For the safety of these divers, these volunteer SC divers are required to operate under the auspices and specific diving guidelines of the SCA. The SCA currently supports a group of over 90 volunteer divers that start with a minimum training level of advanced certification in recreational SCUBA diving. These divers then progress to acquiring Scientific Diving Standards of the American Academy of Underwater Sciences (AAUS) or equivalent standards with cooperating partners (NOAA) to do open-water diving activities under the auspices of the SCA.

Although SCA volunteer divers were critical to this cooperative fisheries research project, the SCA also has a variety of physical resources to support open-water diving activities. The SCA owns a 28-foot Scout -Abaco Series vessel, 'On the Clock/RV,' with two 225-HP Yamaha outboards that was donated by Scouts Boats, Incorporated, in November 2006. This vessel includes electronics and other equipment critical to offshore activities such as the collection of live specimens. This vessel can comfortably carry up to four SCA volunteer divers and the required two SCA crewmembers for offshore dive trips involving trained volunteer divers. SCA staff and volunteers are CPR and Oxygen Provider certified with full emergency equipment in place on the vessel. Additionally, the SCA has a full collection of scuba diving gear and safety equipment used for SCA's daily educational dive programs as well as open-water diving activities. The SCA also has a nitrox blending station as enriched air-nitrox is needed for open-water diving activities.

Methods

The sites selected for observational dives during this project were limited to areas generally considered accessible by private boat recreational anglers and divers departing from boat ramps (e.g., Wappoo Cut Boat Ramp), marinas, private docks and other boating access points in the greater Charleston areas and within about two hours or less of boat traveling time when departing from these points. This accessibility to the general study area, mainly via the Charleston Harbor, is important when considering the potential impacts of fishing and diving on artificial reef sites and HB areas stemming from the apparently large number of both user groups (i.e., divers and anglers), especially recreational saltwater anglers, in the greater Charleston area.

Based upon recommendations by the MRD Artificial Reef Program staff and results of two pretest trips, three different sites were selected for surveying per dive trip: one offshore man-made structure, i.e., 'Y-73,' within a PAR area, one comparable offshore natural HB site, i.e., locally called 'The Gardens', and one nearshore PAR site, i.e., 'The Charleston 60.' Selection of these survey sites was based upon several criteria including their accessibility from Charleston Harbor, a rough proxy for fishing pressure; location-depth characteristics, i.e., 'offshore' (~80-100 ft) site vs. 'near-shore' (~40–60 ft) site and the need to have one HB site comparable to a selected PAR site. The 'Y-73' is approximately 30 miles offshore from the mouth of the Charleston Jetties. It is a 180-ft steel hull tanker ship sitting straight up in sand at 100 ft and it has a 40-foot relief to the wheelhouse. 'The Gardens,' the project's selected HB area, is a limestone out -cropping about 28 miles offshore that has a maximum depth of approximately 85 ft with the ledge relief averaging about 12 ft. 'The Charleston 60', a PAR site, is a 240-ft broken up barge resting in about 60 ft of water about 18 miles offshore. It was hypothesized that 'The Gardens' HB site was generally comparable with the 'Y-73' PAR site based on comparable depth and a proximity of two miles from each other. No comparable HB was chosen to compare with 'The Charleston 60' due to limitations of three dives per day and the desire for consistency during each trip to aid with the seasonality component.

For each survey site selected, two fish assessment trips per month to each of the three selected sites per trip (i.e., a total of three assessment dives per trip) have been attempted starting in July 2007 with plans of continuing these trips through April 2008. During each fish assessment survey at selected sites, the advance trained cooperating volunteer divers have been using the Roving Diver Technique (RDT) (e.g., Schmitt and Sullivan, 1996). RDT is considered a viable fish assessment technique and has been effectively used with trained volunteer divers to collect scientific observations on marine fish communities and assemblages. This methodology has been utilized by NOAA to help with the management of National Marine Sanctuaries (e.g., Pattengill-Semmens and Semmens, 1998) including Gray's Reef, Flower Gardens, Florida Keys, and others. The RDT is a non-stationary *in situ* survey technique used by individual divers to record fish species observed while freely swimming or 'roving' throughout a designed dive site such as an artificial reef. Besides the simplicity of using RDT with a new project involving volunteer divers, it was also selected because of the SC offshore diving environment, especially dive safety issues associated with strong currents and potential poor visibility (e.g., less than five feet). The RDT is compatible with maintaining a close 'buddy team' as required by the SCA as well as mitigating 'task loading' risks for volunteer divers compared to using transect and fixed station type techniques. RDT also allows divers the flexibility to observe and record a broad spectrum of species during each dive, an approach congruent with the qualitative nature of this cooperative research.

Immediately following each survey dive (i.e., during surface intervals), each survey diver records species observed during the dive as well as other biotic (e.g., notes on fish feeding behavior, etc.) and selected abiotic information related to the survey site on a standardized diver fish assessment survey form. Site information is recorded by the surveying diver after each dive also includes assigning recorded species to specific abundance categories (i.e., four $\log_{10}$ categories), survey depth ranges, temperatures and estimated visibility while surveying. The data recorded on the fish assessment survey form were designed to be comparable to the MRD's survey diver assessment form and the data elements in the Reef Environmental Education Foundation (REEF) database. After each survey trip, a SCA Survey Coordinator is responsible for the timely (e.g., within five days) review of observations recorded on each diver's survey form and subsequent entry into a SCA-designed database.

All divers participating in field survey activities were required to receive training on identification of fish species that might be observed during a survey dive. Moreover**,** given the importance of open-water diving skills, only divers with AAUS or equivalent certification were given extensive advanced fish identification training including testing at the end of the training sessions and then evaluated under field (open-water) conditions before being allowed to participate in an assessment survey. Training materials such as an underwater photo books were also created to aid the volunteers in learning to accurately differentiate between the 256 potential fish species that might be observed at a given survey site. Lecture seminars were given to give more hands-on learning options, and finally practice and testing was used to confirm accuracy of the knowledge of fish identification. Candidate divers also had the opportunity to hone their fish identification skills while diving in our Great Ocean Exhibit, the deepest marine exhibit in North America at 42 ft. This 385,000 gallon tank holds about 45 different marine fish species with many species representing pelagic to dermersal species commonly found off of SC.

Results

By the end of February 2008, 110 dive surveys have been completed and 127 different fish species have been observed by SCA volunteer divers. There have been multiple challenges and some incredible sightings during this project. The challenges started with the survey abilities of the volunteers. We started with a small pool of eight divers that were ready to go offshore with a tested quality of fish identification knowledge from the beginning. During the first eight months of this study that number has grown to 24 qualified divers. This larger group to choose from has helped with scheduling due to limited ability to accurately predict weather conditions more than 48 hours before a planned trip. The weather conditions off the coast of SC are variable and severe. Winds, waves, and visibility change rapidly to create potential dangerous dive conditions. The offshore weather buoys that normally can give a fairly reliable five day forecast were broken for the majority of 2007. Several trips were canceled with short notice and one trip had to return to port due to unpredicted seas. Cold water did lessen the number of qualified divers that wear thermally protected in the 55°F water temperatures in the winter. Survey trips were not possible for two months because the SCA boat trailer was stolen and required boat maintenance for offshore trips was not possible.

There were some interesting sightings during the completed surveys. Schools of adult red drum (*Sciaenops ocellatus*) were observed aggregating during fall of 2007 at the near-shore PAR, 'The Charleston 60.' Red drum is a very important inshore recreational fishing species in SC. Rarely seen goliath grouper (*Epinephelus itajara*) were observed in summer 2007 at the deep water PAR survey site. Red lionfish (*Pterois volitans*) were seen at both deep water sites multiple times. These are an invasive species that are spreading rapidly through the western Atlantic. Fish in the batfish family (Ogcocephalidae) that appear to be palefin batfish (*Ogcocephalus rostellum*) were observed during a fall 2007 survey dive at the deepwater PAR site. Survey divers were not able to verify the species on first sighting because the palefin batfish is similar in appearance to the polka-dot batfish (*Ogcocephalus cubifrons*). *O. rostellum* was reported by Bradbury (1980) in deep water trawl samples collected off of the western Atlantic coast. On a subsequent dive trip, SCA also collected a single specimen for identification purposes.

Discussion

Once the field phase of this study is completed, a final report will be prepared that will include: a) a comprehensive list of observed fish species with frequency, abundance and size group estimates; b) comparison of permitted artificial reef vs. natural hard bottom; and, c) comparison of depth-location and seasonality. In addition to the benefits derived from the technical analysis of the survey data being collected, the active involvement of the MRD staff in this project will allow them to better evaluate the utility of a cooperative fishery research project involving trained volunteer SC recreational divers especially with regard to conducting future fish assessment surveys related to the SC artificial reef sites. Furthermore, the experience that the SCA staff has acquired implementing and conducting this study is increasing SCA capabilities to cost-effectively participate in applied cooperative research with the recreational diving community, MRD and other stakeholder groups. Moreover, given the environmental education mission of the SCA, we now have trained more local divers to better understand the habitats and fish species off of the SC coast. Several of volunteer survey divers are knowledgeable about REEF so we believe that their involvement in this project will help motivate them to continue doing REEF surveys when participating in local recreational diving activities and/or when diving out of state. We also believe volunteer divers that participating in this project can also better communicate with our guests at SCA during educational presentations about what they see offshore.

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Feasibility of In Water Recompression for Precautionary and Treatment of Decompression Illness in Remote Locations

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Abstract

Divers taking a precautionary safety stop at 15 fsw would benefit from using enriched air up to 100% oxygen as the breathing gas. This would enhance off gassing of nitrogen. In water recompression (IWR) to treat decompression illness, has been discouraged by the diving medical community until recently. In the absence of a nearby recompression chamber, it may be medically beneficial to recompression in the water. IWR breathing oxygen rich mixtures up to 100% is a much more effective procedure than breathing air. Commercially available equipment were evaluated. Essential equipment for IWR using oxygen include adequate thermal protection, an adequate supply line, supply of oxygen, a closed-circuit rebreather or an open circuit with full face mask with a non return valve. One major problem with IWR using enriched air is providing adequate oxygen supply and storage. Oxygen concentrators with booster pumps are currently commercially available. The cost of the oxygen generation and storage equipment as well as other required supplies compare favorably with the expense of transporting a single diver one time, from a remote dive location to the nearest hyperbaric facility. Precautionary 100% safety stops and IWR are feasible technically, and 100% precautionary safety stops and IWR are economical.

Keywords: bends prevention, decompression, live aboard, safety stop

Introduction

A safety stop is a halt in the planned ascent to the surface. The duration is typically three to five minutes at a depth of 15 to 20 fsw. The goal of a safety stop is to reduce the risk of decompression injury. So called precautionary safety stops allow time for the body to off gas nitrogen and add an extra margin of safety. Precautionary safety stops are usually taken breathing air but are more beneficial with enriched air and likely to be most beneficial breathing 100% oxygen. Divers taking a precautionary safety stop at 15 fsw would benefit from using enriched air up to 100% oxygen as the breathing gas. Safety stops with enriched air up to 100% would be especially useful for divers participating in multiple dives over multiple days such as divers on live aboards and/or dive instructors and/or underwater scientists or commercial divers.

If one compares critical nitrogen in air versus 100% oxygen on a hypothetical dive to 60 fsw for 60 min with a five minute precautionary safety stop, the 100% oxygen dive is comparable from a perspective of critical tissue nitrogen of a dive for only 48 min.

In addition, in water recompression (IWR) has been suggested as a treatment alternative to transporting a diver suffering from mild to moderate decompression illness in a remote location far removed from a recompression chamber (Pyle and Youngblood, 1995; Walker, 2002; Francis, 2005).

It has been recognized that one of the causes of a failure of decompression sickness to respond to recompression is a delay in treatment. IWR can be administered with air or enriched air up to 100% oxygen. IWR employs water as the recompression pressure, and air supply is by compressors on the dive boat. It has been used successfully in Northern Australia and Hawaii (Pyle and Youngblood, 1995); admittedly there are problems with IWR with air. There are compressed air supply limitations with amateur and semi professionals who have SCUBA or simple portable air compressors. Environmental conditions may not be conducive to IWR. Considerable depths may be required, and sea state and boat safety may become issues. Hypothermia is a difficult problem to overcome, and there is always the possibility of sea sickness in the diver and/or attendants.

Comparing oxygen to air as the recompression gas, the former is associated with increased nitrogen elimination gradients, additional nitrogen loading is prevented, and there is increased oxygen to the tissues. Importantly, depths and recompression times are reduced.

Let us consider the pros and cons of IWR. Advantages include immediate treatment with little or no delay and results reported have generally been favorable (Pyle and Youngblood, 1995; Francis, 2005). Problems with IWR include gas supply, environmental conditions, hypothermia and the sea state.

Barriers to IWR may be divided into four categories, namely, professional, logistical, technologic and economic. Professional barriers involve reluctance or refusal on the part of medical personnel involved in treating patients and establishing treatment protocols to accept this treatment concept. Logistical barriers relate to issues such as boat location and safety during the IWR as well as issues relating to sea state during the treatment. Technological barriers involve the available of the appropriate equipment such as oxygen generators and compressors with boosters to make available the quantity of necessary gas at the appropriate pressures available. Lastly, there are perceived economic barriers which relate to the significant expense of the equipment.

Methods

Barriers to precautionary enriched air up to 100% oxygen safety stops and IWR with enriched air up to 100% oxygen as the treatment gas were explored by evaluating currently available oxygen generators, compressors with pressure boosters, and oxygen storage equipment. The cost of these equipments and supplies were compared to the costs of transportation and treatment of mild to moderate decompression sickness in remote locations.

Results

Precautionary safety stops have been advocated by the diving medical community and dive education and training organizations for years. Currently with the widespread use and availability of enriched air, scuba divers have been utilizing enriched air for safety stops in a haphazard manner. With currently available technology to generate large volumes of high percentage oxygen up to 100%, safety stops with 100% for the breathing gas are now feasible. This is for the small group or individual diver as well as scuba divers taking multiple dive multiple day dive trips, even to remote locations. On a typical live aboard, 20 divers would take five minute safety stops on 100% for up to six dives per day. Available technology can produce the quantities of 100% oxygen for large numbers of dives and divers.

There appears to be a greater acceptance of IWR for mild to moderate DCS in remote locations by the hyperbaric medical community as evidenced by the conclusions of a workshop dedicated to this topic (Mitchell et al., 2005). Furthermore at least three different protocols for IWR using 100% oxygen have been published and used (Pyle and Youngblood, 1995; Walker, 2002).

The cost of evacuating and treating an injured diver with mild to moderate DCS ranges from $8,000 for evacuation and $3,000 to $29,000 for the treatment once transported. The cost of IWR including all the necessary equipment for the treatment as well the equipment to provide 100% oxygen for large numbers of divers making precautionary safety stops is approximately $15,000 (Dick Clark, National Baromedical, personal communication, 2008). On the other hand a cost benefit analysis and risk assessment was performed by Drewry which considered hidden costs of medical legal considerations which affect treating physicians' decision making (Drewry, 2005).

Discussion

It is feasible for scuba divers to routinely take precautionary safety stops on enriched air up to 100% oxygen with currently available technology, even for live aboards with divers making multiple dive multiple day dive trips. In addition, it is feasible and is cost effective to utilize IWR on 100% oxygen for mild to moderate DCS in remote locations.

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Scientific Diving in Europe: Integration, Representation and Promotion

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Abstract

In 2000, the European Union (EU) created the European Research Area with the intention of forming a unified area across Europe that would enable researchers to move and interact seamlessly based on a series of aligned working directives. The EU research network presently consists of 33 countries made up of the EU member states (27 countries) plus an additional 6 non-EU member countries that have associated status. The challenge for European scientific diving has been to integrate existing national programmes through a single organisational structure that supports the promotion of recognized diving standards within European science while advancing the wider acceptance of diving as a research tool. Since 2007, scientific diving in Europe has been overseen by the European Scientific Diving Committee that is based on the principle of promoting the European Scientific Diver and the Advanced European Scientific Diver competencies as the primary European scientific diving standards.

Keywords: diving standards, Europe, scientific diving

Introduction

Scientific diving is an indispensable research tool that supports a wide range of aquatic science disciplines including underwater archaeology and European water body management disciplines (Fischer P, personal communication, 2006; Sayer, 2007a) throughout most global environments (Lang and Sayer, 2007; Sayer, 2007a; Sayer et al., 2007a). In addition to surface supplied sampling from European research vessels, medium and deep water landers, and the application of remotely operated and autonomous underwater vehicles, scientific diving is an essential tool that supports cutting-edge science worldwide both in marine and freshwater environments (Fischer et al., 2007; Lang, 2007; Sayer, 2007a; Sayer, 2007b; Sayer et al., 2007a). Diver-supported aquatic research allows for high-quality, highly-selective, accurately-repeated and ecologically-compatible research (Bussmann et al., 2007; Keskinen and Arponen, 2007; Kuklinski, 2007; Sayer, 2007a; Schröder A and Krone R, personal communication, 2007). Today, scientific diving is often considered an essential tool for many research projects, predominantly in depth ranges of between 0 and 50 m water depth but with the technical capability of now going deeper and longer (Lang and Smith, 2006; Sayer, 2006). As such, diving is a research tool that is employed widely throughout Europe in support of a large number of high-quality research programmes (Sayer, 2007a).

Scientific diving throughout the world has an extremely good safety record (Carter et al., 2005; Lang, 2005; Sayer, 2005; Sayer and Barrington, 2005; Dardeau and McDonald, 2007; Sayer et al., 2007b). However, through its very nature, many national legislators view occupational scientific diving as carrying a higher than normal risk (Sayer, 2004; Sayer and Forbes, 2007). Because of this, many countries insist on scientific divers having varying levels of training and qualifications in order to dive as part of their work. Although these qualifications have many commonalities between nations, it has been evident for some time that national considerations may impede the ability to use scientific diving easily between all nations that may partner pan-European research programmes. As well as imposing financial penalties on programmes wishing to engage in trans-national diving research programmes, different national approaches may now infringe European Union (EU) working directives (e.g., Directive 2005/36/EC that came into force in 2007).

As of the beginning of 2008, there are 33 countries that are eligible for European science funding with the potential for undertaking trans-national scientific diving projects. These are the present 27 EU member states (Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Republic of Cyprus, Romania, Slovakia, Slovenia, Sweden, Spain, United Kingdom) plus six member candidate or research-associated states (Iceland, Israel, Lichtenstein, Norway, Switzerland, Turkey). Scientific diving as a research tool and/or an 'at work' activity is approached differently in many of the 33 countries and may range from highly organised and co-ordinated activities integrated fully into that nation's health and safety legislation to an activity undertaken without any organisational framework. In addition, some nations may have specific diving at work legislation that fails to recognize certification and/or standards from other countries.

The need to develop an international and resilient research platform for scientific diving within the EU has become established because of the increasing need to address scientific issues on pan-European scales while advancing scientific excellence in diving-supported programmes within the EU-research framework. In addition, a more integrated approach will establish an organisation and organisational structure for scientific diving within the developing European Research Area (ERA). In all circumstances, it is essential to ensure that all advances in scientific endeavour and achievement are achieved within acceptable safety levels. For these reasons, leading scientists who employ diving techniques within eight European countries (UK, France, Sweden, Finland, Poland, Italy, Belgium and Germany) started an EU-wide initiative in 2007 to establish a pan-European platform to support scientific diving. As well as promoting and enhancing scientific excellence within the field of diving-supported aquatic research, the initiative also sought to establish harmonized rules and guidelines. Based on two international workshops in Berlin and Bremerhaven (Germany), the European Scientific Diving Committee (ESDC) was established and formally constituted in October 2007 in Bremerhaven.

This account outlines the structure and operational basis of the ESDC, the practical competency standards for scientific diving in Europe, and the proposed methodology for promoting scientific diving within the European research community.

Discussion

European Standards for Scientific Diving

The requirement to establish standard competencies throughout Europe was foreseen, and in the late 1980s scientists who used diving in their research sought to initiate the harmonization of the rules and procedures for scientific diving in Europe. In 2000, during the final meeting of that group in Banyuls-sur-Mer, France, this effort finally resulted in the development of two European scientific diving standards: that of European Scientific Diver (ESD), and of Advanced European Scientific Diver (AESD) (Table 1). The quality and widespread acceptance of these draft standards by much of the

European scientific community has resulted in them already becoming adopted within the health and safety legislation of some EU countries.

European Scientific Diving Committee (ESDC)

Since the publication of the 2000 draft standards, there have been an increasing number of aquatic-aligned EU projects on worldwide relevant topics like global change and biodiversity, but also an increasing number of international relevant archaeological projects with respect to the UNESCO world heritage program. This increase in diving-supported research highlighted the need to develop an international and resilient research platform for scientific diving within the EU. Doing so would act to facilitate scientific excellence in diving-supported programmes within the EU research framework. In addition, it would establish a formal organisation and organisational structure within the developing ERA. In all circumstances, it was essential to ensure that all advances in scientific endeavour and achievement were achieved within acceptable safety levels.

For these reasons, in 2007, leading scientists who employ scientific diving techniques within eight European countries (UK, France, Sweden, Finland, Poland, Italy, Belgium, and Germany) started an EU-wide initiative to establish a pan-European platform to support scientific diving. As well as promoting and enhancing scientific excellence within the field of diving-supported aquatic research, the initiative also sought to establish harmonized rules and guidelines. Based on two international workshops in Berlin and Bremerhaven, Germany in 2007, the European Scientific Diving Committee was established and formally constituted in October 2007 in Bremerhaven. The main objectives of this newly founded ESDC are:

1. To advance underwater scientific excellence in Europe through:
   (a) the facilitation of conferences, workshops, courses and publications where scientific diving is promoted as a research tool;
   (b) the encouragement and support for European funded research networks that employ scientific diving;
   (c) the continual improvement and extension of the methodology of diver-supported research beyond the actual state-of-the-art methods and to seek out and validate new technologies which underpin future gains in knowledge of underwater science.

2. To promote safety in scientific diving across Europe through:
   (a) facilitating a pan-European framework that exists to promote industry best practice in scientific diving;
   (b) promotion and support for the establishment of national scientific diving committees where they don't exist;
   (c) the development and maintenance of a European database of scientific diving activities.

3. To encourage international mobility in the European scientific diving community through the implementation of a practical support framework by:
   (a) promoting the widespread recognition of the existing ESD and AESD as the minimum standards for scientific diving by assuring their acceptance as the primary qualifications for scientific diving in Europe;
   (b) becoming established as the recognised European body with responsibility to provide advice and guidance on the acceptance of existing standards within national and international legislative processes;
   (c) facilitating, promoting and maintaining communication with and between present national scientific diving organisations and the National Scientific Diving Committees.

The ESDC is working toward a format whereby the status of membership is indicative of the present structure that supports scientific diving at the national level. The three different levels of membership are: full members, associated members, and committed members.

1. Full members (FM): FM must have implemented the ESD and AESD qualifications as the primary standards for scientific diving in their country. FM must accept ESD/AESD certificates from other 'full member' states. FM must have in place a committee that represents their national scientific diving community and has a recognised status with a national authority that has responsibility for vocational scientific diving.

2. Associated members (AM): AM must have in place a committee that will represent their national scientific diving community, which formally represents the national scientific diving committee. AM must be supported by, or seeking support from, a relevant national authority and must demonstrate that there is an intention to implement the ESD and AESD qualifications as the primary standards for scientific diving in their country.

3. Committed members (CM): CM should demonstrate the support of their national scientific diving community, and the intention and mechanism to develop a national committee that will represent their scientific diving community. CM should furthermore demonstrate an implementation framework by which the ESD and AESD qualifications can become assured standards for scientific diving in their country and recognised by relevant national authorities.

The Development of the ESDC

A main goal of the ESDC for the near future is its recognition as the primary EU panel for scientific diving across Europe with the responsibility for promoting underwater science. An EU-wide coordination of diver supported research activities (presently mainly conducted at the national level) will provide the framework for significant synergistic opportunities with concomitant enhanced quality and quantity of aquatic research across Europe. In bringing together the national member organization for scientific diving, the ESDC wants to:

- Share information on scientific diving across Europe by initiating regular workshops;
- Identify national and international problems with respect to the application of this key method in aquatic science and to find appropriate solutions;
- Develop common strategies for synergistic diver-supported research across Europe;
- Develop strategies to facilitate improved access and efficient use of scientific diving infrastructure across Europe;
- Compile and publish position papers to give advice on strategic and scientific policy related to aquatic science and technology at the European level.

Besides the necessary conceptual work with respect to the development of EU harmonized guidelines for scientific diving (e.g., for conducting scientific dive missions from European research vessels, from land based European research stations, or in extreme environments, such as the polar regions), the ESDC shall, in particular, promote and initiate benchmark projects in the field of diver-supported aquatic science. To achieve this, the ESDC will become established as the recognised forum of European experts in scientific diving in order to provide a think tank based synergistic environment for prospective developments in the future of this research discipline.

Based on the above goals, it is the intention that the ESDC will initiate and support national but EU open training programmes (basic and advanced skills) for students and scientists in addition to special training courses for benchmark techniques in research diving.

Discussion

Overseeing and working across Europe toward achieving high standards of scientific delivery using diving techniques will present a considerable challenge given the range of nationalities and national approaches to occupational scientific diving. However, EU working directives and the EU research funding frameworks present methods by which co-ordination and integration may be accommodated. It is hoped to build rapidly on the solid foundations of agreed scientific diving competencies to deliver a strong, productive and safe scientific diving community in Europe.

In October 2008, scientific diving in Europe was accepted to become an approved Panel of the Marine Board of the European Science Foundation. Scientifc diving in Europe will, therefore, from now on be overseen by the Marine Board - European Scientific Diving Panel (MB-ESDP)

Table 1. A summary of the main competency requirements for the European and Advanced European Scientific Diver standards. The main differences between the two standards are highlighted in bold italics.

EUROPEAN SCIENTIFIC DIVER (ESD)	**ADVANCED EUROPEAN SCIENTIFIC DIVER (AESD)**
An ESD is a diver capable of ***acting as a member of*** a scientific diving team. They may attain this level by either a course or by in-field training and experience under suitable supervision or by a combination of both these methods.	An AESD is a diver capable of ***organising*** a scientific diving team. They may attain this level by either a course or by in-field training and experience under suitable supervision or by a combination of both these methods.
- show proof of ***basic*** theoretical knowledge and a ***basic*** understanding of: 1. diving physics and physiology, the causes and effects of diving-related illnesses and disorders and their management; 2. the specific problems associated with diving to and beyond ***20 m***, calculations of air requirements, correct use of decompression tables; 3. equipment, including personal dive computers and guidelines as to their safe use; 4. emergency procedures and diving casualty management; 5. principles of dive planning; 6. legal aspects and responsibilities relevant to scientific diving in Europe and elsewhere.	- show proof of theoretical knowledge and a ***comprehensive*** understanding of: 1. diving physics and physiology, the causes and effects of diving-related illnesses and disorders and their management; 2. the specific problems associated with diving to and beyond ***30m***, calculations of air requirements, correct use of decompression tables; 3. equipment, including personal dive computers and guidelines as to their safe use; 4. emergency procedures and diving casualty management; 5. principles ***and practices*** of dive planning ***and the selection and assessment of divers***; 6. legal aspects and responsibilities relevant to scientific diving in Europe and elsewhere; 7. ***dive project planning.***
- be fully competent with/in: 1. diving first aid, including CPR and oxygen administration to diving casualties; 2. SCUBA rescue techniques and management of casualties; 3. the use and user maintenance of appropriate SCUBA diving equipment.	- be fully competent with/in: 1. diving first aid, including CPR and oxygen administration to diving casualties; 2. SCUBA rescue techniques and management of casualties; 3. the use and user maintenance of appropriate SCUBA diving equipment ***including dry suits and full-face masks;***

	4. ***basic small boat handling and electronic navigation;*** 5. ***supervision of diving operations***.
- be fully competent with: 1. search methods; 2. survey methods, both surface and sub-surface, capable of accurately locating and marking objects and sites; 3. the basic use of airbags and airlifts for controlled lifts, excavations and sampling; 4. basic rigging and rope work, including the construction and deployment of transects and search grids; 5. underwater navigation methods using suitable techniques; 6. recording techniques; 7. ***acting as surface tender for a roped-diver;*** 8. sampling techniques appropriate to the scientific discipline being pursued.	- be fully competent with: 1. search methods, ***such as those utilizing free-swimming and towed-divers together with remote methods suitable for a various range of surface and sub-surface situations;*** 2. survey methods, both surface and sub-surface, capable of accurately locating and marking objects and sites; 3. the basic use of airbags and airlifts for controlled lifts, excavations and sampling; 4. basic rigging and rope work, including the construction and deployment of transects and search grids; 5. underwater navigation methods using suitable techniques; 6. recording techniques; 7. ***roped/tethered diver techniques and various types of underwater communication systems such as those utilizing visual, aural, physical and electronic methods;*** 8. sampling techniques appropriate to the scientific discipline being pursued.
- show proof of having undertaken ***70*** open-water dives to include a minimum of: 1. ***20*** dives with a scientific task of work such as listed above; 2. 10 dives between ***15 and 24 m;*** 3. ***5*** dives ***greater than 25 m;*** 4. 12 dives in the last 12 months, including at least 6 with a scientific task of work.	- show proof of having undertaken ***100*** open-water dives to include a minimum of: 1. ***50*** dives with a scientific task of work such as listed above; 2. 10 dives between ***20 and 29 m;*** 3. ***10*** dives ***between 29 m and the national limit;*** 4. 12 dives in the last 12 months, including at least 6 with a scientific task of work. 5. ***20 dives in adverse conditions such as currents, cold or moving water.***

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The Difficulties of Sampling in Underwater Caves in the Bahamas: an Exercise in Ingenuity and Survival

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Abstract

Currently flooded horizontal, fracture, and vertical caves in the Bahamas, commonly referred to as blue holes, are very dangerous underwater systems to dive and study. Caves with their main entrances in the ocean out on the carbonate platform, are subject to tidal cycles and storm surges that can result in sudden (in <1 minute) changes in flow direction, from outflow to inflow that can trap divers within the caves. Horizontal and vertical caves that have their main entrances on land, offer different challenges including silting composed of biological silt, anthropogenic pollution in the form of sewage, as well as high concentrations of microbial toxins. Un-tethered methods were designed for collection of water samples for microbial and geochemical analyses, as well as a rock corer powered by compressed air from a spare scuba bottle. These methods allow maximum collection opportunities in minimum amounts of time, and with minimal disturbance of the environment.

Keywords: bacteria, cores, diving, sterile, vacutainers

Introduction

Diving in caves in Florida, Australia, and the Bahamas each present different environmental challenges for the scientific diver. The submerged marine and inland caves in the Bahamas however, have provided some of the greatest physical and biological challenges of all (Benjamin, 1970; Palmer, 1986). Caves with their main entrances in the marine environment on the carbonate platform are subject to high tidal water flow (Schwabe and Carew, 2004). These flows become further exaggerated when the entrance is restricted. As tide is rising large volumes of ocean water are forced through these restricted entrances resulting in the formation of tidal vortexes (Figure 1). When the cave systems are draining, they form a dome of water on the surface above the entrance. The cause of this phenomenon is related to the water levels within the island never catching up with the tidal levels outside on the platform; in other words, one or the other is always lagging behind. This endless tidal dance, although detrimental to human visitors, is of vital importance to all the marine life forms that have taken up residence in the flooded caves. The tidal lag is caused by tidal water piling up on one side of the island, against low permeable rocks. The diurnal flood of marine water brings in life supporting food, whereas on the ebb tide outflow flushing out oxygen depleted and waste-laden waters. Each cavern with its marine openings on the carbonate platform has its own tidal clock. Some flow directions lag behind the tide by two to four hours so it is necessary, for safety reasons, to become familiar with the specific cave of interest. Storm events can also change the flow direction in a very short time frame (as little as 45 sec). During low tide, when water is draining out of a cave, sudden storm events can push water onto the platform. This generates a hydraulic head that is greater than the one inside the island, thus causing the flow in the cave to reverse, and water floods into the cave, creating a tidal vortex. However, as soon as the storm waters retreat off the platform and the hydraulic head is reduced, just as quickly, the flow of the cave returns to its original state prior to the storm. Because divers in a cave cannot be aware of what the weather is doing on the surface, this phenomenon can be deadly.

Figure 1. Tidal vortex forming in the entrances of caves on the high tide.
Photo credit: Stephanie Schwabe.

Horizontal and vertical caves (Schwabe and Carew, 2004) present environmental dangers that can be equally as lethal to an unsuspecting diver. Vertical caves, referred to as black holes, (Schwabe and Herbert, 2004) house very large populations of bacteria, (5 tons dry weight), that can float on the major density interface between marine and brackish water. These bacteria produce large amounts of hydrogen sulphide as waste from organic decomposition and they reduce pH to levels as low as 2 (Schwabe and Herbert, 2004). Aside from the disorientation caused by diving in zero-visibility water, these often very hot, acidic, black waters are challenging to a diver, especially when the sampling protocol requires samples from within this environment. We are not aware of the safe limits for exposure to hydrogen sulphide, so expedience when working within this layer is a must. In horizontal caves, bacteria produce very large amounts of biofilm. This biofilm sticks to all rock surfaces within the marine section of the water column and accumulates in metre thick layers on the floor of the cave. A diver entering into such a system will have crystal clear visibility heading in, however, when heading out of the cave, the diver will find that the guideline will disappear into a brown wall. The only way out is to forge ahead blindly using the guideline to feel the way out. Psychologically, not many divers are comfortable doing this.

Anthropogenic pollution is another major problem within the waters that flow through the caves. On the outer islands it is not uncommon for septic systems to be located right next to a cave entrance with the overflow pipes leading into the cave water. Depending on the amount of effluent entering the system, an unsuspecting diver may experience full body septicaemia within 24 hours as it happened with my late husband. Without immediate treatment, the diver may die. Villages often use this same water for drinking and, generally during droughts, it is not uncommon to hear about several cases of gastroenteritis.

Collecting water and rock samples within such dynamic environments requires a great deal of thought about the methods and the equipment to be used. The objective on most of these scientific dives is to collect sterile water samples from precise locations within the water column, as well as to obtain rock cores. Another important requirement for the water samples is that they be collected as quickly as possible. It is important to be able to say that your samples represented a small and specific time frame within a constantly changing water column. To solve this problem, sterile red-top blood tubes, or vacutainers, which are under vacuum, were used along with silicon tubing and intravenous solution bags. All of these items can be sterilized and reused. They are robust, so are not easily punctured by the sharp rocks and they withstand the pressure in deep environments. They are also easy to handle and organize.

Rock cores were generally collected far from the cave entrance and in highly decorated caves. To do this required designing an un-tethered drilling system. The drill, an air-wrench used formerly to loosen lug nuts from cars tires, had its internal metal components replaced with machined stainless steel. All paper washers were replaced with plastic and a stabilizing base was designed to support the drill shaft during the drilling process. Cave walls are not smooth and without this base, which was made from PVC, the likelihood of snapping the diamond coated drill shaft during use was great.

Methods

Water Sampling

The water column within horizontal, vertical, and the land based component of fracture caves (Schwabe and Carew, 2004) are highly stratified. Stratification layers have been observed to be as thick as 4 or 5 cm or as thin as 1 or 2 cm (Figure 2). These layers are visible because they act as traps for particulate organic matter (POM), and are zones where macro- and microfauna become trapped (Schwabe, 1999). Although the major density interfaces are known to be re-established within 24 hours of being disturbed, it is not known how quickly these finer layers re-establish themselves after a disturbance, so it is important, that minimal disturbance occurs if these layers are to be individually sampled.

Figure 2. Silicon water sample tubes attached to a line attached to the ceiling and floor. Note the stratification of the water column. Photo credit: Stephanie Schwabe.

To prepare the site for water sampling, one end of the line from a cave-reel was attached to the ceiling of the cave, usually within the freshwater zone and the other end was secured to the floor in the saline zone (Figure 2). Prior to installing the pre-cut silicon sampling tubes (4.8 mm ID, supplier BDH, UK, USA), the internal volume of the longest of the tubes was determined. The tubes were then filled with filtered, sterile, deoxygenated, distilled water and capped at one end to prevent cave water from entering. The caps come from the three-way-stopcocks (Figure 3) that were used later on in this set up. At pre-selected and measured depths the sampling end of the tubes were then attached to the line in the cave with nylon cable ties, in a criss-crossed pattern. The other ends of the tubing (which were several metres long) were secured to a horizontal base line at the opposite side of the chamber. The criss-crossed pattern of securing the sampling tubes was done to ensure that the end where the cave water enters the tube is parallel to the layers in the water and not drooping into another layer.

Figure 3. Three-way-stopcock used to redirect sample flow into either a red-top tube or IV bag. Photo credit: Stephanie Schwabe.

When it was time to collect the water samples, each tube was flushed with cave water from the intended layer to be sampled. Knowing the volume of the longest sample tube, it was easy to determine when cave water filled the tube. A separate BDH, plastic disposable syringe (100 mL) was used for each collection tube. Water samples for geochemical analysis were collected in 0.5 litre polyethylene 'intravenous bags'. Any hospital supplier supplies intravenous bags. These bags arrive with sterile 0.5% NaCl solution. To prep the bags for the field, the saline solution was drawn out and the bags flushed four times with sterile distilled water to remove all remaining salt. The injection port on the IV bag was cut away and a sterile 3-way stopcock (Sherwood Medical) was attached with two nylon cable ties. After flushing the bag, a sub-sample was removed and conductance was measured. It was found that 100 mL of distilled water, flushed through the empty bags three times, was enough to remove all residual salt solution. Four additional flushes were conducted to ensure zero contamination. Following the flushing, all water and air was evacuated from the bag.

A gas permeability test was performed on one bag prior to use in the cave. This test was done to determine whether oxygen would permeate the bag, and if it did, how much time frame we had to work with if the sample was anoxic. Water was boiled and cooled with filtered nitrogen to remove oxygen. This water was then reduced with a few drops of 12% w/v sulphide solution, followed with a few drops of Resazurine (Sigma Chemicals) used as a redox indicator. The bag was then filled to capacity and left on the lab bench in ambient conditions. Approximately three hours later the water within the bag began to turn pink, indicating diffusion of oxygen into the bag. Hence, samples were measured for oxygen content immediately upon removal from the cave environment. Samples were usually processed within 15-30 min, so diffusion of oxygen into the sample would be minimal.

After flushing the tubes, one of the intravenous bags was attached to the sample tube with a three-way-stopcock following removal of the end caps. Water was drawn up into a 100 mL syringe and the stopcock was opened to the IV bag, and the sample expelled into the bag. The syringe was removed and the bag was shaken and the water was sucked out again and expelled into the water surrounding the diver. This procedure was conducted three times for each bag to avoid any dilution by any water that may have been left in the bag. At this point, the sample tube, the syringe and the IV bag all contained sample water and the collection could begin in earnest. Keep in mind that the diver is no where near the sample site when the sampling begins. The IV bag was filled to maximum (600 mL). Used bags were flushed with sterile distilled water, then flushed with 100 mL of 95% w/v ethanol, and flushed again three times with sterile distilled water.

Vacutainers (Red tops)

Smaller volumes of water samples for bacterial counts and bacterial activity were collected using 10 mL BD/Sterile interior/ no additive/red top vacutainers (Figure 4). These can also be purchased from any hospital supplier. Tubes were taped together in strips using silver cloth tape (duct tape), and labelled for different treatments. Do not label the tube because the white label on the tube will come off underwater and you will have no idea what your sample is. The duct tape is placed in such a way as to show the maximum fill level. The tubes, which are under vacuum, are more so at depth. This is important to note because overfilling of the tubes at depth means that the tube will expel their tops before reaching the surface. Although the tubes at the surface can hold 10 mL, at depth it is recommended that the tubes not be filled over 6 mL. Not overfilling also allows room for any type of preservative being added to the sample.

Figure 4. Red-top tubes called 'vacutainer' shown here, are typed into long strips of cloth or duct tape. The duct tape is labelled, not the tube. Photo credit: Stephanie Schwabe.

When the tubes are taped and labelled, a carabiner is attached to the end of all the strips of tape through a hole punched through the duct tape. One can either attach a weight to the carbiner or the tubes strips can be clipped to the diver. The tubes will float straight up in the water column, making it very easy to recognize the differently labelled tubes and making them easier to handle. These tubes were used for a variety of purposes, though primarily for collecting samples for acridine orange direct counts (AODC). These samples were used for determining bacterial numbers in the water. Before sample collection the tubes contained 1 mL of 37% filtered formaldehyde. Some tubes were marked for specific isotope use (isotopes added later). The addition of isotopes was for measuring bacterial activity. Some samples were collected for geochemical analyses, such as hydrogen sulphide. These tubes contain 1 mL of a 20% solution of zinc acetate. Zinc acetate binds with hydrogen sulphide and produces a white precipitate called zinc sulphide. The only substances that these tubes cannot be used with are acids, because a silicon layer, which is used to keep platelets from sticking to the inside of the tube, will dissolve and contaminate the sample.

Rock Corer

Rock core collection was made possible using a rebuilt Clarke air model pneumatic wrench (Figure 5) purchased from a local hardware store. The original metal components within the unit were composed of iron and aluminium, which are not compatible with exposure to seawater. So, all those metal parts were replaced with machined stainless steel. Because the unit is open to water, lubricants are not needed. The air hose for the drill is fitted with an adapter that allows the hose to be connected to the first stage of a scuba regulator. This permits the use of 12 to 15 litre scuba cylinders as the air source for driving the modified air wrench drill corer. Another hose is attached to the exhaust port on the air wrench. At the end of this hose a dispersion unit is attached. This dispersion unit is built from a piece of PVC pipe approximately 8 cm in diameter and approximately 4 cm deep (Figure 6). One end is sealed with a fitting to accommodate the exhaust hose, while the removable exhaust end has a large-diameter plastic screen. Inside this unit a large pore sponge which has been cut to fit the exhaust compartment is inserted.

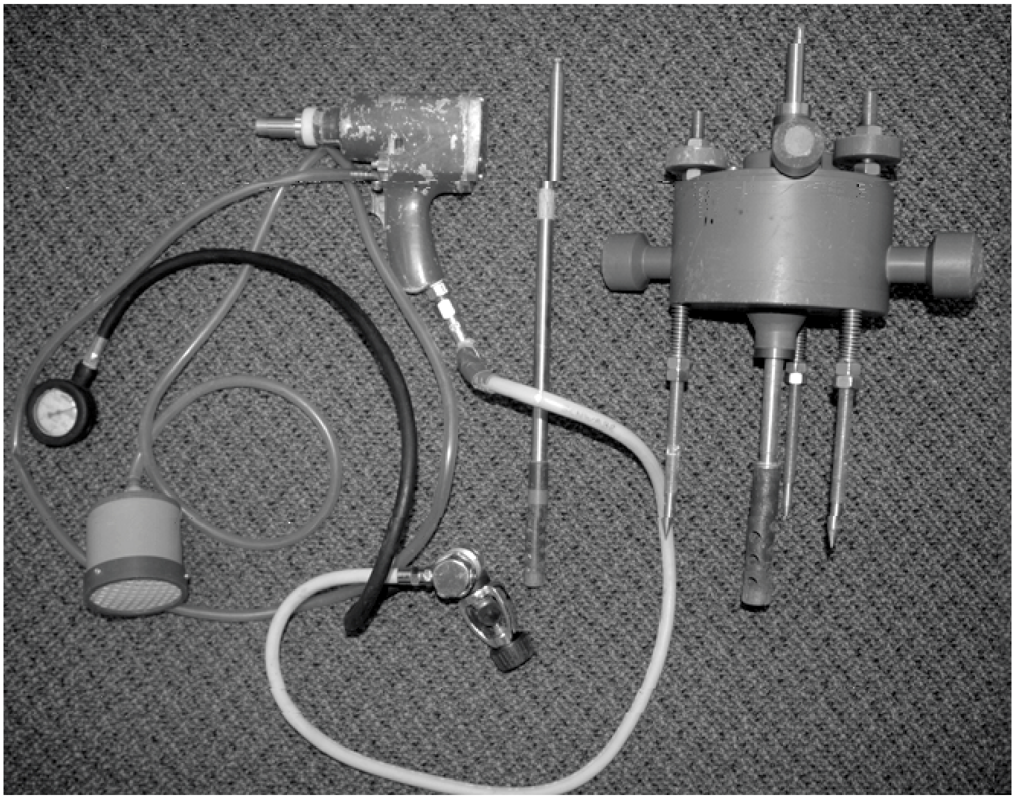

Figure 5. Pneumatic Wrench used to drive the corer. Photo credit: Stephanie Schwabe.

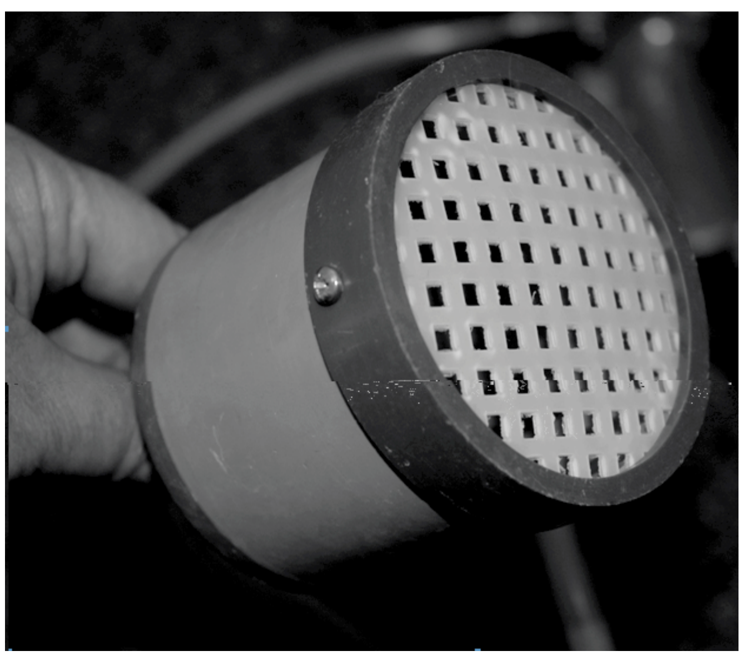

Figure 6. Air diffuser for the pneumatic wrench. Photo credit: Stephanie Schwabe.

The pore size of the sponge must be large or the power of the drill will be reduced, and it is likely that the exhaust hose will be blown off. When in use, this exhaust unit will float above the operator, well out of the way of the sampling area. The object of having this dispersion unit attached to the end of the exhaust hose is to reduce the impact of gas bubbles on the cave ceiling, which will displace cave biota and sediments and thus reduce visibility. Visibility will be reduced in time anyway, and it is for this reason that drilling should be the last project worked on in a cave, especially if you are measuring particulate organic carbon (POC), total organic carbon (TOC), or dissolved organic carbon (DOC). This drill uses a lot of air and you can only count on recovering one core. In addition the release of such a large amount of air in one area will change the geochemistry of the immediate surroundings. Drilling is not a cave friendly activity.

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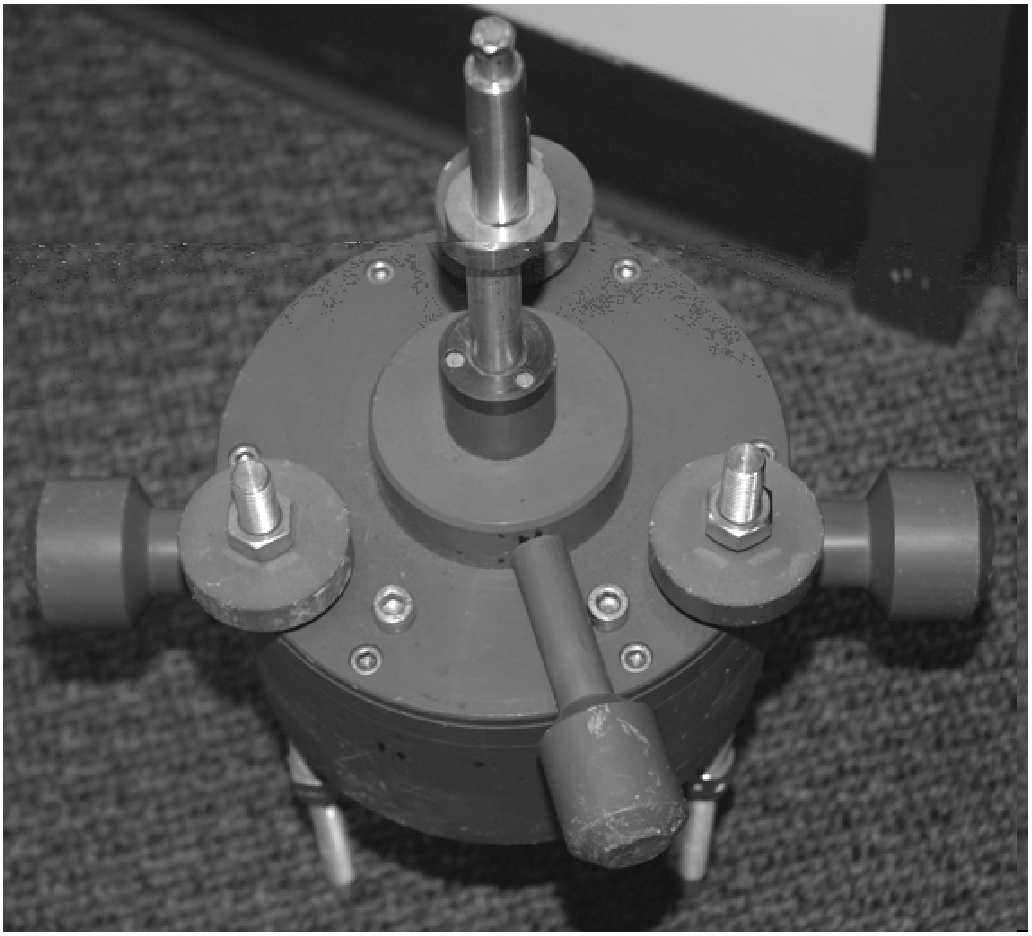

Figure 7b. View from the top of the corer base. The arm is the cam, which moves between the two hexagonal nuts. Photo credit: Stephanie Schwabe.

To remove the rock core from the wall, first remove the base, then insert a 10 cm long stainless steel tube, made to exactly fit the rock core and gently tap on the side of the tube. This will liberate the core from the cave wall. The rock cores were stored for transport in 30 mL, sterile, disposable polypropylene, clear tubes. As soon as possible, after exiting the cave drain the cave water from the storage tubes and insert a sterile polypropylene sponge (supplier BDH).

Discussion

Flooded cave environments can be particularly difficult areas to study in detail even under the best of circumstances and conditions. Cave diving is the world's most dangerous sport (Exley, 1994), and although I do not cave dive for the sport of it, it in no way lessens the danger and if anything, makes the job at hand even more difficult. It is the desire to be successful and even more importantly, stay alive, that gets the creative juices flowing. To design good sampling methods, several things need to be taken into account: 1) expense, 2) ease of handling in potentially tight or small spaces, 3) sterile collection methods that can stand up to tight scientific scrutiny, 4) reusability, and 5) robustness. What is most important, however, is the flexibility of the sampling methods, allowing use in different cave environments (e.g., different chamber sizes, depths, visibility, and highly ornamented rooms).

Automatic water samplers, which are commercially available, would still have to be placed by a diver in a cave environment and would still not allow the degree of freedom provided by using silicon tubes. Sampling can be performed vertically throughout the water column every 20 cm in a very short period of time, in a complex environment. In most cave environments the water body is hydrologically active with a flow ranfci-5(from34 e t2er)]TJ.0007 Tc.0118 Tw226834 0 Tdk(nbesOnree os th)-5(onm)

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Survey Technique for Underwater Digital Photography with Integrated GPS Location Data

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Abstract

This underwater survey technique developed by the U.S. Environmental Protection Agency Region 10 Dive Team is an inexpensive method of conducting impact analysis and shoreline inventory of submerged aquatic resources by means of geo-located photo documentation. This technique is capable of identifying, mapping, and recording the x and y coordinates of submerged aquatic resources in low visibility waters with minimal error. The geo-referencing of these objects is accomplished by a two person SCUBA dive team surveying the underwater environment by documenting the seafloor with an underwater digital camera taking photos at regular intervals along a contour of the shore. An inexpensive recreational Wide Area Augmentation System enabled Global Positioning System (GPS) device is diver-towed in a raft directly above the dive team which records positions throughout the dive. Commercial software is later used to relate the GPS information to the digital photos resulting in geo-located digital photos that can be viewed on a map or in a Geographic Information System for later analysis of the seafloor environment.

Keywords: geo-located photographs, submerged aquatic resources

Introduction

As the U.S. Environmental Protection Agency (EPA) continues to heavily focus on water and sediment quality issues, expanding the agency's capabilities relating to marine and benthic surveys is vital. New techniques for geo-referencing underwater digital photos, developed by the EPA Region 10 Dive Team, provide location data of previously unobtainable accuracy for specific sample sites, hazards, and marine resources.

Identifying, mapping and recording the exact locations of submerged aquatic resources and unwanted hazards in low visibility waters throughout the Northwest has always proved challenging. The EPA Region 10 Dive Team has the task of documenting and geo-locating these objects which stem from projects such as the following: drum dumping investigations, pre-capping and pre-dredging surveys, chemical product seepage from groundwater to surface water, pipe outfall compliance inspections, various reconnaissance and recovery operations, sampling, and ecological shoreline inventories and assessments. Involved in any of these projects is the need to record the physical location of submerged objects for future mapping and analysis through the use of Geographic Information Systems (GIS).

Assisted by the use of underwater digital photography a diver can descend, survey the project area, and photo document any objects of interest for future analysis. Unfortunately, the diver has not been able to accurately record where those photos were taken under the water because traditional global positioning system (GPS) devices do not work beneath the water's surface.

Poor underwater geo-positioning accuracy has usually caused fundamental difficulties in accomplishing the mission of EPA dive projects, which typically include delineating areas of contamination, identifying areas needing localized cleanup, or identifying areas which otherwise need to be revisited. Other types of missions that we have found difficult to accomplish because of poor underwater positioning technology include determining locations of an anchor line, an illegally scuttled ship, an unknown sewer overflow outfall, and a protected species habitat.

The challenge presented to the EPA Region 10 Dive Team was to devise a method of recording a diver's location and path underwater while simultaneously photo-documenting the seafloor project area with a digital camera in a waterproof housing. This kind of survey must be performed without the use of acoustic based underwater positioning systems, which have proved to be costly, involving lengthy setup procedures, requiring cumbersome underwater observation recording consoles, and subject to performance errors due to haloclines, thermoclines, and acoustic shadows. Although commercial systems are available that place a GPS unit in a waterproof housing with a long extension to a surface antenna (Sound Ocean Systems, 2008), our method is even simpler, more cost-effective, and just as efficient. The simplicity and improved accuracy of geo-referenced photos used with recreational (i.e., less than $1,000 in cost) GPS units has shown that better data can be made available at a fraction of the cost and time invested.

Methods

To address this underwater geo-location challenge the 'Survey Technique for Underwater Digital Photography with Integrated GPS Location Data' was created. This procedure is conducted by a two person dive team who surveys the underwater environment in search of relevant submerged aquatic objects and documents them with digital photos. An inexpensive recreational GPS device is towed by a diver in a raft directly above the dive team which records time-stamped positions throughout the dive. Commercial software is later used to relate the GPS information to the digital photos resulting in geo-referenced digital photos that can be viewed on a map or Geographic Information System (GIS) for later analysis of the benthic environment. This technique has been successfully used previously in a terrestrial environment with a high level of success (Directions Magazine, 2007).

The result of this collaborative effort between EPA GIS staff, dive team, scientists, and field support personnel is a system that demonstrates an inexpensive method of relating a common timestamp of photos of submerged aquatic objects of interest and a GPS track log. This produces a GIS shapefile, defined as follows: "A shapefile stores nontopological geometry and attribute information for the spatial features in a data set. The geometry for a feature is stored as a shape comprising a set of vector coordinates" (Environmental Systems Research Institute, 1998). The shapefile produced shows point locations of each photo's physical x and y coordinates.

The technology behind this survey technique consists of: a recreational grade Wide Area Augmentation System (WAAS) GPS device placed in a water tight drybag (Figure 1, we use a Garmin 60CSX), attached to a four foot long Styrofoam raft equipped with a dive flag (Figure 2); a reel of line to connect the diver to the raft; a digital still camera (or video camera with still capability) with time-stamping capabilities within an underwater housing; an inexpensive digital photo GPS integration software package (e.g., GPS-Photo Link version 4); and, a laptop running GIS software (such as ArcGIS 9).

Figure 1. GPS device inserted in drybag. Photo credit: EPA Region 10 Dive Team (used with permission).

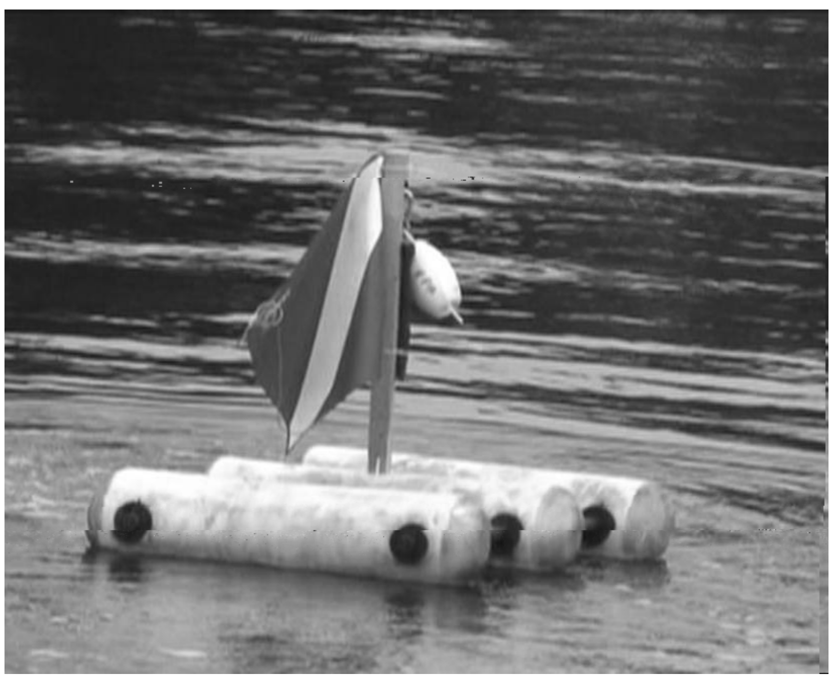

Figure 2. GPS device carried atop diver towed raft, augmented with a dive flag for safety purposes.
Photo credit: EPA Region 10 Dive Team (used with permission).

The survey procedure:

1. GPS device is placed in a drybag and clipped atop a diver-towed raft (Figures 1 and 2).
2. Before the dive the GPS device screen is photographed by the underwater digital camera to record the exact difference in device clocks.
3. Divers descend, tighten the scope on the tow line to bring the raft vertically overhead, take a photograph to document the survey start point, and survey the underwater environment by following a compass bearing, a depth contour, or directions from surface support, while recording results via the underwater digital camera.
4. During the dive the GPS device continuously records the location of the divers below through the GPS device's track log. The divers tighten the scope on the tow line to ensure the raft is directly overhead for all critical pictures. At the conclusion of a transect, the divers take a photo to document the location of the end of the survey.
5. After the dive the track log data and the digital photos are offloaded to a laptop located on the dive boat for additional processing.
6. An inexpensive digital photo GPS integration software package is used to relate the GPS data time stamps to the digital photo time stamps, and create a geospatial data set with hyperlinks to photographed locations.

After the dive, the data is exported into a shapefile format, and the photo-hyperlinked geospatial dataset is brought into a GIS application along with a time- and coordinate- stamped copy of the photos (Figures 3 and 4).

Figure 3. Example of a georeferenced photo of eelgrass.
Photo credit: EPA Region 10 Dive Team (used with permission).

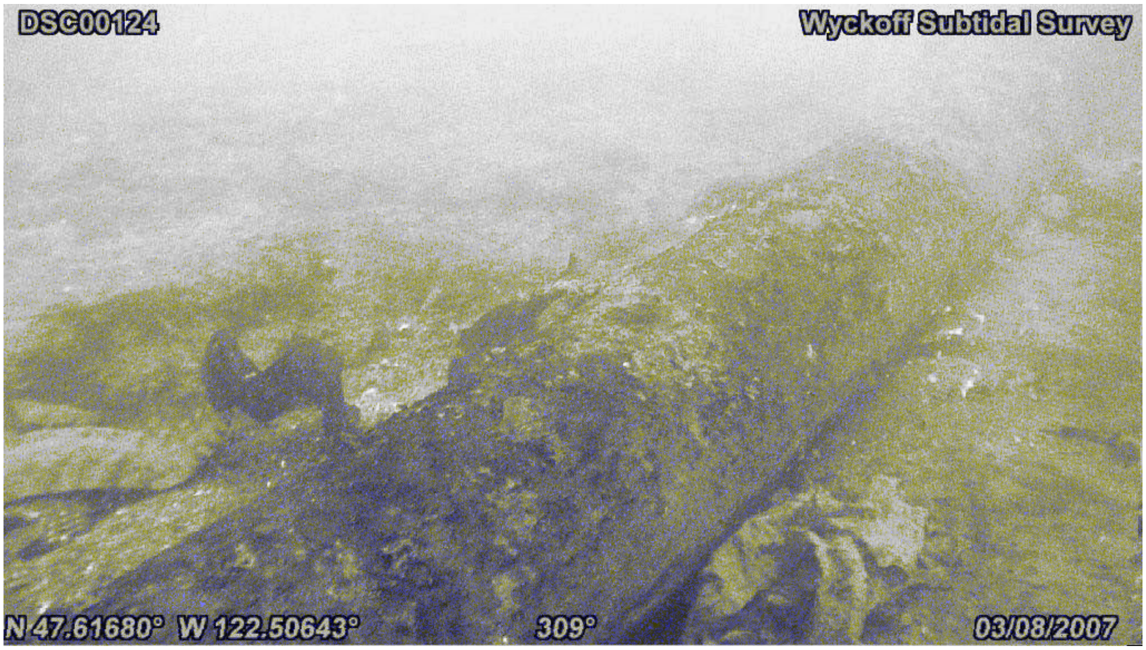

Figure 4. Example of a georeferenced photo of bottom debris.
Photo credit: EPA Region 10 Dive Team (used with permission).

Once in the GIS application a user can click on a seafloor point to see the photo from that corresponding location. In addition, a track log of the path the divers traveled underwater can be displayed in the GIS application for further navigation, verification, and ground truth purposes.

Results and Discussion

The availability of new, inexpensive, innovative and effective tools that facilitate marine and benthic survey work is critical to addressing water quality issues. SCUBA-based underwater geo-located digital photography is a new technique for locating specific hazards and marine resources with previously unobtainable accuracy at such low cost. This survey technique has already been implemented in EPA's Puget Sound Regional Priority Project Area (Figure 5), and is scheduled to be used in the Columbia River Basin Regional Priority Area in the future (U.S. Environmental Protection Agency, Region 10: the Pacific Northwest, 2008).

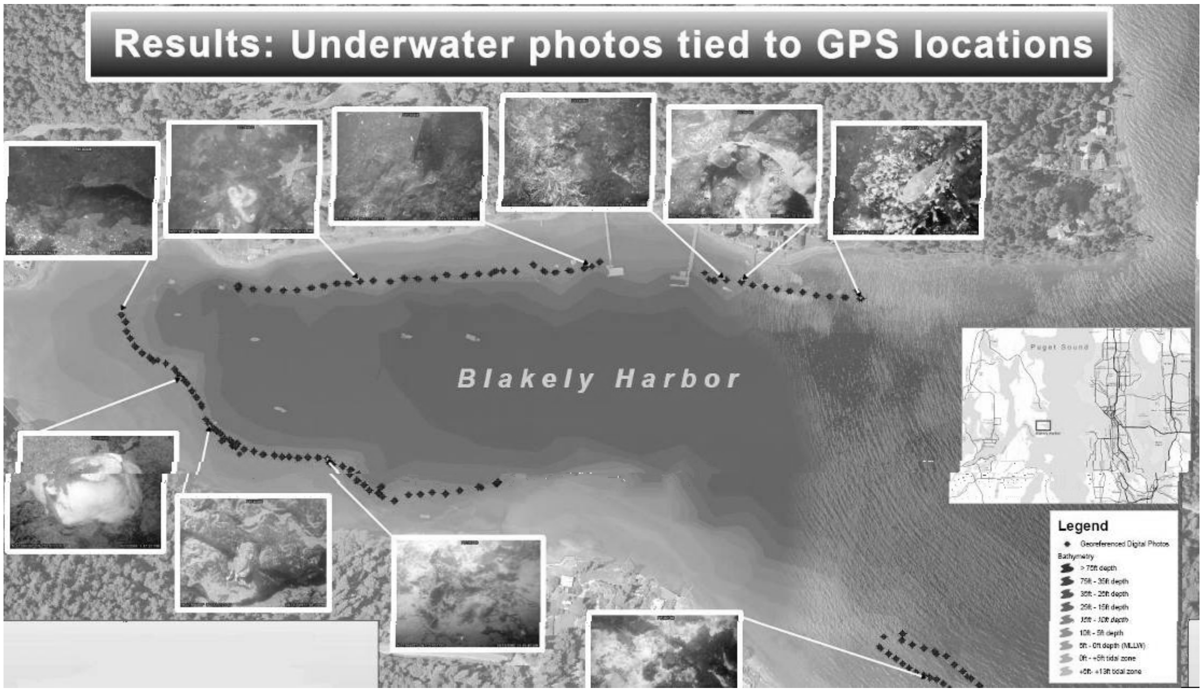

Figure 5. Example of results of underwater survey technique.
Photo credit: EPA Region 10 Dive Team (used with permission).

The outcomes of this survey technique are photo-hyperlinked geospatial datasets consisting of water resource information including: National Pollutant Discharge Elimination System outfalls locations (EPA. National Pollutant Discharge Elimination System, 2008), water, soil, and biota sampling locations, illegal dumping locations, or benthic impact analysis. These data are easily fed into project, regional and national EPA databases such as the Water Discharge Permit Compliance System (EPA. Water Discharge Permits (PCS), 2008), STORET repository for water quality, biological, and physical data (EPA. STORET, 2008), or Water Quality Exchange (EPA. Water Quality Exchange (WQX), 2008).

Cost and usability of this navigation and photo linking technology is also a major advantage of this survey technique. The cost of our GPS receiver is approximately $400. The off-the-shelf GPS-Photo Link software by GeoSpatial Experts retails for approximately $200. The raft was fashioned from previously acquired foam pontoons and supplies for approximately $100. While other acoustic based underwater navigation and tracking systems advertise 'submeter accuracy' for a long baseline (LBL) system (Desert Star Systems' AquaMap/ DiveBase Seafloor sytem) or in the case of ultra short baseline systems (USBL) 'up to 0.15 degree' accuracy (LinkQuest's TrackLink 5000 system), most retail starting at $13,000 and can cost over $100,000. In addition, these have proven more difficult to setup during EPA Region 10 dive operations, prone to failure (e.g., from haloclines, thermoclines, physical obstructions, battery drain on sonar buoys or diver units, etc.), and problematic to interpret and extract the resulting location data. In addition to the qualification of the advertised accuracies of LBL and USBL being highly dependent on field conditions, they are also highly dependent on ancillary equipment accuracy. For example, the digital compass component can introduce significant error to a USBL system that propagates with increasing distance between the surface tracking platform and underwater target. For this reason, it is not uncommon for manufacturers to qualify USBL accuracy excluding heading errors (Applied Acoustics Engineering, 2007).

'The Survey Technique for Underwater Digital Photography with Integrated GPS Location Data' is a more effective tool for achieving project goals and extracting underwater locations into a usable GIS compatible format. It is also much more efficient and cost-effective. GIS data are easily exported due to the simplicity and compatibility of data integration tools and shapefile export functionality included in the off-the-shelf digital photo GPS-Photo Link software. In addition, such a system costs less than five percent of acoustic technology alternatives, saving increasingly scarce budget resources to better focus on other project needs. Logistically, we find that more dive time is available due to the limited time required to setup and remove the equipment when out on the water. While line scoping of the GPS float may diminish the accuracy of this technique at depths greater than 30 ft, error introduced by line scoping is also endemic to LBL system setup in open water, particularly when deploying transducers to compensate for haloclines or thermoclines mid-water column.

'The Survey Technique for Underwater Digital Photography with Integrated GPS Location Data' has been tested, utilized, proven successful and accurate within the last year on a handful of regional dives throughout the Puget Sound including a habitat survey at Blakely Harbor, Washington, a pre-capping debris survey at the Wyckoff Superfund Site, Washington, a habitat survey at Henderson Inlet, and an ordnance/habitat survey at the Jackson Park Superfund Site (U.S. Environmental Protection Agency, Region 10: the Pacific Northwest, Dive Team, 2008). More experimentation will be required for diving depths greater than 30 ft, but on shallower dives less than 30 ft we have observed the overhead raft to be directly above the diver's photographed object, and the WAAS enabled GPS to be approximately at a three to five meter level of accuracy. Further, depth and accuracy limitations to this technique relating to line scoping issues are endemic also to LBL acoustic based systems, which rely on triangulation of three or more buoyed transponders, making the ease of data acquisition in this survey technique a true advantage.

Expanded use and awareness of GIS throughout the EPA's environmental assessment and dive team communities has also been an added benefit from this survey technique. EPA Region 10 divers now have ArcGIS installed on their field laptops for mapping and tracking support in the field and on the dive boat. In addition more regional field staff is now using GIS products including: aerial imagery, base map data, and EPA databases to compliment the geo-referenced location of their digital photos on other non-diving related projects.

Disclaimer: This paper is an illustration of the application of an underwater survey technique and does not represent the official view of the U.S. Environmental Protection Agency. Mention of any specific brand or model instrument or material does not constitute endorsement by the U.S. Environmental Protection Agency.

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US Environmental Protection Agency, Region 10: the Pacific Northwest, Dive Team. Jackson Park Superfund Site, Silverdale, WA. yosemite.epa.gov/r10/OEA.NSF/webpage/Dive+Team+Projects

US Environmental Protection Agency, Region 10: the Pacific Northwest, Dive Team. Shoreline Inventory/Assessment. yosemite.epa.gov/r10/OEA.NSF/webpage/Dive+Team+Projects

US Environmental Protection Agency, Region 10: the Pacific Northwest, Dive Team. Wyckoff Pre-capping Debris Survey; Bainbridge Island, WA. yosemite.epa.gov/r10/OEA.NSF/webpage/Dive+Team+Projects

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Proceedings of the American Academy of Underwater Sciences 27th Symposium.Dauphin Island, AL: AAUS; 2008.

DNA Sequencing of 18s Ribosomal RNA Genes of the Northern Star Coral (*Astrangia poculata*) Collected from New Jersey Artificial Reefs.

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Abstract

The purpose of this work was to investigate the distribution and molecular evolution of the Northern Star coral *Astrangia poculata* on artificial reefs and shipwrecks in the local coastal ocean. This region is generally devoid of natural hard structure habitat, consisting primarily of gently sloping sandy plains. Most corals live in tropical to sub-tropical waters above 20ºC and cannot tolerate high turbidity. The Northern Star Coral is an exception in that it is found from the sub-tropics to the temperate zone on hard surfaces. Northern Star Coral colonies can be encrusting, massive or branching. Individual coral colonies were sampled from seven collection sites offshore of Atlantic City, New Jersey at depths from 20-30 m. Coral samples containing at least 5-10 corallites per sample were collected by scuba diver. Between 10-30 mg of tissue was extracted from individual polyps using a Qiagen DNA extraction kit (Qiagen Corp). DNA was amplified by the polymerase chain reaction (PCR) using eukaryotic universal primers for the small subunit (18s) ribosomal RNA gene. PCR products were cloned in pGemT (Promega) and plasmid preparations were cycle sequenced to determine the exact DNA base sequence of the individual being typed. This DNA sequence information was aligned and analyzed to determine the genetic relationship of the individuals using a reference *A. poculata* sequence from GenBank. In general, genetic variation between the local sites was found to be very low, but will serve as a baseline for collections over a wider geographic range.

Keywords: DNA sequencing, NJ artificial reef

Introduction

A prominent member of the invertebrate assemblage colonizing shipwrecks and artificial reefs in the coastal waters offshore of New Jersey is the Northern Star coral *Astrangia poculata*, synonyms *A. danae* and *A. astreiformis*, Phylum Cnidaria, Class Anthozoa (Peters et al., 1988). This region is generally devoid of natural hard structure habitat, consisting primarily of gently sloping sandy plains. Most scleractinian corals, subclass Hexacorallia, Order Scleractinia, live in tropical to sub-tropical waters above 20ºC and cannot tolerate high turbidity. However, an exception is the Northern Star Coral which is found from the sub-tropics to the temperate zone on the Northwest Atlantic coast. Northern Star Coral colonies can be encrusting, massive or branching but are not considered to be reef forming as they generally only cover existing structures. The individual coral skeletons or corallites are well defined, circular, compact, and up to 10 mm in diameter. The polyps or living coral tissue may be translucent to dark brown depending on the presence and number of zooxanthellae (Peters et al., 1988). The corals may reproduce both asexually by budding within established colonies and by sexual reproduction with planktonic dispersal followed by settling on hard surfaces such as rocks, pilings and shipwrecks. The purpose of this study was to look at genetic differentiation of star coral in relation to their wide ecological amplitude and their habit of colonizing a substrate that is extremely patchy.

Methods

Coral Collection

Individual coral colonies were sampled from seven collection sites offshore of Atlantic City, New Jersey, USA (39°23'N: 74°26'W) at depths from 20-30 m on shipwrecks and ships sunk as artificial reefs (Figure 1). The sites, followed by their sinking dates are: Jet Trader- 2005, Lemuel Burrows-1942, Almirante-1918, John Marvin-1992, Sea Girt-1990, Car Float-unknown and Glory (possibly Lake Frampton)-1920. Coral samples containing at least 5-10 corallites per sample were collected by scuba diver (Figure 2). Corals were kept in aquaria with filtered seawater and aeration until sampling (Figure 3).

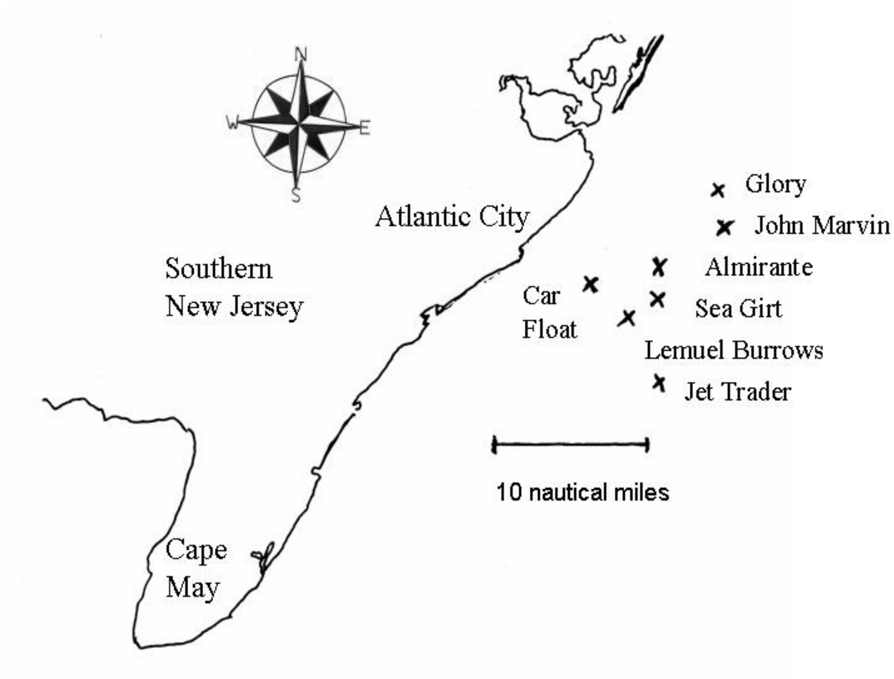

Figure 1. Dive sites for *Astrangia poculata* collection off of the southern New Jersey, USA coast

Figure 2. *Astrangia poculata* collection from deck plates on the wreck of the Almirante.
Photo credit: David Roche (used with permission).

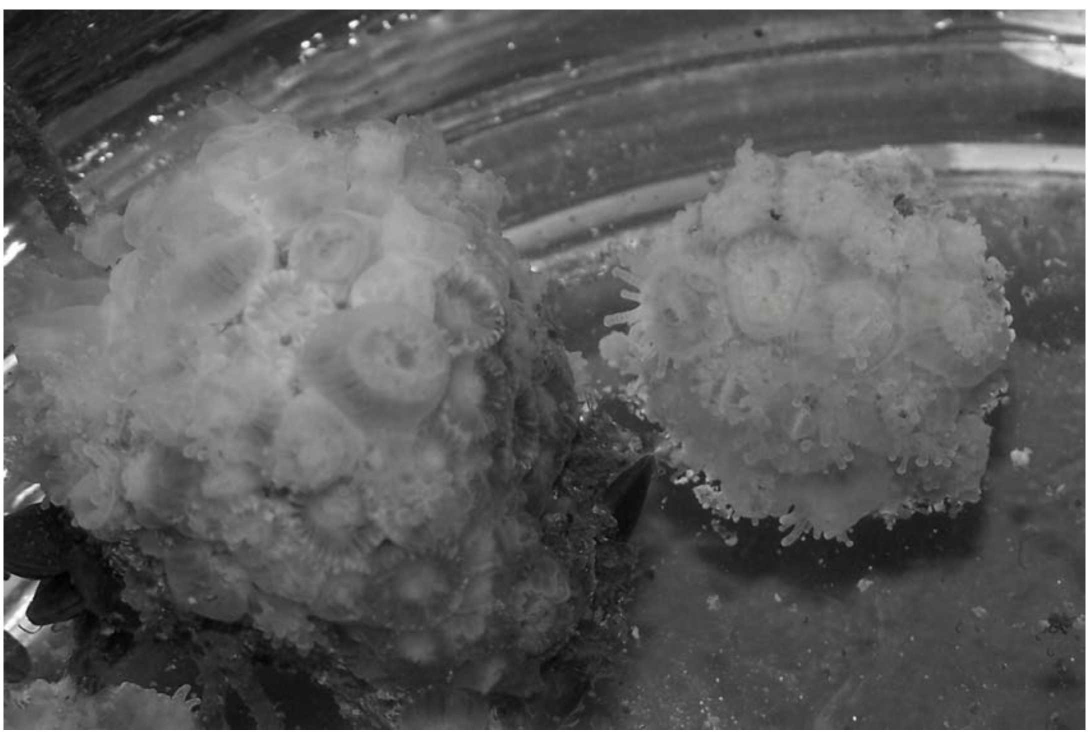

Figure 3. *Astrangia poculata* colonies with extended polyps. Photo credit: Peter Straub (used with permission).

DNA Extraction and Amplification

Between 10-30 mg of tissue was extracted and purified from individual polyps using a DNeasy Blood and Tissue extraction kit (Qiagen Corp., Valencia, CA). Genomic DNA was eluted in sterile water and quantified by UV spectrophotometer at 260 nm. DNA was amplified by the polymerase chain reaction (PCR) using eukaryotic universal primers for a portion of the small subunit (18s) ribosomal RNA gene with primers NS1 (White et al., 1990) and Euc 516r (Amann et al., 1990). The amplification by PCR was performed in 50 µl reactions with 100 ng template DNA, 5 UN Taq polymerase and 1X coraload buffer (Qiagen Corp., Valencia, CA), 200 µM each dNTPs, 0.2 µM each primer NS1 and Euc 516r. Conditions for PCR were as follows: denature 1 min at 94ºC; followed by 35 cycles of 94ºC for 1 min, 52ºC for 1:30 min and 72ºC for 2:00 min; ending with a final 10 min at 72ºC to ensure complete synthesis.

Purification and Cloning of PCR Products

PCR products were analyzed by agarose gel electrophoresis to ensure amplification of single products and then purified using a column cleanup with a Wizard SV Gel and PCR Clean-up kit (Promega, Madison, WI) to remove primers and template. Purified PCR products were then ligated into the plasmid pGemT Easy using a pGemT Easy cloning kit (Promega, Madison, WI) taking advantage of the non-template 'A' nucleotide addition commonly added to PCR products by Taq polymerase and single 'T' nucleotide overhangs on the prepared pGemT Easy vector. Ligation products were transformed into ultra-competent JM109 *E. coli* cells (Promega, Madison, WI) by heat shock for 50 s at 42ºC, grown for 60 min in LB broth with shaking. Transformants were then plated on LB agar medium supplemented with 100 µg/ml ampicillin and 80 µg/ml X-Gal and 0.5 mM IPTG for blue-white color selection and grown overnight at 37ºC. Individual white colonies were picked and grown overnight at 37ºC in 5 mL broth cultures of LB medium with 50 µg/ml ampicillin. Plasmid DNA was prepared using a column method from 1.5 mL of bacterial cultures with a Wizard Plus Miniprep DNA Purification System (Promega, Madison, WI). Plasmid DNA, eluted in water, was quantified on a UV spectrophotometer at 260 nm and diluted to100 ng/µl for DNA sequencing.

DNA Sequencing

A Genome Lab Dye Terminator Cycle Sequencing quick start kit (Beckman-Coulter, Fullerton, CA) was used with 200 ng of plasmid preparations for each reaction plus T7 or SP6 flanking sequencing primers (Promega, Madison, WI). Sequence reactions were subjected to 30 cycles of: 96ºC for 20 s, 50ºC for 20 s, and 60ºC for 4 min. After cycling, sequence reactions were purified by ethanol precipitation, dried and resuspended in sequence loading solution as per the Genome Lab instructions. Cycle sequence reaction products were separated and sequenced on a Beckman Coulter CEQ 8000 automated capillary genetic analyzer.

Sequence Analysis

Individual DNA sequence results were edited in Vector NTI (Invitrogen, Carlsbad, CA) to remove vector sequence and to resolve conflicts in forward and reverse sequences to produce consensus sequence for each template. Individual sequences were uploaded to the BLAST server at the National Center for Biotechnology Information and compared with known sequences deposited in GenBank for confirmation of identity. Individual confirmed consensus sequences were aligned using the Align-X application of Vector NTI (Invitrogen, Carlsbad, CA) and checked manually. The DNA sequence information was aligned and analyzed to determine the genetic relationship of the individuals using a reference *Astrangia danae* (=*A. poculata*) sequence from Genbank [AY039209] (Podar et al., 2001) and nine other Scleractinian coral sequences. Alignments were exported to PAUP 4.10 (Swofford,

2003) for phylogenetic analysis via MacClade (Maddison, 2005), and a Maximum Parsimony tree was inferred based on these 554 base pairs.

Results

DNA of sufficient purity was recovered from most samples to readily amplify and obtain a ca. 550 bp PCR product. After cloning of the PCR product and DNA sequencing, both BLAST search and nucleotide alignment indicated that all of the samples collected belong to a Scleractinian coral, most likely *Astrangia poculata.* All sequences match the Northern Star Coral with no more than one or two base pair differences per sequence in this region, with very little variability either between or among populations (Figure 4). The inferred Maximum Parsimony tree constructed in PAUP includes both our collected coral sequences and available sequences from a number of related coral species within the Subclass: Hexacorallia; Order: Scleractinia (Figure 5).

Discussion

The DNA extracted, amplified and sequenced was identified as coral tissue. Despite using 'universal' eukaryotic primers, we did not clone or sequence any zooxanthellae specific DNA. This may be due to the fact that *A. poculata* has been reported as a facultative symbiotic partner or that our primers were not specific enough to detect the zooxanthellae symbiont due to an overabundance of host DNA. This suggests that symbiont-specific primers may be useful to amplify zooxanthellae DNA in this species. The coral DNA sequences from this study were aligned with one another and with nine other species of Scleractinian corals. In published studies, the use of nuclear 18S rDNA (Bernston et al., 1999) and 16 S mitochondrial rDNA (Romano and Palumbi, 1996) have been shown to effectively discriminate between Orders of the Class Anthozoa. The closest DNA sequence matches for the collected specimens, based on the maximum parsimony analysis, were with the three species *Astrangia poculata*, *Phyllangia mouchezii*, and *Fungia scutaria*. Both *A. poculata* and *P. mouchezii* are in the same family, Family Rhizangiidae, Suborder Faviina. *F. scutaria* is classified in Suborder: Fungiina, Family: Fungiidae. Given the morphological identification of our collected specimens as *A. poculata*, we are confident that the DNA identification as *A. poculata* is concordant. Neither *P. mouchezii* (east North Atlantic-Mediterranean) or *F. scutaria* (Indo-Pacific) are found in the Northwest Atlantic. Given these results, it was not possible to discriminate the corals below the level of Order Scleractinia. This would suggest that a more variable region of the small subunit rRNA, possibly including the internally transcribed spacer region may be a useful approach to defining an area with enough variability to discriminate between the populations. In addition, collection of samples of *A. poculata* from a wider geographical area could identify areas of greater variability in DNA sequence to the analysis.

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                                 201                                                                                              300
     Montastraea annularis (195) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGAGCTGGCGATGTTTCAT
      Cirrhipathes lutkeni (200) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTGCTTTGGTGATCCATAATAACTGATCGAATCGCATGGCGTTGAGCTGGCGATGTTTCAT
          Fungia scutaria (200) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
           Pavona varians (200) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTCATTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
   Enallopsammia rostrata (200) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
      Phyllangia mouchezii (200) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
   Corynactis californica (201) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTGTTTGGTGATTCATAGTAACTTCACGAATCGCACGGCCTCGCGCTGGCGATGTTTCAT
      Tubastraea coccinea (199) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
           Corynactis sp. (199) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTGTTTGGTGATTCATAGTAACTTCACGAATCGCACGGCCTCGCGCTGGCGATGTTTCAT
        Astrangia poculata (200) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
                 Glory 1-1 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
                 Glory 2-1 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
                Glory 20-2 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
              Sea Girt 2-1 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
              Sea Girt 2-3 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
              Sea Girt 2-4 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
           John Marvin 1-1 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAGCTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
           John Marvin 5-1 (180) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
           John Marvin 5-3 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
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       Lemuel Burrows 20-2 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
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       Lemuel Burrows 21-2 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
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            Car Float 22-1 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
            Almirante 21-2 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
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            Almirante 24-1 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
            Almirante 24-2 (181) TGTATTTATTAGATTAAAAACCAATGCGGGTTCTGCCCGGTTCTTTGGTGATTCATAGTAACTGATCGAATCGCACGGCCTTGCGCTGGCGATGTTTCAT
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Figure 4. Alignment of 22 NJ artificial Reef samples with 1 full length *Astrangia poculata* (MA) and 9 other Scleractinian species (only part of alignment shown).

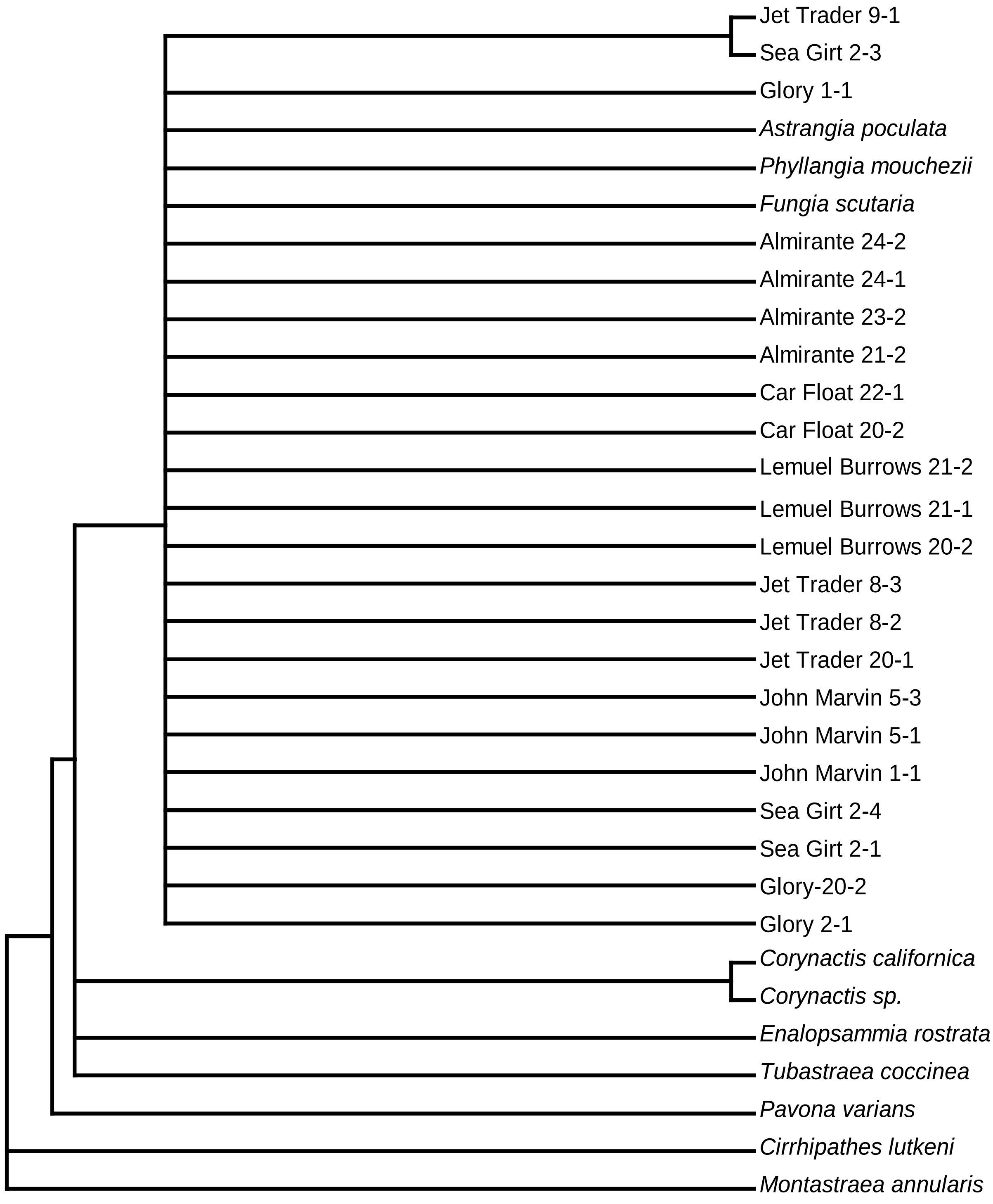

Figure 5. Maximum Parsimony analysis of 554 base pairs of the 18S rRNA gene from collected samples, as well as several related coral sequences

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University of Tasmania Field Safety System

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Abstract

Development and 'mechanics' of the web-based system developed by the University of Tasmania (Australia), for management of marine and terrestrial field operations, including diving.

Keywords: scuba diving

Introduction

In 2001 the University of Tasmania (UTAS) identified a need for an integrated system for managing field operations, with the goals of reducing the inherent paperwork burden, and improving the efficiency of the approval process – particularly for diving operations. A web-based platform was chosen for this implementation, due to its inherent ability to allow access to personnel across a diverse range of sites.

Discussion

By way of background information, UTAS has been conducting research diving operations since the early 1970s. Numbers of divers on the University diving register have averaged between 60 and 110 scientific divers during the past 10 years, and of these, around 25-30% were very active (completing 50-150 dives per year). During this time UTAS has averaged around 1,500-2,000 dives per year.

Within Australia (and New Zealand), an organisation called Standards Australia has been tasked by the Federal government with forming subgroups of 'industry experts' in order to formulate national standards across an enormous range of fields.

Although an Australian standard is not legally required to be met by an organisation unless it has been called up specifically by their state's occupational health and safety legislation, it is extremely likely that this document would used in court in case of a serious incident or accident - to measure the duty of care applied by an employer. This <u>has</u> happened on several occasions, with negative outcomes for the organisation concerned where they did not measure up to standard concerned.

The Standards Australia document AS/NZS 2299.2 was released in 2002, and is the national standard for scientific diving. This document sets out quite prescriptive requirements for conduct of scientific diving operations, and the record-keeping burden resulting from fully meeting the requirements of this standard was one of the things that provided considerable impetus for development of the Tasmanian University Field Safety System® (TUFSS®). TUFSS® has been specifically built to ensure that all possible AS2299 requirements are covered by the system if it is used to its full capacity (including requirements from both the AS/NZS2299.1: 2007 commercial diving standard and the AS/NZS2299.2: 2002 scientific diving standard). The TUFSS® Risk Assessment module has been designed around the AS/NZS4360: 2004 Risk Management Standard. Additionally, the field and boating operations modules have been designed to reflect best practice record keeping and reporting requirements for these types of research operation.

TUFSS® has been in development since 2003, and in that time has evolved to include integration of the following areas:

- Personnel registration - including checks on currency of qualifications, and automated reminders to personnel to update these as required;
- Project registration and risk assessment - including checks on currency of these, and automated reminders to personnel to update them as required;
- Equipment and vessel maintenance records - including checks on service currency, and automated reminders to appropriate personnel to update them as required;
- Terrestrial, boating and diving operational requirements, including submission of approvals/trip notifications (as required), plus qualification and medical checks on attending personnel, and notification of nominated contacts;
- Post-dive reporting requirements, including dive logging and generation of AAUS statistics reports.

The system has been operational within UTAS since 2004. Initially this was for diving operations only, but subsequent upgrades have built on this capability considerably, adding improvements and new functionalities as described above. TUFSS® is in regular use by several hundred staff and students to provide occupational health and safety support across a diverse range of field and laboratory projects.

In 2005, the commercial arm of UTAS (UTAS Innovations) evaluated TUFSS® as a candidate for commercialisation – in large part due to numerous requests for access to the software by other universities and research organisations. Since that time, the program code has been recompiled to facilitate such commercialisation, with several features built into the software to support a broader user base outside the University. The formal release of TUFSS® was in March 2008 (v1.005).

Further information on TUFSS® can be found on the UTAS Innovation Ltd. TUFSS® website: www.tufss.com.au

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Performing Multiprobe Profiles and Hydrographic Description of the Water column in an Anchialine Cave in Quintana Roo

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Abstract

In anchialine coastal caves, the underlying marine water layer beneath the density interface is hypoxic and food limited due to the absence of light and reduced transport of material from the sinkholes. Cave fauna survive under these conditions displaying great adaptation. This study describes the use of multiprobe data loggers (e.g., Hydrolab DS3) to generate high-resolution profiles of environmental factors in the anchialine cave system Crustacea in Quintana Roo, Mexico. Cave diving techniques, advanced Nitrox and decompression procedures were required to achieve the goals of this study over the course of twelve dives and approximately 26 h of observations and underwater work. Salinity profiles show the strong stratification with a brackish upper water lens, a bottom marine water zone and a mixing zone or halocline between those. There is a thermal inversion with the bottom marine water warmer than the upper brackish lens. The water column within the cave is hypoxic (2.54 to 0.2 $mg \cdot L^{-1}$). The suitable use of an internally logging multiprobe is shown to be of great value in the collection of detailed environmental data which then contributes to the understanding of coastal groundwater habitats and helps strengthen conservation policies through ecological information.

Keywords: groundwater, halocline, hypoxia, Mexican Caribbean coast, thermal inversion

Introduction

A distinct feature of coastal anchialine aquifer systems is the presence of freshwater overlying denser salt water, with a density interface separating the two which acts as a physico-chemical boundary. Karst cave systems form along carbonate coastlines from dissolution driven by hydrogeochemical and biogeochemical process. The dissolution produces organized and interconnected voids that efficiently drain meteoric water to the coast. The term anchialine is used herein to define the coastal cave systems with an inland opening and subsurface connections to the sea.

Anchialine groundwater ecology is a relevant domain of research. Many anchialine systems are known to host rich fauna (Stock et al., 1986), mainly crustaceans of marine ancestry (Iliffe, 2000), and these ecologies remain poorly documented similar to those of the deep seas (Glover and Earle, 2004). Of particular note, the underlying marine water in anchialine limestone caves is usually hypoxic (3 to <1 mg·L^{-1}) (Yager, 1994; Yager and Humphreys, 1996; Iliffe, 2000) seriously limiting the presence of most fauna.

There is also a pressing demand for research on groundwater ecology since much of the world's population depends on groundwater resources, of which ~25% of the world's population is specifically reliant on karst aquifers (World Water Assessment Program, 2006). In the state of Quintana Roo along the Caribbean coast of the Yucatan Peninsula, the freshwater layer is frequently of drinking water quality (salinity less than 1 mg·L^{-1}) and the inhabitants are almost solely reliant on groundwater for the daily basic activities (Doehring and Butler, 1974; Marín and Perry, 1994).

The aim of this paper is to describe the use of a self-contained multiprobe in generating profiles of the stratified water column within an anchialine system, and the data resulting from that exercise. The anchialine cave system Crustacea was chosen for a broader study due to the increasing stress on the site from urban expansion and tourism. A segment of the explored cave conduit was selected for profiling of the water column with four stations along a 289 m transect running nearly parallel to the coast at depth of 9-19 m. Crustacea is located at 0.5 km inland from the coastline in the NE Yucatan Peninsula, Mexico. The flooded cave links three sinkholes, and has a total surveyed length of 3,626 m (Quintana Roo Speleological Survey, 2008).

Methods

Cave Diving

Full cave diving, advanced Nitrox and decompression procedures were required to achieve the study goals. The dives were performed by a team of two divers using back mounted double manifolded aluminum 80 tanks with Hogarthian cave diving configuration and a third aluminum 80 tank mounted as a stage bottle using a EANx32 gas mix. Equivalent air depth (EAD) at 19 m using EANx 32 is 15 m, no decompression limit is 75 min on Bühlmann's ZHL-16 Dive Tables, and most of the dives were over 120 min bottom time so decompression was mandatory. A distinctly marked and cleaned tank with 100% oxygen was clipped to the primary reel line at 6 m depth within the open sinkhole water pool at the entrance of the anchialine system for shared decompression as nitrogen release, as an added safety margin given the physical labor and task loading on these dives, their duration, moderately deep depth, and the many sequential days of diving.

Multiprobe Use

In situ profiles in four selected sampling stations (A,B,C,D) were performed with a Hydrolab DS3 data logger to record depth, conductivity which is equated to salinity, temperature, and dissolved oxygen. The multiprobe was programmed to the maximum instrumental frequency of 0.2 Hz, or once every five seconds to achieve the best description of the stratified hydrographic structure of the water column. The 1.2 m long multiprobe was clipped D-rings at the hip and upper chest on the cave diving 5-point harness during travel and unclipped for hand-held use at each station to generate the profiles. The diver rose to the ceiling and achieved a mean descent rate of 2.3 cm/sec principally using breath hold buoyancy, and thus allowing the sensors to thermally compensate and stabilize the readings particularly while crossing through the salinity and thermal stratification zone. It is recommended to read the operating manual for parameter specifications prior to use of any multiprobe data logger (Table 1). The data sets from the Hydrolab DS3 multiprobe were downloaded after each dive, compiled, plotted and compared against the use of another multiprobe by Yager and Madden (Yager and Madden, 2002) in the same cave system.

Table 1. Hydrolab DS3 parameter specifications (Hydrolab, 1991)

Parameter	Range	Accuracy	Resolution	Response time
Temperature	-5 to 50°C	±0.15°C	0.01°C	<1 min
Conductivity	0 to 100 $mS \cdot cm^{-1}$	±1% of range	4 digits	<1 min
Salinity	0 to 70 ppt	± 0.2 ppt	0.1 ppt	<1 min
Dissolved O_2	0 to 20 $mg \cdot L^{-1}$	±0.2 $mg \cdot L^{-1}$	0.01 $mg \cdot L^{-1}$	<1 min

Results

Data Record and the Diving Descent Rate

Extended bottom time was necessary to perform slow and controlled vertical profiles from which detailed hydrographic description of the water column could be obtained (Figure 1 and 3, Table 3). Ceiling-to-floor profiles were initiated with a pause for ~30 sec near the ceiling (Figure 1) allowing the probe to thermally equilibrate and for the diver to stabilize themselves. This was followed by a slow and constant mean descent rate of 2.3±0.2 $cm \cdot s^{-1}$ which should allow the sensors to compensate [redacted]

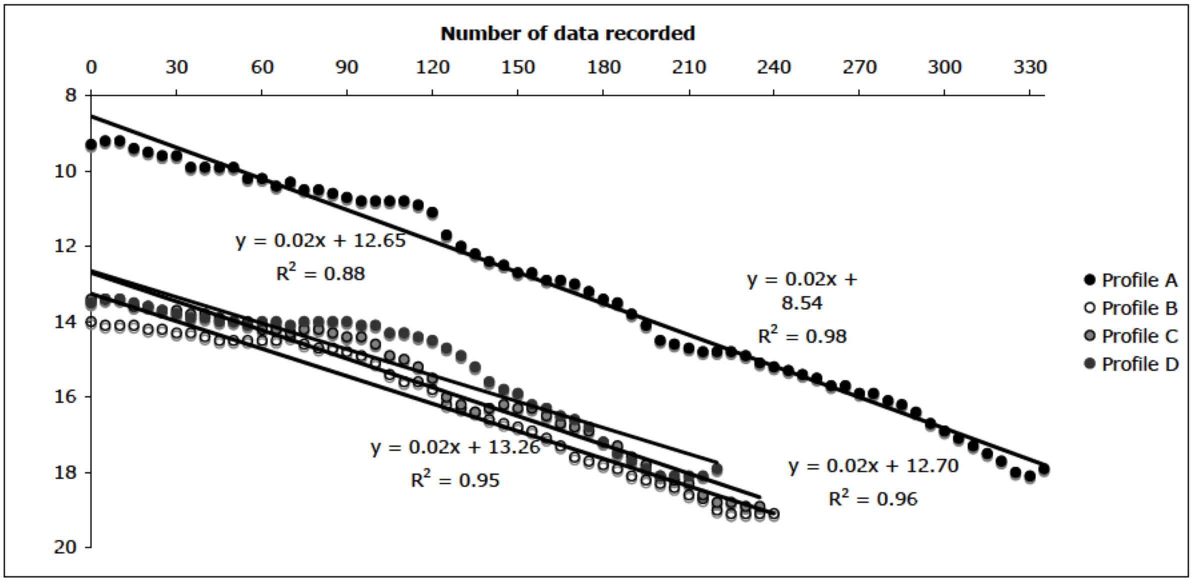

Figure 2. Similar slopes between profiled station show slow and constant descent rate, data record every five seconds

Table 2. Data recorded summary of water column profiles in four sampling stations

Station	Number of data recorded	Profile length cm	Descent speed cm·s^{-1}	Number of data recorded		
				Upper water lens	Intermediate water lens	Bottom water lens
A	68	880	2.62	42	11	15
B	49	510	2.12	6	14	29
C	48	550	2.34	10	11	24
D	45	460	2.13	7	17	19

Hydrographic Description of the Water Column

General salinity data of the cave shows a strong stratification with three water masses (Figure 3). The first water mass is fresh (<3 g·L^{-1}) from the surface to 6 m depth, the second is brackish ~10 g·L^{-1} from 9.4 to 14.6, and the third is marine ~35 g·L^{-1} from 15.9 to bottom. Steep salinity gradients or [redacted]

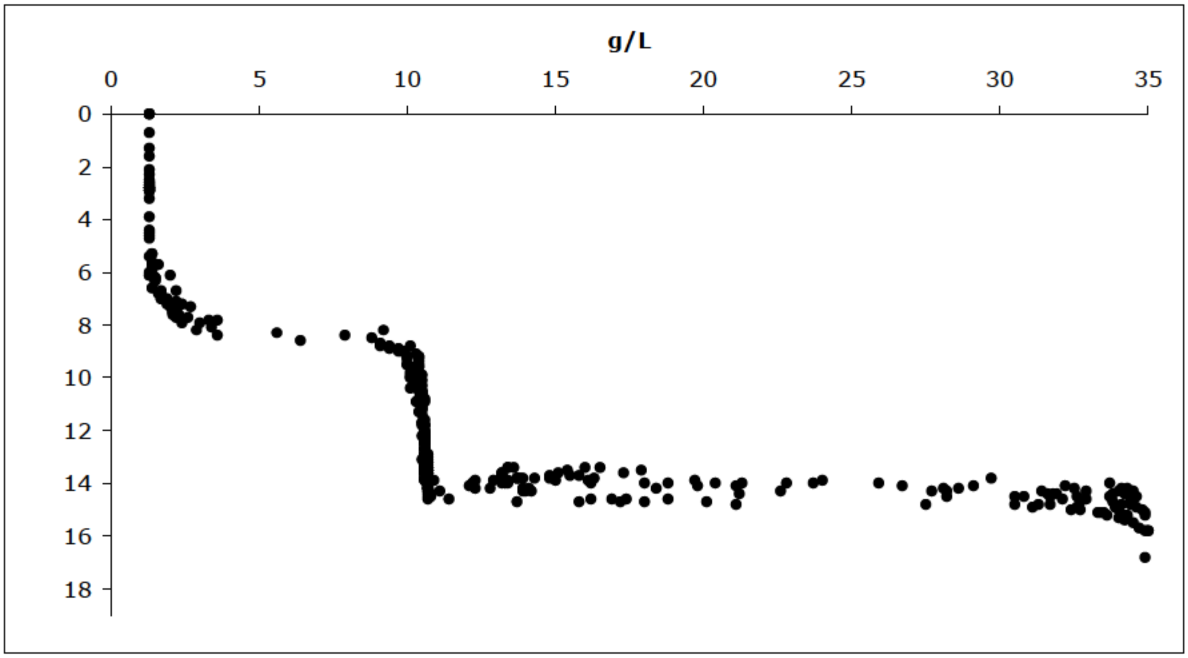

Figure 3. Salinity record along the dive data record every five seconds from the cave system Crustacea

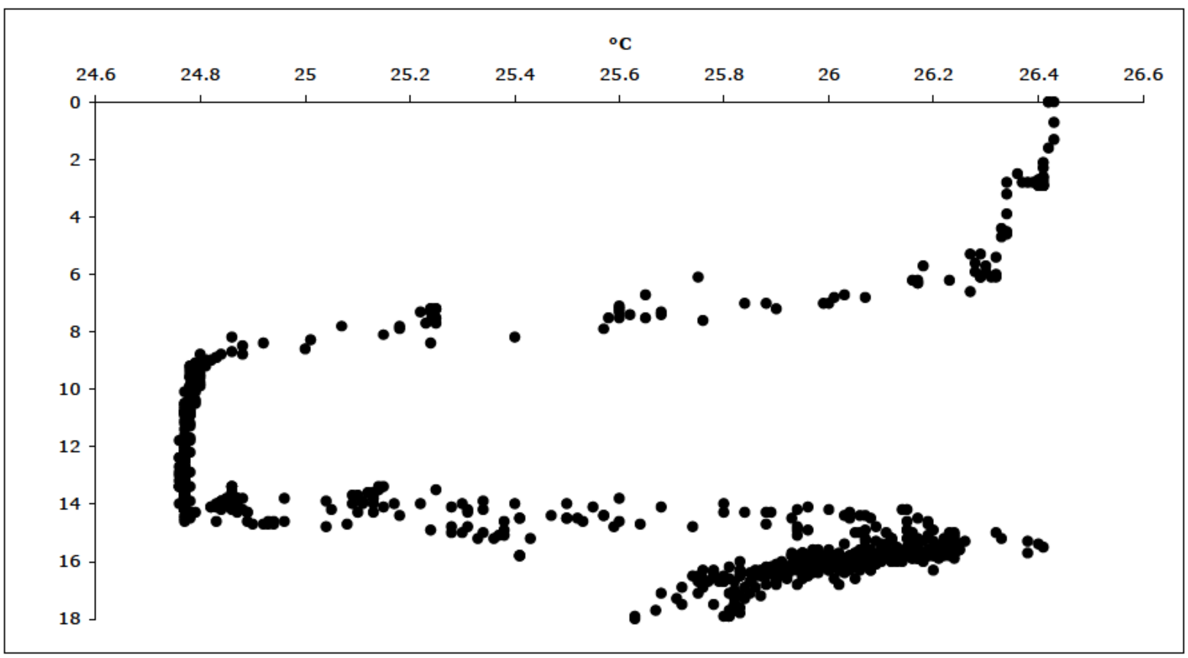

Figure 4. Thermal record along the dive data record every five seconds from the cave system Crustacea

Table 3. Values for salinity, temperature and dissolved oxygen profiles by stations and depth range in Crustacea

		Depth m	Salinity $g \cdot L^{-1}$	Temperature ^oC	Dissolved. O_2 $mg \cdot L^{-1}$
Station A	Epi	9.2 - 14.6	10.52 ± 0.11	24.77 ± 0.00	0.13 ± 0.07
	Cline	14.6 - 16.3	31.09 ± 5.5	26.07 ± 0.44	0.08 ± 0.07
	Hypo	16.3 - 18.9	35.46 ± 0.09	25.8 ± 0.18	0.01 ± 0.00
Station B	Epi	13.9 - 14.2	12.30 ± 0.25	24.8 ± 0.01	0.16 ± 0.02
	Cline	14.2 - 14.7	28.71 ± 7.03	25.47 ± 0.32	0.32 ± 0.10
	Hypo	14.7 - 18.9	35.49 ± 0.18	25.86 ± 0.11	0.03 ± 0.06
Station C	Epi	13.9 - 14.2	13.27 ± 0.13	24.85 ± 0.00	0.2 ± 0.03
	Cline	14.2 - 15.2	26.50 ± 7.66	25.39 ± 0.40	0.27 ± 0.07
	Hypo	15.2 - 18.9	35.61 ± 0.10	25.89 ± 0.11	0.04 ± 0.02
Station D	Epi	13.4 - 13.9	15.68 ± 1.07	25.14 ± 0.04	1.83 ± 0.81
	Cline	13.9 - 14.7	24.29 ± 6.22	25.44 ± 0.35	0.92 ± 0.67
	Hypo	14.7 - 18.9	35.55 ± 0.18	25.94 ± 0.14	0.03 ± 0.01

Discussion

Data Record and the Diving Descent Rate

The plotting and interpretation of data allows for the description of the hydrographic structure and discrimination of boundaries within the system. These results were compared and differences described against different methods of usage of other multiprobe (Yager and Madden, 2002) in the same cave system. It is notable that the work of Yager and Madden (2002) was a pioneering assessment for Crustacea focusing on the biology and that preliminary effort set the baseline for other studies such as this one.

Yager and Madden (2002) obtained whole dive data by fixing the multiprobe in between the manifolded tanks throughout the dive with the instrument set to a one minute sampling interval (Figure 5). In contrast, we clipped the multiprobe to accessible D-rings and thus were able to detach it for profiling use at selected stations, while the sampling interval was every five seconds (Figure 1). Salinity profiles from both data sets (Figures 3 and 6) show the stratification with a fresh to brackish upper water lens, a mixing zone or halocline, and bottom marine water zone. However only with the targeting profiling and higher sampling rate is it possible to see the finer structure of the water column, notably the two distinct stepwise increases in salinity through the halocline. It is notable that Figure 1 does not show the return to the surface since the instrument memory reached capacity prior to the end of the dive (in this case, with 881 records or 73 min). Handling the multiprobe during the dive give us the chance to perform vertical profiles in selected sampling station, while attaching the probe to the tanks is a viable approach for broad scale assessments.

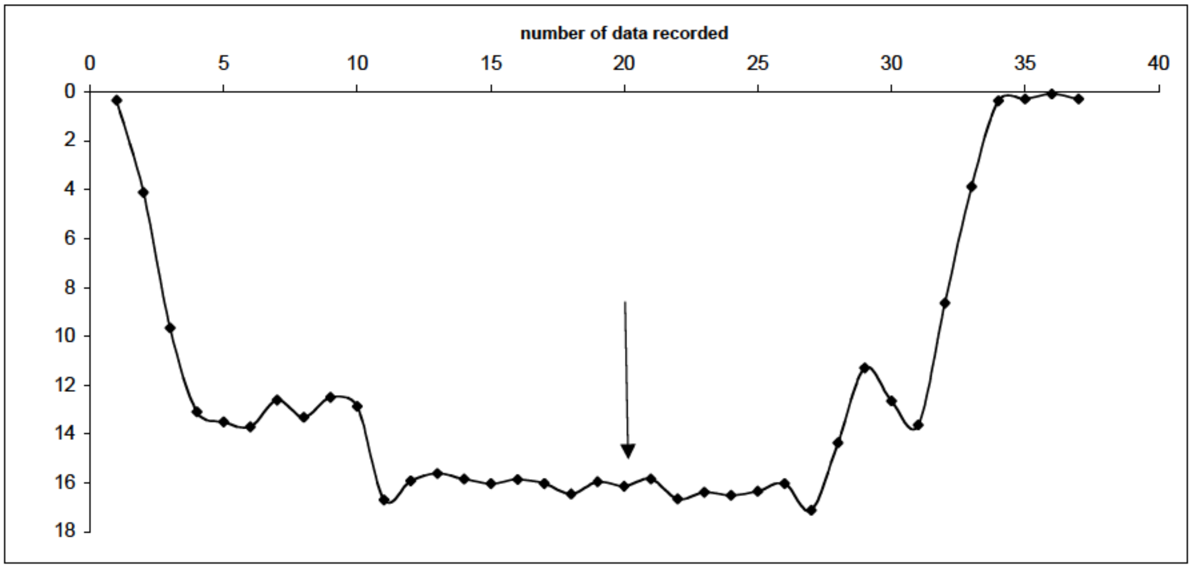

Figure 5. Dive profile and data record at one minute interval, arrow indicate mid-point in the dive at the point of furthest penetration into the cave system Crustacea (Yager and Madden, 2002)

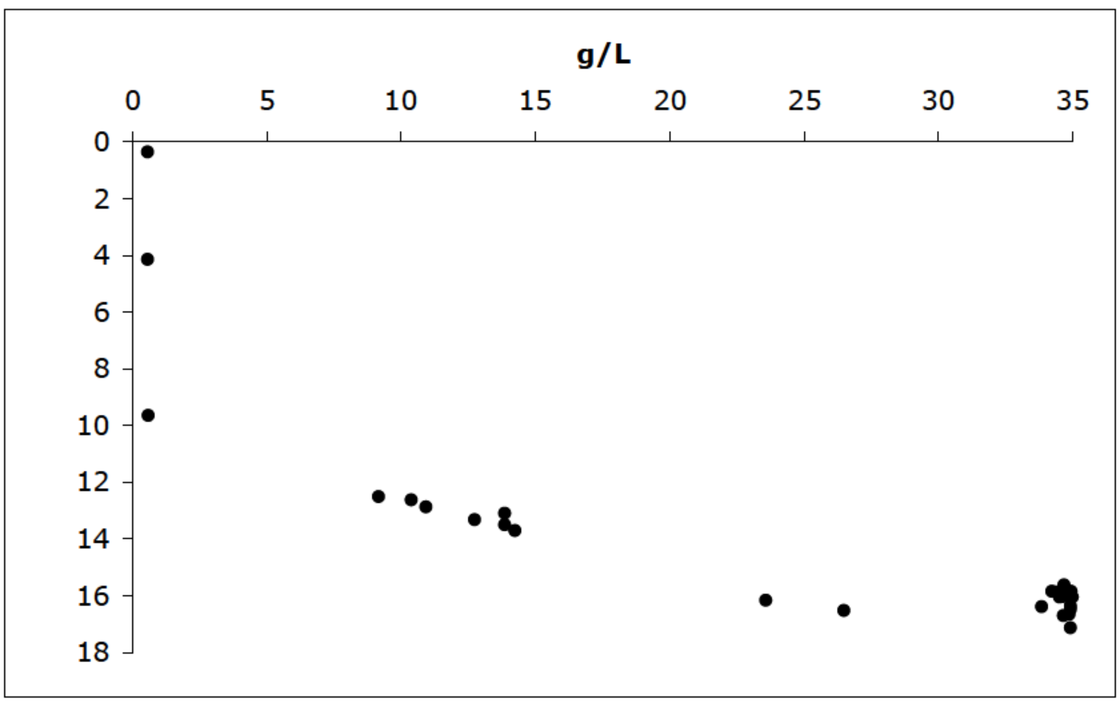

Figure 6. Salinity record along the dive data record at one minute interval from cave system Crustacea (Yager and Madden, 2002)

Table 4. Comparison of data set Yager and Madden (2002) and this study

	Yager and Madden (2002)	This study
Bottom time in minutes	37	>120
Number of data points	37	881
Number of data points within interface	9	792
Profiles	0	4
Descent speed at interface $cm \cdot s^{-1}$	~5	2.3
Slope	0.16	0.02

Hydrographic Description of the Water Column

Temperature maxima within and below the halocline as observed in this data (Figure 3B) have been described for sites in the Yucatan (Beddows et al., 2002; Stoessell et al., 2002; Beddows, 2004) and for a site in the Bahamas (Schwabe and Herbert, 2004), with examples including both open water and overhead anchialine systems. Stoessell et al. (2002) review three possible explanations for thermal anomalies also called thermal excursions: biological activity by microorganisms, solar heating and geothermal convective cells. It must be noticed that the three processes are of very different scale, from 10^2-10^4 for geothermal convective cells to 10^{-2} to 10^{-4} for microorganisms, and could be complementary rather than exclusive. Stoessell et al. (2002) suggest a geothermal convective cell as the main explanation for such temperature maxima. However Beddows et al. (2002) and Beddows (2004) discriminates between thermal maxima contained within the halocline in open sites which is ascribed to heliothermic heating, thermal maxima immediately below the halocline as is the case here in Crustacea which is ascribed to rapid shuttling of warm marine water through the coastal caves, while she assigns importance to geothermal convective cells only in much deeper saline water circulation.

Biological activity has been explained in two ways. Stoessell considers that the heat is generated *in situ* by exothermic chemical reactions like sulfate reduction or sulfide oxidation in bacteria at the halocline, while Schwabe and Herbert (2004) working in open water Black Holes argue a explanation based on Pfennig (1967) and Culver and Brunskill (1969) where massive anoxigenic phototrophic bacterial production at halocline dissipate light energy absorbed by photosynthetic pigments as heat. This latter explanation cannot be applicable in the case of the Crustacea data since the profiles are from inside the cave where there is no incident sunlight.

In Crustacea the water column is hypoxic (2.54 to <1 $mg \cdot L^{-1}$) as had been reported for other anchialine caves (Yager, 1994; Yager and Humphreys, 1996; Yager and Carpenter, 1999; Iliffe, 2000). Meteoric and marine water are two sources of dissolved oxygen for the water in anchialine caves. The diminished dissolved oxygen (DO) content in water is attributed to a lack of contact with the atmosphere, the absence of photosynthetic activity, organic matter remineralization, and zooplankton and nekton respiration. Quantification and characterization of organic matter from anchialine caves has rarely been undertaken. Biological oxygen demand of anchialine cave water in Bermuda is very low <0.25 mg $O_2 \cdot L^{-1}$ but increases at the halocline (Iliffe, 2000). In Danube wetlands groundwater ecosystems DO is strongly dependent on the atmospheric temperature and total organic carbon (Danielopol et al., 2000). When meteoric or shallow marine water enters in the cave, we can assume that the DO is close to 100% saturation. Therefore once inside the cave, the dissolved oxygen must be consumed by respiration by microorganisms and stygobitic fauna. It is clear that ongoing work with simple dissolved oxygen profiles may provide significant insight to organic matter content and water column-sediment respiration rates and how these relate to cycling of nutrients and the overall functioning of groundwater ecosystems (Stigebrand et al., 1996).

The method development for obtaining detailed water column profiles of multiple parameters and the results of those profiles contribute to the Mexican anchialine cave research program in order to advance the overall understanding of coastal groundwater habitats, pinpoint gaps in knowledge and encourage further integrative studies to set a baseline of solid ecological information and help strengthen conservation policies for decision makers.

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Evaluation of Atmospheric Oxygen Concentrators as a Source of Oxygen and Oxygen Rich Mixtures for Treatment of Diving Accident Victims in Remote Areas

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Abstract

The difficulty of obtaining oxygen for the treatment of diving accident victims in remote areas has been a long-standing problem. The normal method for transporting oxygen is in metal high-pressure cylinders. These cannot be carried on airliners, and contain insufficient oxygen for the long-term on-site treatment of diving accident victims or for evacuation to a treatment facility. Oxygen concentrators offer the potential of providing a long duration supply of oxygen in remote areas or aboard ships. These devices selectively remove nitrogen from air, and produce a breathing gas mixture containing up to 95% oxygen. The flow rates, and pressure output of current portable units are insufficient for use with the demand type, or free flow oxygen masks currently used to provide pure oxygen to accident victims. However, when used with oxygen conserving delivery systems, they will provide sufficient flows of oxygen for the on-site administration of up to 90% oxygen, and for the long duration evacuation of diving accident victims, or other patients in need of augmented oxygen. Recently developed PSA units are small, lightweight, and can operate on batteries or 12 volt DC electricity, thus making them practical for use in the field. This study evaluated three different concentrators providing breathing gas to a high efficiency mask and to a semiclosed-circuit rebreather. Significant findings were: 1) High flow rates with lower oxygen content produced higher alveolar oxygen concentrations than low flow rates with high oxygen content, 2) Using the same supply gas, the semiclosed rebreather produced over twice the alveolar oxygen concentrations as did the high efficiency mask, 3) At the same output flow and oxygen concentration a threefold difference in electrical power was measured between different models of concentrators, and 4) One model produced sufficient pressure to conduct in-water recompression to a depth of 20 ft.

Keywords: safety, scuba

Introduction

The difficulty of obtaining oxygen for the treatment of diving accident victims in remote areas has been a long-standing problem for the NOAA Diving Program. The normal method for transporting oxygen is in metal high-pressure cylinders. These cannot be carried on airliners, and contain insufficient oxygen for the long-term on-site treatment of diving accident victims or for evacuation to a treatment facility. Chemical production of oxygen by "oxygen candles" likewise produces insufficient oxygen. Atmospheric oxygen concentrators offer the potential of providing a long duration supply of oxygen in remote areas or aboard ships. These devices concentrate the oxygen in air, and provide a constant supply of low pressure oxygen. They have been successfully used as an oxygen source for the production of divers' oxygen enriched breathing gas (Wells, 1991; Wells and Moroz, 1993; Wells and Moroz, 1994).

PSA oxygen concentrator

The most appropriate type of concentrator for the purposes addressed here are 'pressure swing adsorption' (PSA), units (shown at left). These devices selectively remove nitrogen from air, and produce a breathing gas mixture containing up to 95% oxygen. They are currently used as a replacement for high-pressure oxygen cylinders to provide oxygen-enriched mixtures in the homes of patients who require additional oxygen. These electrically powered devices provide a continuous, slow flow of 95% oxygen. The flow rates, and pressure output of current units are insufficient for use with the demand type, or free flow oxygen masks currently used to provide pure oxygen to accident victims. However, calculations show that when used with oxygen conserving delivery systems, they will provide sufficient flows of oxygen for the on-site administration of up to 90% oxygen, and for the long duration evacuation of diving accident victims, or other patients in need of augmented oxygen. Recently developed PSA units are small, lightweight, and can operate on batteries or 12 volt DC electricity, thus making them practical for use in the field. When used with the oxygen conserving delivery systems identified below, they provide viable methods of providing oxygen for on-site administration and evacuation.

The purpose of this study was to evaluate currently available oxygen concentrators, and oxygen delivery systems, and to provide recommendations to NOAA on the most efficient and cost effective combinations of the systems.

Three PSA oxygen concentrators of different size, weight, and power requirements were evaluated; the Excel, Healthdyne, and Eclipse. Specifications for each of the units are in Table 6 in the Results section below, where these units are discussed.

Two oxygen delivery systems were evaluated. The Hi-OX mask is a partial rebreathing open circuit system and was selected because of its high efficiency relative to similar simple mask systems (Bouak, 2004; Somogy et al., 2002). The design of this mask results in the inspiration of the high oxygen supply gas during the initial portion of the inspiration cycle from a flexible reservoir, and when that is depleted, the latter portion of the inspired gas is made up of surrounding air. The high oxygen mixture thus enters the alveoli first, while the air fills the respiratory dead space. This results in higher alveolar oxygen concentrations than would result if air were mixed with the inspired gas throughout the respiratory cycle.

The closed-circuit diving oxygen rebreather, Minolung, was selected because of its gas-tight design. Since the gas mixture used contained a minimum of 5% nitrogen rather than pure oxygen, it had to be tested in a semiclosed-circuit mode.

The fundamental difference between the above delivery systems is that the resulting alveolar oxygen concentrations at a particular flow/oxygen concentration setting are dependent on the respiratory minute volume, tidal volume, and breathing pattern for the Hi-OX mask, while for the rebreather, alveolar oxygen concentrations depend primarily on the oxygen consumption of the individual and are less dependent on breathing pattern and respiratory minute volume.

Methods

Tests of the volumetric output, oxygen concentration, and power requirements of the oxygen concentrators were accomplished in the same fashion for all three units. Each unit has its own flow indicator. The Excel and Healthdyne have standard 'ball' type flow meters, while the Eclipse has a digital meter. To eliminate differences between the respective individual flow meters, a 'standard' meter was installed in series with output meters and the readings of this meter are those reported here. The gas from the flow meter then passed through a sample cell containing a fast response oxygen sensor attached to a Teledyne oxygen analyzer. The analog output of the analyzer provided input to an A-D converter, and was recorded on a laptop computer equipped with appropriate software to graph the results. The graphs showed when steady state oxygen concentrations were obtained. Power requirements at each of the flow settings were obtained with a wattmeter.

Forced end expiratory oxygen concentration ($FEEO_2$) was used as a measure of alveolar oxygen concentration produced by the various combinations of gas flow rates and oxygen concentrations with the Hi-OX mask. Both $FEEO_2$ and the oxygen concentration of the inspiration 'bag' of the rebreather were recorded. Subjects received brief training in the procedure for obtaining FEEO2. The test procedure consisted of the subject removing the mask and performing a forced exhalation through the gas sampling cell. At the end of the exhalation, the subject placed his fingers over both ports of the cell and held them there for one minute. After approximately 15 sec a stable reading was obtained, and this value was recorded as the $FEEO_2$.

Results

Gas flow and oxygen percentages for the three concentrators are shown in Table 1 and Figure 1. Note that flow rates listed for the Excel and Healthdyne extend beyond the rated values, and beyond the flowmeters on the units, 3 and 5 L/min respectively. The digital flowmeter on the Eclipse limits flow to the rated value of 3 L/min.

Table 1. Flow vs. O_2 concentration

Flowrate L/min	Excel % O_2	Eclipse % O_2	Healthdyne % O_2
1	95.3	94	94.2
1.5		94.6	
2	92.5	96.5	94.2
2.5		96.3	
3	83.5	95.5	93.7
4	70.5		88
5	61.8		73.5
6	55.5		37.2
7	49.8		
8	45.8		

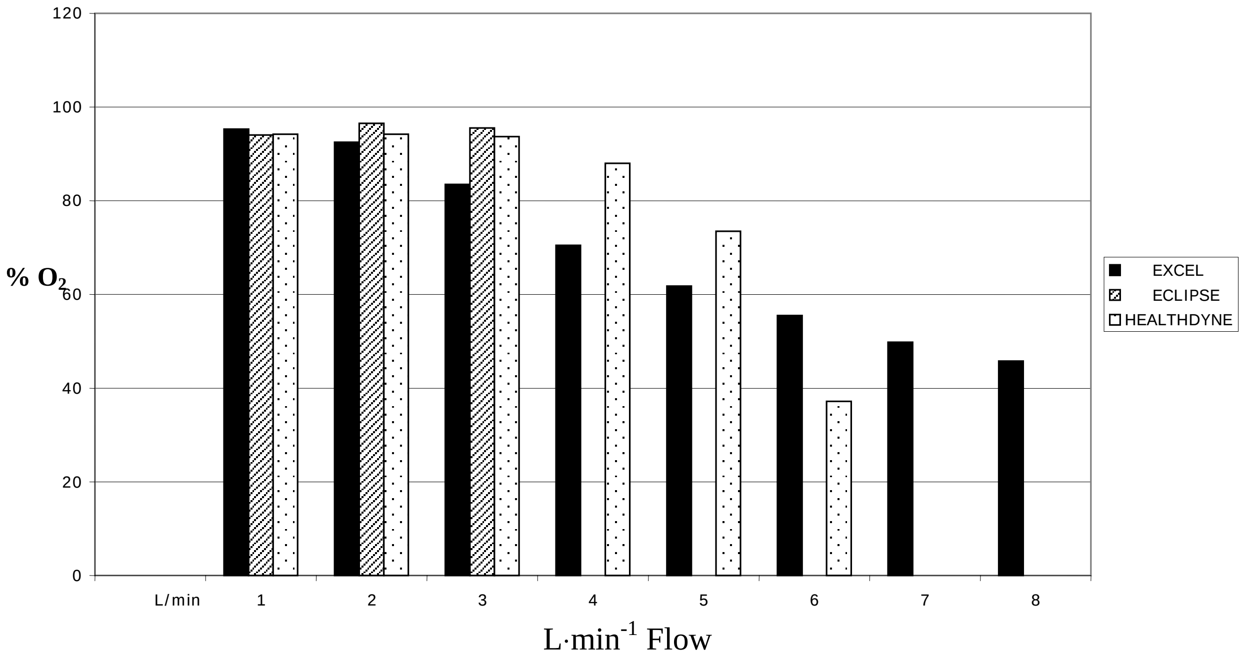

Figure 1. Flow vs. %O_2

Power requirements as a function of gas flow rates are shown in Figure 2 and Table 2. Note that the power consumption for the Excel and Healthdyne is independent of gas flow, while that of the Eclipse is dependent on gas flow.

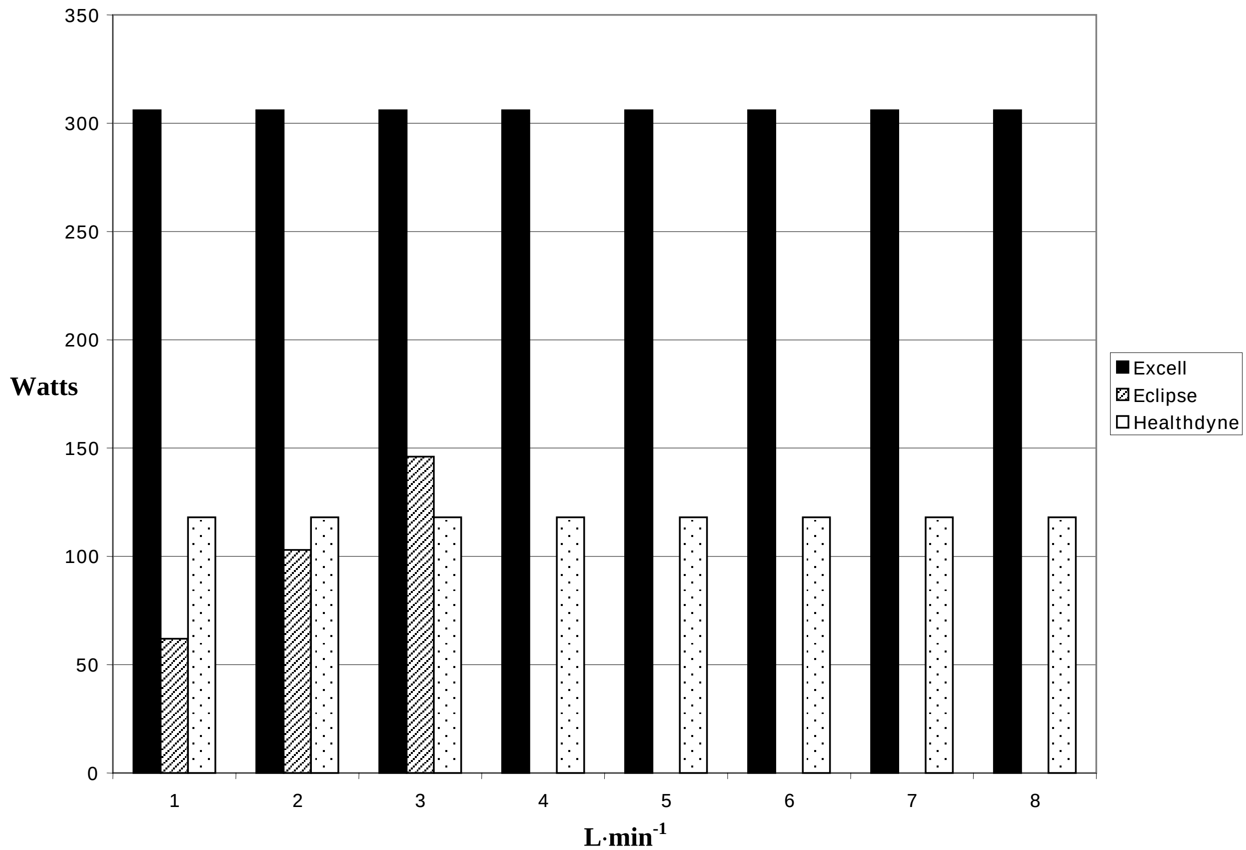

Figure 2. Watts vs. flow

Table 2. Watts vs. flow rates

$L \cdot min^{-1}$	Excell watts	Eclipse watts	Healthdyne watts
1	306	62	118
1.5		76	
2	306	103	118
2.5		116	
3	306	146	118
4	306		118
5	306		118
6	306		118
7	306		118
8	306		118

The $FEEO_2$ values for three subjects using the Hi-OX mask, as a function of gas flow rates and oxygen percentage, are shown in Figure 3 and Table 3 for gas supplied by the Excel concentrator. It is significant that the highest $FEEO_2$ values measured were at high flow rates and low oxygen concentrations, a combination that is not currently used in the administration of supplemental oxygen from oxygen concentrators. These values were obtained at flow rates in significant excess of the rated volumetric output of the concentrator and in excess of the maximum reading of their flow indicators. This observation could have a significant impact on how oxygen concentrators are used in the future.

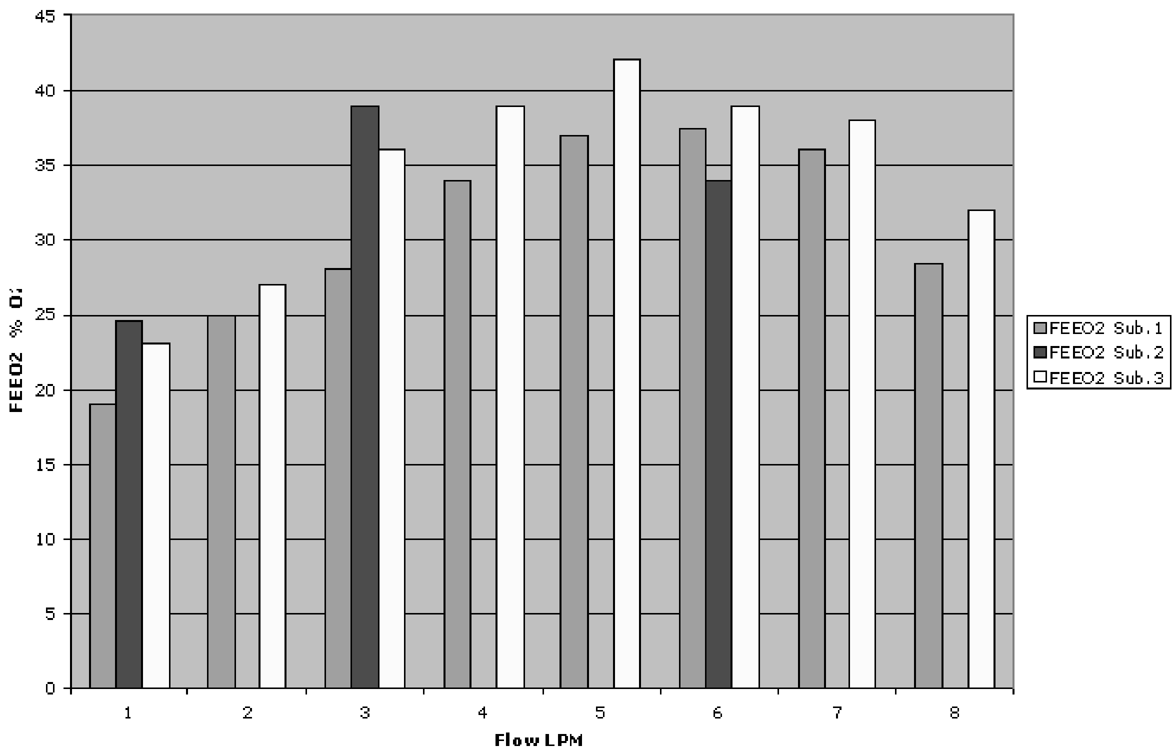

Figure 3. $FEEO_2$ vs. flow

Table 3. $FEEO_2$ vs. O_2 % and flow

L/min	% O_2	$FEEO_2$ Sub.1	$FEEO_2$ Sub.2	$FEEO_2$ Sub.3
1	95.3	19	24.5	23
2	92.5	25		27
3	83.5	28	39	36
4	70.5	34		39
5	61.8	37		42
6	55.5	37.5	34	39
7	49.8	36		38
8	45.8	28.5		32

Table 4 shows the results of the rebreather tests conducted using the Eclipse as an oxygen source. Shown are the supply flow rates and percent O_2, the oxygen % of the inhalation bag, the $FEEO_2$, and the subject. Note that the $FEEO_2$ values listed are all approximately twice those listed in Table 3 for the Hi-OX mask when supplied with a similar gas mixture and flow rates. Table 5 shows data obtained on the same subject on the same day, using the same gas source (Eclipse) when delivered by the Hi-OX mask and rebreather. Again, the rebreather produced twice the $FEEO_2$ of the mask.

Table 4. Rebreather Flow, Bag % O_2, and $FEEO_2$

L/min	Supply % O_2	Bag % O_2	$FEEO_2$	Subject
2	92.5	84	77	1
3	93.5	86.5	79	1
3	93.5	88.5	72.5	2
3	93.5	84.5	73	4

Table 5. $FEEO_2$ Rebreather vs. Hi-OX Mask

L/min	% O_2	$FEEO_2$	Delivery	Subject
3	93.5	37.5	Hi-OX	3
3	93.5	73	Rebreather	3

Table 6 shows the size and weights of the respective units. The Eclipse is the only unit tested that is equipped with a battery. The battery is internal and does not change the size of the case. Weight of the Eclipse is given with and without the battery. The battery is charged in place through the 120 volt AC cord and a 12 volt DC power cord is provided. As is evident from Table 2 the power requirements of the Excell and Healthdyne are small enough for these units to be powered by a 12 volt DC source through a small DC to AC inverter.

Table 6. Unit Dimensions

	Height (in)	Width (in)	Depth (in)	Weight (lb)	Volume (cu in)
Eclipse	18	11.5	7	17.97 with batt. 14.48 w/o batt.	1,449
Excell	18	11.5	11.5	29	2,380
Healthdyne	27	12	18	54.4	5,832

Table 7 shows the relationship between flow settings and output pressure. Note that while the output pressures of the Excell and Healthdyne decrease at higher flow rates, that of the Eclipse increases. Also note from Table 2, that the power requirements of the Excell and Healthdyne are constant at all flow rates while that of the Eclipse increases with increasing flow. This is because the compressor 'speed' of the Excell and Healthdyne is constant at all flow rates, while that of the Eclipse increases with increasing flow. The higher output pressure (9 psig) of the Eclipse offers the potential of using this unit for 'in-water' recompression of divers using a semiclosed-circuit rebreather to a depth of 20 ft.

Table 7. Flow settings vs. output pressure (in psig)

Flow Setting ($L \cdot min^{-1}$)	1	1.5	2	2.5	3	4	5
Eclipse	3	4.5	5.5	9	9		
Excell	2.5		2.5		2.2	2	1.5
Healthdyne	4.2		4.2		4.2	4	4

During a separate study, some day to day variation in the O_2 concentration of the Eclipse was noted. The other units were not used. Careful calibration of the O_2 analyzer showed these variations to be true. The initial tests of all units were conducted at temperatures of 72-78°F. When the Eclipse was used in Key Largo, FL, at temperatures of 92-96°F, a significant decrease in O_2 concentration was measured at the respective flow rates. The unit produced a maximum O_2 concentration of 90%. When the measurements were repeated in an air conditioned space at 73°F the original flow/O_2 values were obtained. This simple observation suggests that the O_2/flow values are dependent on the ambient temperature. While the decrease of approximately 3.5% O_2 for a temperature increase of 20°F is rather small, it may warrant further study. Since the other units tested also work on the PSA basis, known to be temperature sensitive, it is highly probable that their output is also temperature sensitive.

Discussion

1. Using the same high oxygen concentrations and gas flow rates, the rebreather produced twice the forced end expiratory (alveolar) oxygen concentrations as the Hi-OX mask and uniform inspired gas mixtures of 84 - 87 % oxygen.

2. When size, weight, power requirements, and gas output are considered, the Eclipse oxygen concentrator is clearly the most desirable of the units tested for use under remote field conditions.

3. Higher alveolar oxygen concentrations can be achieved with the Hi-OX mask by breathing a high flow (5-6 L/min) gas mixture with low (55%) oxygen content than with a low flow (1-2 L/min) gas mixture with high (93+%) oxygen content. The high flow rates listed above exceed the manufacturer's specifications for the Healthdyne and Excel and similar units. They are 'off scale' on their flowmeters, and are not currently used for oxygen administration. A 'change in procedure,' using the same oxygen concentrators, could result in a significant increase in alveolar oxygen concentration of patients.

4. The Eclipse concentrator provides sufficient pressure, flow, and oxygen concentration to be used for 'in water' recompression, with a semiclosed-circuit rebreather, to a depth of 20 ft.

*Subjects 1 and 4 are the same individual. They are separated by three months in time, a program of physical fitness, and a weight loss of 10 lbs, and thus listed as different subjects.

Acknowledgments

The authors thank subjects David Abbott, Nancy Roche, and Morgan Wells for their time and effort, the NOAA Undersea Research Center of the University of North Carolina, Wilmington for facility support in Key Largo, Florida, and the following manufacturers: Oxlife Inc., Sequal Technologies Inc., and Healthdyne for the loan of the units used in this study.

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AAUS 2008 Symposium Program - Scripps Institution of Oceanography

Friday, March 14th

0830 - 0855 Volunteer Diving
Mitchel Tartt
NOAA Office of National Marine Sanctuaries, Silver Spring, MD

0900 - 0925 Diving with Captive Elasmobranchs
Vallorie Hodges
Oregon Coast Aquarium Newport, OR

0930 - 0955 What Can the U.S. Navy Contribute to Science Diving?
John Clarke
Naval Experimental Diving Unit, Panama City, FL

1000 - 1025 Break

1030 - 1055 In-Water Recompression for the Treatment of Decompression Illness in Remote Locations
Ralph Potkin[1] and J. Morgan Wells[2]
[1]Cedars Sinai Medical Center; [2]Undersea Research Foundation

1100 - 1125 Measurement of Fatigue Following 18 msw Open Water Dives Breather Air or EAN36
Scott Chapman
San José State University, San José, CA

1130 - 1155 Survey Technique for Underwater Digital Photography with Integrated GPS Location Data
Tim Siwiec and Sean Sheldrake,
United States Environmental Protection Agency, Region 10, Seattle, WA

1200 - 1255 Lunch

1300 - 1325 Invertebrate Communities Associated with Crustose Coralline Algae in the Eastern Aluetian Islands
Héloïse Chenelot, Stephen Jewett, Max Hoberg
School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks, AK

1330 - 1355 Reef Check California Subtidal Survey Methods - Adding Science to Safety
Cyndi Dawson
Reef Check, Pacific Palisades, CA

1400 - 1425 Translocation and Homing of Rockfishes from Offshore Oil Platforms in the Santa Barbara Channel
Kim Anthony[1], Christopher .G. Lowe[1], and Milton .S. Love[2]
[1] California State U. Long Beach; [2] Marine Science Institute, U. of Calif. Santa Barbara

1430 - 1455 Only the Strong Will Survive: Red Tides as Community-Structuring Forces in the Eastern Gulf of Mexico

Jennifer Dupont[1] and Casey Coy[2]
[1] University of South Florida College of Marine Science; [2] The Florida Aquarium

1500 - 1525 Break

1530 - 1555 Exploration of the West Florida Shelf Blue Holes: Investigation of Physical and Biological Characteristics

James K. Culter
Mote Marine Laboratory, Sarasota, FL

1600 - 1625 Scuba Techniques for the Alaska; Monitoring and Assessment Program (AKMAP) of the Aleutian Islands

Stephen Jewett[1], Reid Brewer[2], Heloise Chenelot[1], Roger Clark[3], Douglas Dasher[4], Shawn Harper[1] and Max Hoberg[1]
[I] Institute of Marine Science, University of Alaska Fairbanks, Fairbanks; [2] Marine Advisory Program, University of Alaska, Unalaska; [3] Eagle Mountain; [4] Alaska Department of Environmental Conservation, Fairbanks, AK

1630 - 1655 Effects of Tegula Grazing on the Productivity and Reproductive Potential of the Giant Kelp, *Macrocystis pyrifera*, in Central California

Selena McMillian
Moss Landing Marine Lab, Moss Landing, CA

Saturday, March 15th

0800 - 0825 Hydrobiogeochemical Description of Remipede Habitat in a Coastal Karst Cave

Olmo Torres-Talamante, Elva Escobar, P, Beddows and Jill Yager
Universidad Nacional Autónoma de México

8030 - 0855 Assessment of Fish Communities Associated with SC Permitted Artificial Reef Sites with Comparable Natural Hard Bottom Sites by South Carolina Aquarium Volunteer Divers

Arnold Postell
South Carolina Aquarium
Charleston, SC

0900 - 0925 Diver Eco-Tourism and the Behavior of Reef Sharks

Phillip Lobel
Boston University

0930 - 0955 DNA Sequencing of 18s Ribosomal RNA Genes of the Northern Star Coral (*Astrangia poculata*) collected from New Jersey Artificial Reefs

Peter F. Straub and Tara L. Harmer Luke
Biology Program, Natural Sciences and Math, Richard Stockton College
Pomona, NJ

1000 - 1025 Break

1030 - 1055 The Invasion History, Status and Potential Impact of the Indo-Pacific Lionfish in the Western Atlantic

Paula Whitfield[1], Roldan Muñoz[1], Brian Degan[1], Doug Kesling[2], Wilson Freshwater[3], Andy David[4], Stacey Harter[4], James Morris[1] and Jennifer Potts[5]
[1] NOAA National Ocean Service, National Centers for Coastal Ocean Science, Center for Coastal Fisheries and Habitat Research, Beaufort, NC, [2]National Undersea Research Center, University of North Carolina Wilmington, Wilmington, NC, [3]Center for Marine Science, University of North Carolina at Wilmington, Wilmington, NC, [4]NOAA NMFS SEFSC, Panama City Laboratory, Panama City, FL, [5]NOAA NMFS, SEFSC, Beaufort Laboratory Beaufort, NC

1100 - 1125 Dive Logistics of the Turner to Wakulla Cave Traverse

Dawn N. Kernagis[1], Casey McKinlay[2], Todd R. Kincaid[3]
[1]Duke University, Durham, NC
[2]Global Underwater Explorer, Gainsville, FL
[3]Halett-Kincaid, Inc. Reno, NV

1130 - 1155 Southeast Florida Coral Reef Restoration Research

David S. Gilliam[1], Alison L. Moulding[1], Vladimir N. Kosmynin[2], Vanessa I.P. Brinkhuis[1], Elizabeth A. Goergen[1], Allison S. Brownlee[1], Adam St. Gelais[1], and Richard E. Dodge[1]
[1] National Coral Reef Institute, Nova Southeastern University Oceanographic Center; [2] Bureau of Beaches and Coastal Systems, Florida Department of Environmental Protection

1200 - 1255 Lunch

1300 - 1325 The Subtidal Safari; Broadcasting Research and Educational Dives to Live Audiences Using Videoconferencing

Tomas Bird and Anne Stewart
Bamfield Marine Sciences Centre

1330 - 1355 Atmospheric Oxygen Concentrators as a Source of Oxygen and Oxygen Rich Mixtures for Treatment of Diving Accident Victims in Remote Areas

J. Morgan Wells[1] and David A. Dinsmore[2]
[1] Undersea Research Foundation; [2] NOAA Diving Program

1400 - 1425 Tasmanian University Field Safety System (TUFSS)

Simon Talbot
University of Tasmania

1430 - 1455 Scientific Diving in Europe Integration, Representation and Promotion

Martin Sayer[1,2], Philipp Fischer[1,3] and Jean-Pierre Feral[1,4]
[1] European Scientific Diving Committee; [2] UK National Facility for Scientific Diving, Scottish Association for Marine Science, UK; [3] Alfred Wegener Institute, Helgoland, Germany; [4] Centre d'Oceanologie de Marseille, Marseille, France

1500 - 1525 Break

1530 - 1555 The Difficulties of Sampling in Underwater Caves in the Bahamas: An Exercise in Ingenuity and Survival

Stephanie Schwabe
Marine and Environmental Studies Department, University of San Diego, CA

1600 - 1625 Developing a Training Program for Scientific Diving Environmental Health and Safety Professionals

Neal W. Pollock
Divers Alert Network and Center for Hyperbaric Medicine and Environmental Physiology, Duke University Medical Center, Durham, NC

1630 - 1655 Midwater Fish Assemblages Associated with Petroleum Platforms on the San Pedro Shelf

Christopher J.B. Martin and Chris G. Lowe
Department of Biological Sciences, California State University, Long Beach, CA