GREAT LAKES FISHERY COMMISSION

2016 Project Completion Report[1]

A HYPERBARIC HOLDING & TRANSPORT VESSEL FOR COLLECTION OF DEEPWATER FISHES FOR RESEARCH AND BROODSTOCK DEVELOPMENT

by:

Owen T. Gorman
U.S. Geological Survey
Lake Superior Biological Station
Ashland, WI 54806

and

Trevor D. Keyler
Department of Biology
University of Minnesota
Duluth, MN 55812

March 2016

[1] Project completion reports of Commission-sponsored research are made available to the Commission's Cooperators in the interest of rapid dissemination of information that may be useful in Great Lakes fishery management, research, or administration. The reader should be aware that project completion reports have not been through a peer-review process and that sponsorship of the project by the Commission does not necessarily imply that the findings or conclusions are endorsed by the Commission. Do not cite findings without permission of the author.

CONTACT INFORMATION:

Owen T. Gorman, U.S. Geological Survey, Lake Superior Biological Station, 2800 Lake Shore Drive East, Ashland, WI 54806, Tel. 715-682-6163; Email otgorman@usgs.gov

ABSTRACT:

Barotrauma is an array of physiological responses that are manifested when organisms are subjected to a rapid reduction in barometric pressure. Fish brought to the surface from deepwater (> 30 m) quickly succumb to the effects of barotrauma and the severity of barotrauma increases with depth of capture. In 2012, a program to collect deepwater fishes from Lake Superior was initiated to supply live fish for laboratory research on foraging ecology of Siscowet Lake Trout. The challenge was to collect fish from depths > 100 m and keep them alive despite the effects of severe barotrauma. Protocols were developed to increase the short- and long-term survival of deep-caught fishes suffering from barotrauma, but without rapid recompression and controlled decompression (RRCD). Mortality was high in ciscoes and Lake Whitefish (100% within 2-5 days) but relatively low in more resilient species, e.g., Siscowet Lake Trout and Burbot (< 20% over 30 days), though recovery was prolonged (≥ 30 days). To improve survival and health of deep-caught fishes, a hyperbaric apparatus that performs RRCD on deepwater fishes was designed in 2013, constructed in 2013-2015, and tested in fall 2015. The hyperbaric apparatus for fish (*HAfF*) consists of two stainless-steel 50-gallon capacity pressure vessels mounted in a stainless-steel transport frame and a hyperbaric control system (HCS) to regulate RRCD in the vessels. The vessels are capable of rapid recompression to 7 atmospheres (equivalent to 70 m depth) and are insulated to limit temperature change to < 2°C over 6 hours. Decompression is accomplished by manually adjusting pressure regulators in the HCS to decrease vessel pressure in steps (decompressions stops). The HCS allows custom mixing of oxygen, nitrogen, and air to achieve a desired level of dissolved oxygen prior to recompression of fish. Physical parameters (temperature, pressure, dissolved oxygen, conductivity, and pH) are measured inside each vessel by a multi-parameter instrument and remotely displayed and logged on a computer tablet. Video cameras mounted inside each vessel provide live images and record fish movement and behavior under infrared lighting. The *HAfF* can be moved and loaded onto a truck bed with a forklift capable of lifting 1500 kg and loaded onto a ship with a crane with similar lifting capacity. While in transit to a laboratory facility, conditions inside the *HAfF* vessels can be monitored remotely from inside the vehicle cab and adjustments to the HCS can be easily made during short vehicle stops to adhere to a decompression schedule. The *HAfF* was field tested during two deepwater fish collecting trips in October and November 2015. Fish treated with RRCD did not show improved survival compared to fish not decompressed, although treated fish showed reduced signs of barotrauma. 75% of Lake Trout and 60% of Burbot survived long-term (> 30 days) when treated with RRCD but only 25% of sculpins and 10% of Ninespine Stickleback survived long-term and no coregonids (ciscoes and Lake Whitefish) survived beyond 3-4 days. Siscowet and Burbot treated with RRCD recovered quickly compared to untreated fish (7 days vs. ~30 days). Our initial trials showed the *HAfF* worked as designed but additional research is required to refine protocols for live collection, RRCD, and recovery of deepwater fishes. We anticipate that the *HAfF* will be a useful research tool for understanding barotrauma and the physical limitations of diel migration in deepwater fishes, and may serve as a useful tool for live collection of deepwater ciscoes for broodstock development and wildstock propagation initiatives aimed at recovery of extirpated ciscoes in the Great Lakes.

INTRODUCTION:

Barotrauma is an array of physiological responses that are manifested when organisms are subjected to a rapid reduction in barometric pressure. Fish collected from deepwater (> 30 m) quickly succumb to the effects of barotrauma (Feathers and Knable 1983; Rummer and Bennett 2005) and the severity of barotrauma increases with depth of capture (Casillas et al. 1975; Rogers et al. 1986; St. John and Syers 2005; Hannah et al. 2008). Collectively, these symptoms have been referred to as catastrophic decompression syndrome (CDS) by Rummer and Bennett (2005). Recognition of the deleterious effects of barotrauma on deepwater fish stocks subjected to strong fishing pressure is highlighted in rockfish stocks off the west coast of the U.S. (Pribyl et al. 2009, 2011, 2012b). These stocks underwent rapid depletion despite catch-and-release rules because nearly 100% of released fish died of the effects of barotrauma. Losses due to barotrauma in rockfish stocks were addressed by encouraging the use of various deep-release devices that allowed sport fishers to release fish at capture depth.

Recompression by returning fish to capture depth may alleviate some symptoms of barotrauma, e.g., distended swim bladder, but other physiological damage, e.g., torsion and volvulus of stomach and intestine, internal bleeding, hematomas, if extensive, are not resolved and can lead to death (Feathers and Knable 1983; Rummer and Bennett 2005; Morrissey et al. 2005; Jarvis and Lowe 2008). Bruesewitz et al. (1993) found that Burbot with barotrauma that were vented survived equally to Burbot that were recompressed and Keniry et al. (1996) observed increased survival of yellow perch with barotrauma that were not recompressed but swim bladders were vented. In a comprehensive review of published studies on alleviating the effects of barotrauma in fishes, Wilde (2009) contends that regardless of interventions, nearly all fish subjected to barotrauma will perish, usually in a matter of hours to days. Wilde's (2009) analysis challenges the efficacy of deep-release strategies to improve survival of rock fish (Pribyl et al. 2009, 2011, 2012b), though Pribyl et al. (2012a) found that rapidly recompressed rockfish in a laboratory hyperbaric vessel showed 100% survival after 31 days. But real-world conditions may show different outcomes. Experimental field studies by Brown et al. (2008) showed deep-released and surface released fish had the same levels of survival. Assessing survival of fish subjected to barotrauma is problematic because of the difficulty in tracking the fate of released fish in the wild. Moreover, survival of fish subjected to barotrauma is complicated by an array of contributing factors, e.g., depth of capture, injury sustained in capture, time out of water, handling, etc. Pribyl et al.'s (2012a) laboratory study of induced barotrauma in rockfish suggested that the effects of handling outweighed that of barotrauma. Thermal stress is an often overlooked factor contributing to mortality of deep-caught fishes though the physiological effects of thermal stress in fish are well-known (Heinicke and Houston 1965; Heath 1973; Mazeaud et al. 1977). Thermal stress can exacerbate the effects of barotrauma; deepwater fishes that are acclimated to cold water and are suddenly exposed to warmer conditions in surface waters or holding tanks suffer increased mortality (Jarvis and Lowe 2008).

An experimental approach is needed to determine the best means of improving long-term survival of deep-caught fish subjected to barotrauma. To address that need, we developed collection, handling and recovery protocols that improve the survival of deep-caught fishes subjected to barotrauma. Moreover, we designed, constructed, and tested a transportable hyperbaric apparatus for fish (*HAfF*) that can be placed on a research ship, rapidly recompresses deepwater fish shortly after capture, allows controlled decompression, and can be transported from the ship to a laboratory and thus serve as an essential research tool for the study of barotrauma in fishes.

OBJECTIVES:

1. Construct a hyperbaric vessel for recompression, decompression, holding, and transport of deepwater fishes.

The Hyperbaric Apparatus for Fish (*HAfF*) is capable of rapid recompression and controlled decompression (RRCD) of fish was designed and constructed in 2013-2015 and tested in late 2015. The *HAfF* consists of two 50-gallon hyperbaric vessels mounted in a transport frame, pressure control system, internal video systems, and multi-parameter water quality probes. During two field tests in October and November 2015, the *HAfF* performed as designed. Collection trips are planned in 2016 to refine protocols for RRCD of deep-caught fish subjected to barotrauma.

2. Develop field collection, handling, and recompression/decompression protocols to maximize survival of deepwater fishes (Kiyi, Bloater, Shortjaw Cisco, Siscowet Lake Trout, Burbot, and Deepwater Sculpin).

Methods for field collection and handling of live deepwater fishes without RRCD were developed over the course of 5 cruises in 2012-2013. High levels of long-term survival were achieved for Lake Trout and Burbot ($\geq$ 80% over > 1 year), and sculpins (40-50% for 6 months), but no coregonids (ciscoes and Lake Whitefish) survived more than 5 days. Although initial RRCD trials conducted in October and November 2015 did not show improved long-term survival of Lake Trout, visible symptoms of barotrauma were greatly reduced and recovery was shortened from more than 30 days to less than a week. Additional research is required to refine RRCD protocols.

3. Develop recuperative treatment protocols to promote recovery and long-term survival of captive deepwater fishes.

Protocols for collection, recovery, and care of captive deepwater fishes were developed in 2012-2013. Captive Lake Trout, Burbot, and Deepwater Sculpin were successfully rehabilitated after 30 days of recovery and maintained in a laboratory facility for up to three years, but all captive coregonids died within five days. Our initial trials showed that RRCD did not improve survival of deep-caught fishes though recovery time in Lake Trout was reduced from ~30 days to less than a week. We recognize the need to improve environmental conditions in our recovery facility, e.g., providing more space for fish. Additional research is planned to refine recovery and handling with the goal of improving recovery and long-term survival of deepwater fishes.

4. Develop recommendations for live collection of deepwater fishes for use by the Great Lakes fishery community.

In this report we describe collection, recovery, and care protocols without decompression that allowed us to maintain Lake Trout, Burbot, and sculpins in captivity for up to three years, though these protocols have not been effective in improving survival of coregonids beyond 3-5 days. In late 2015, two field trials with the *HAfF* were conducted to evaluate initial RRCD protocols. Though survival was not improved in these trials, future research is planned to refine RRCD and collection and recovery protocols.

METHODS:

Design, Construction, and Operation of Hyperbaric Apparatus for Fish (*HAfF*)

The initial concept design for the *HAfF* was developed by Owen T. Gorman (OTG) in February 2013, refined in March-April 2013 and finalized in September 2013 (Figure 1; Appendix A). Each of the two vessels were designed to hold 50 gallons (189 liters) of water and recompress fish to a pressure of 7 atmospheres and meet or exceed ASME standards for pressure vessels. The vessels were constructed of type 314 stainless-steel alloy to provide corrosion resistance in the presence of oxygen and salt. The vessels were equipped with an insulation jacket to slow warming of chilled water volume. Ports and fittings were provided for instrument probes to monitor water chemistry and pressure and for video cameras. Each vessel is equipped with a pressure gauge, valves for venting and discharging, and a safety relief valve to prevent over-pressurization. A stainless-steel frame to contain the pressure vessels, hyperbaric control system, and high-pressure gas bottles was designed to be moved by forklift or crane and be transported by vehicle (truck) on highways (Figure 2). Empty weight of the *HAfF* is 1,500 lbs (560 kg), weight with the two vessels filled with 50 gallons (189 liters) of water is 2,700 lbs (1,007 kg), and weight fully equipped with two high-pressure gas bottles is 3,000 lbs (1119 kg).

A solicitation for bids to construct the *HAfF* pressure vessels and transport frame was disseminated in May 2013 and a contract was awarded to Alloy Products Corporation of Waukesha, Wisconsin in June 2013. OTG served as the contract technical representative to oversee the engineering design and construction of the *HAfF* vessels and transport frame. After several design modifications, the contract was finalized in September 2013. The pressure vessels and transport frame were manufactured in December 2013 and the finished product was delivered in January 2014. Total cost was $28,311 and funded by USGS.

The *HAfF* hyperbaric control system (HCS; Figure B.1) was designed and constructed by OTG between May 2014 and August 2015. A gas mixer (Superflash® 2-Gas Adjustable Gas Mixer) was custom-made to regulate gas flow, pressure, and mixture of oxygen and nitrogen/air and supplies gas to independent hyperbaric control subsystems for each vessel. Each subsystem is equipped with a precision adjustable pressure regulator to regulate the supply pressure to each vessel and works in tandem with a precision adjustable relief pressure regulator. To avoid wasting gas during operation, the regulators are non-bleeding. The two regulators work together to allow precise increases or decreases in vessel pressure and can be set so that gas is held in the vessels or is allowed to flow through the vessels. The dual-regulator design prevents accidental rapid depressurization of the vessels, which would be harmful to fish. Each subsystem can be operated to aerate water in the vessels via ceramic air stones (Pentair P4® Micro Bubble Oxygen Diffuser MBD-300) in conjunction with a rotameter to measure and regulate gas flow. Aeration can be used to pressurize the vessels by gas infusion, a feature that allows increasing the dissolved oxygen level in the vessels as required. The HCS is equipped with a number of safety features to

prevent over-pressurization (safety relief valves), back-flow of gas or liquid (check valves) and water traps to protect relief regulators from expelled water and condensation. Shut-off valves allow isolation of each section of the HCS to maintain vessel pressure in the case of an interruption of supply pressure, such as when high-pressure gas bottles are changed out. A detailed description of the HCS is provided in Appendix B. Total cost of the parts and materials to build the HCS was $7,860; $7,547 was funded by GLFC and $313 was funded by USGS.

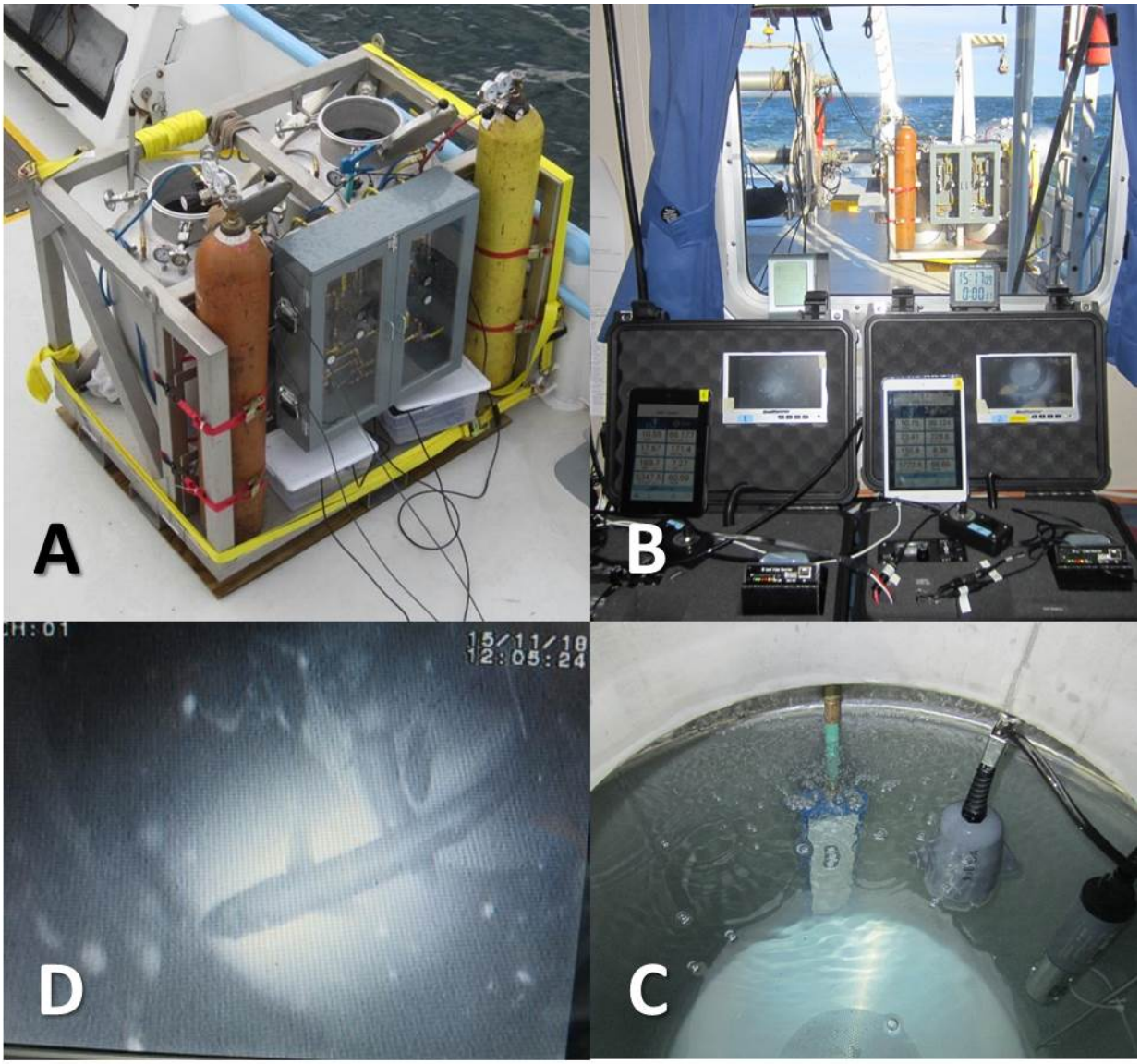


Figure 1. Hyperbaric Apparatus for Fish (*HAfF*). A. Completed *HAfF* on board U.S. Geological Survey Research Vessel Kiyi. B. View of *HAfF* and remote consoles from inside R/V Kiyi. Remote consoles display video images from an internal Seaviewer® camera and data from In-Situ SmarTroll® multi-probe instruments. C. Inside view of pressure vessel from open hatch showing aerator, video camera and internal multi-probe instrument. D. Video frame of fish inside a *HAfF* vessel. Photo credit: Owen Gorman.

Figure 2. Transportability of the Hyperbaric Apparatus for Fish (*HAfF*). A. *HAfF* being loaded onto truck with forklift. B. *HAfF* on truck to deliver fish to the laboratory at University of Minnesota-Duluth. C. Fish being transferred from *HAfF* to coolers at the lab. D. *HAfF* being transferred from R/V Kiyi to truck by crane. Photo credit: Owen Gorman.

In-Situ SmarTROLL® MP multi-parameter instrument probes were installed inside each vessel to measure and record pressure, temperature, conductivity, pH, dissolved oxygen, and oxidation reduction potential (ORP) (Figure 1). This device communicates wirelessly to a computer tablet to log measured parameters to .csv files. A Seaviewer Sea-Drop® underwater video camera was installed inside each vessel to display and record fish behavior to .avi files on a dedicated remote console (Figure1). Remote viewing and logging capability allowed monitoring conditions inside the pressure vessels from inside the research ship while the *HAfF* was placed on the deck during collection cruises, or from inside the cab of a truck when transporting the *HAfF* to a distant laboratory. The instrument logs and video files provide complete records of the RRCD sequence. The cost of the multi-parameter instruments was $8,080 and funded by USGS. The video system cost was $3,910 and funding was evenly split between USGS and GLFC.

Provisional operational protocols were developed for the completed *HAfF* in August 2015 and the *HAfF* was tested for functionality at the Lake Superior Biological Station in September 2015. Field trials aboard the USGS research vessel Kiyi were conducted on October 27 and November 18, 2015. The *HAfF*, loaded with live fish, was transported via truck to the University of Minnesota-Duluth where fish were transferred after decompression to a refrigerated wet lab facility for observation and recuperation. Operational protocols for the *HAfF* HCS were finalized after the second field trial in November 2015 (Appendix B).

Field collection of fish

Fish were collected from the demersal stratum of Lake Superior with bottom trawls towed by the U.S. Geological Survey *R/V Kiyi* at depths of 60-120 m in the Apostle Islands between Stockton, Michigan and Madeline islands in 2012, 2013 and 2015. Most sampling was conducted during daylight hours, although in fall 2012 some night sampling with bottom and midwater trawls was conducted. We limited our sampling to seasons when water surface temperatures were < 10°C to avoid thermal shock to fish captured by trawls from the demersal stratum where temperatures were typically < 5°C. This criterion limited most of our sampling to spring and fall months.

Trawls were towed on contour at target depths (60-120 m) for 10 minutes; the short duration was intended to minimize the time fish are held in the trawl. Trawls were retrieved at normal speeds as there is no feasible means of slowing ascent sufficiently to avoid barotrauma (Rummer and Bennett 2005). The ascent from the deep (> 30 m), cold demersal stratum (2-4°C) to warmer surface waters (6-12°C) imposes severe barotrauma (Feathers and Knable 1983, Rummer and Bennett 2005) and thermal stress on fish (Heinicke and Houston 1965; Heath 1973; Mazeaud et al. 1977; Jarvis and Lowe 2008). Visible symptoms of barotrauma in fish collected by bottom trawls from depths of ≥ 100 m include distended body cavities due to expanded swim bladders, herniated body walls, bulging eyes, and hemorrhaging in fins, skin, and eyes (Figure 3). To reduce stress and initial mortality, fish were transferred as quickly as possible from trawls to temporary holding tanks (100-liter coolers) containing chilled (2-6°C), oxygenated water with 0.5% NaCl, and treated with 0.26 ml/*l* Stresscoat® and 25 mg/*l* MS-222 (anesthetic). These measures follow recommendations from Carmichael et al. (1984) for reducing stress in transported fish. As quickly as possible after fish were transferred to holding tanks, swim bladders of fish were vented with a 14-gauge syringe needle sterilized in 70% ethanol and the wound was dressed with Betadine® iodine tincture. From experience we learned that fish not vented within 5 minutes upon landing would be dead. Ciscoes appear to be especially susceptible to the effects of over-inflated swim bladders as many were dead or nearly so upon transfer from the trawl. Fish that survived this initial intervention to reduce stress and symptoms of acute barotrauma assumed an upright position and appeared to respire normally. For collections made before October 2015, live fish were not decompressed and were transferred from the holding tanks to 75-gallon (284-liter) transport tanks or 100-liter coolers where oxygen aeration was continuously administered until delivery to the University of Minnesota-Duluth (UMD) laboratory. In October and November 2015, live fish were transferred to 50 gallon (189 liter) *HAfF* pressure vessels for RRCD without further aeration while in transit to the UMD laboratory. Water in the transport tanks and *HAfF* vessels was treated in same way as water in the holding tanks except that a lower dosage of MS-222 was administered (2 mg/*l*) for sedation. Collection and handling protocols used in this study are provided in Appendix C.

Recovery and care of fish

Fish were transported by truck from the *R/V Kiyi* dock in Ashland, Wisconsin, to a laboratory facility at the UMD, some 120 km distant, in large tanks, coolers, or *HAfF* vessels. The ambient temperature in the UMD lab was maintained at 5-6°C. A diel photo period of 14 hr light and 10 hr dark was maintained to simulate a circadian rhythm; daytime light level was reduced to that found mid-day at a depth of 45 m in Lake Superior (0.0005 lux). To avoid light disturbance on fish, caretakers worked in infrared or red light illumination. Live fish were transferred to four 150-gallon (568-liter) tanks with water treated with 0.5% NaCl, 0.26 ml /*l* Stresscoat®, and 5 mg/*l* MS-222 and given continuous oxygen aeration for 8 days. Water in tanks was circulated by power filters with biological media at a rate of 185 gal/hr (700 *l*/hr), but without carbon filtration (to avoid removal of anesthetic). Siscowets and Burbot were placed in separate tanks from other species to avoid unwanted predation. At intervals of 4-12 hours, temperature, pH, salinity, and dissolved oxygen (DO) were measured and dead fish were removed and frozen for later enumeration. Ammonia, nitrate, and nitrite levels were measured daily. After 7 days of recovery, atmospheric aeration and carbon filtration was resumed. If fish were active and symptom-free of the effects of barotrauma, food was offered after 3-7 days, though most surviving fish did not meet this criterion or did not consume food until 30 days of recovery. Logs of water quality, fish mortalities and health, and feeding schedules were maintained for each tank. Dead fish were identified to species, measured for total length (mm) and weighed to nearest gram. Recovery and care protocols used in this study are provided in Appendix C.

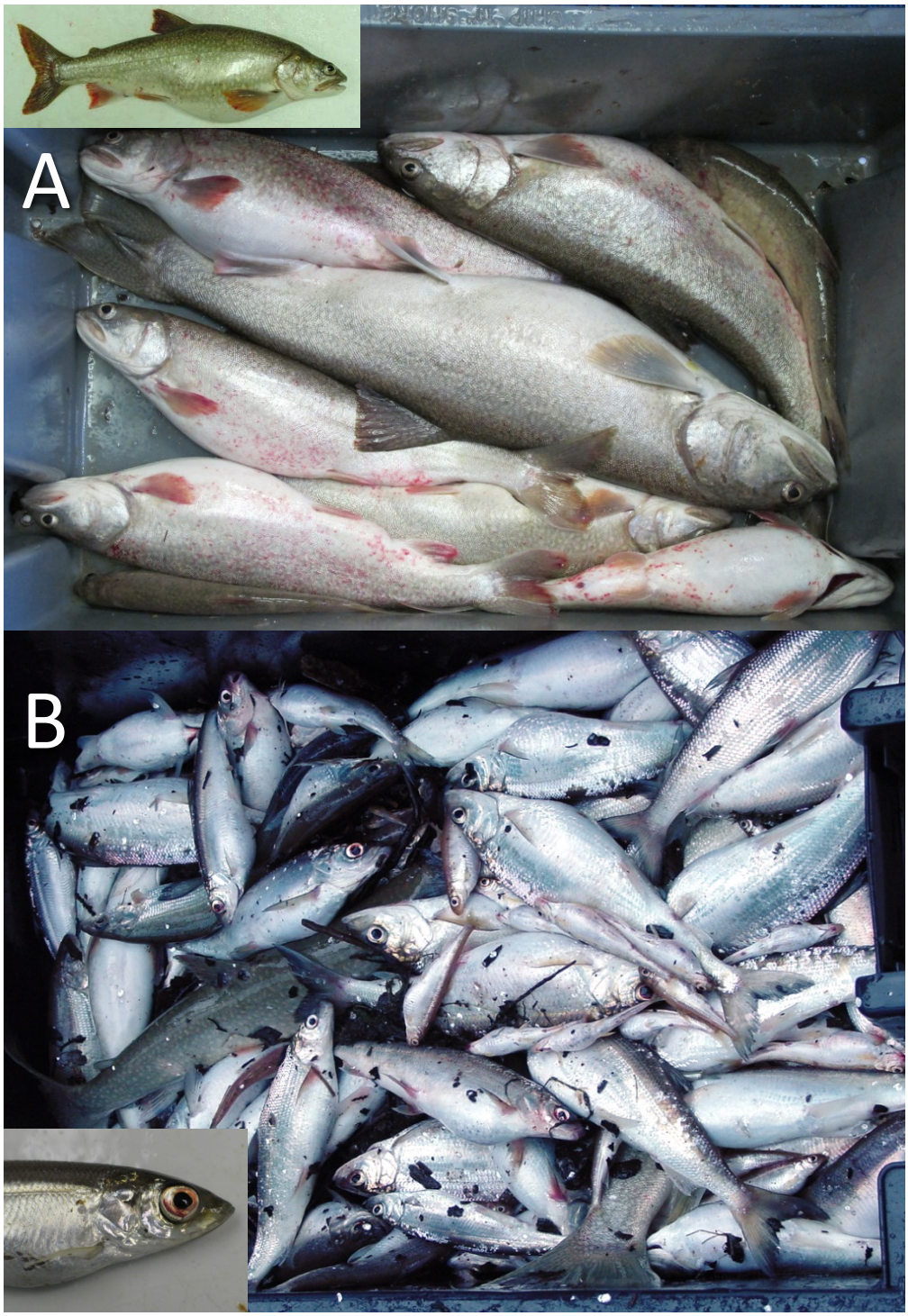


Figure 3. Visible symptoms of severe barotrauma in Lake Superior fishes collected by bottom trawls from depths > 100 m. Photos were taken immediately after emptying fish from the trawls. A. Siscowet Lake Trout, 293-706 mm TL collected from a depth of 183-216 m (inset, Siscowet Lake Trout). The largest individual showed fewer signs of barotrauma than others. B. Mix of Bloater, whitefish, pygmy whitefish, Deepwater Sculpin, and Siscowet Lake Trout collected from a depth of 107-144 m (inset, Bloater). Visible symptoms of severe barotrauma include distended body cavities due to expanded swim bladders, herniated body walls, bulging eyes, and hemorrhaging in fins, skin, and eyes. Because of grossly distended body cavities, ciscoes lose a large proportion of the scales covering their sides and bottom. Photo credit: Owen Gorman.

Decompression of fish

Live fish to be treated with RRCD were transferred from holding tanks to *HAfF* pressure vessels and logging of pressure and water quality parameters and video recording was started. The vessels were pressurized to 7 atm (103 psi; 7.2 kg/cm^2) and the decompression sequence was initiated. The provisional decompression schedule held the vessels at 7 atm for 0.5 hours and decreased the pressure by 0.5 atm every 0.5 hour (decompression stop) and was fully depressurized after 7 hours. Decompression stops were initiated while the *HAfF* was onboard the *R/V Kiyi* and continued while being transported by truck to the UMD lab by making periodic vehicle stops to make scheduled decompression stops. Revised operational protocols for the *HAfF* are provided in Appendix B.

Assessment of fish mortality

Mortality curves for each species were expressed as proportion remaining. Fish collected fall 2012, summer and fall 2013, and spring and summer 2015 were not decompressed and served as a baseline for comparison with fish that would be treated with RRCD in fall 2015.

RESULTS:

1. Construct a hyperbaric vessel for recompression, decompression, holding, and transport of deepwater fishes.

Following development of RRCD protocols for the *HAfF* in August 2015, tests were conducted at the U.S Geological Survey Lake Superior Biological Station (LSBS) to evaluate functionality in September 2015. Overall, the *HAfF* performed as designed, though there were some problems with individual components. Initial pressure tests revealed a design problem with the precision pressure regulators; a small amount of gas was allowed to bleed constantly with the default factory configuration in order to achieve more precise pressure regulation. However, a bleed rate of ~8 liters/hour per regulator at an input pressure of 135 psi was judged to be excessive. After consultation with the manufacturer, it was decided that the bleed feature was not necessary for precise pressure regulation when used in conjunction with a precision relief regulator. With parts provided by the manufacturer, the pressure regulators were modified to be non-bleeding. During initial pressure tests, one of the In-Situ SmarTROLL® MP instruments flooded, destroying the device and had to be replaced. Examination of the instrument revealed a defective O-ring in the oxygen sensor as the likely cause. During the first field trial on October 27, 2015, a precision relief regulator began to leak and required frequent adjustments to maintain pressure. That problem was resolved by rebuilding both relief regulators and sealing leaky diaphragms. Also on the first field trial, one of the video cameras flooded because of a failed O-ring, interrupting the video recording. That issue was resolved by rebuilding the camera with new parts provided by the manufacturer. All components performed flawlessly during the second field trial on November 18, 2015.

The transportable design of the *HAfF* was demonstrated during field trials (Figure 2). The *HAfF* was easily moved and placed onto a truck by forklift and lifted on and off the ship by crane and transported to UMD to deliver fish. While the RRCD sequence was initiated aboard the ship, the remote monitoring and video systems operated flawlessly and continued while the *HAfF* was in transit by truck to UMD. Decompression stops were adjusted in route to UMD by making brief vehicle stops. Fish were easily removed from the *HAfF* vessels with a custom-design net, and transferred to tanks in the wet lab facility.

2. Develop field collection, handling, and recompression/decompression protocols to maximize survival of deepwater fishes (Kiyi, Bloater, Shortjaw Cisco, Siscowet, Burbot, and Deepwater Sculpin).

Methods for field collection and handling of live deepwater fishes without recompression were developed and refined over the course of 8 cruises in 2012-2015. We found that survival from the stress of capture, handling, and barotrauma varied widely by species (Table 1; Figures 4, 5). Lake Trout and Burbot showed the highest rates of survival; we observed nearly 100% long-term survival (> 30 days) in larger fish (> 300 mm TL) and poor survival for smaller fish, all of which died within a week following capture. Sculpins (Slimy, Spoonhead, deepwater) showed variable survival; long-term survival varied from 23% to 73% across the 2013-2015 catches.

Table 1. Survival of deepwater fishes collected from the Apostle Islands Region of Lake Superior at depths > 100 m. Treatments: N – not decompressed; D –decompressed. Mortalities of Siscowet and Burbot were fish < 300 mm TL; all larger fish survived > 30 days. Deepwater ciscoes include Bloater, Kiyi, and Shortjaw Cisco.

				Days since capture									
Species	**Treatment**	**Collection date**		0	1	2	3	4	5	6	7	14	24
Deepwater ciscoes	N	November 2012	Number	57	38	27	8	3	0	0	0	0	
			% remaining	100%	67%	47%	14%	5%	0%	0%	0%	0%	
Deepwater ciscoes	N	June 2013	Number	33	10	0	0	0	0	0	0	0	
			% remaining	100%	30%	0%	0%	0%	0%	0%	0%	0%	
Deepwater ciscoes	N	November 2013	Number	16	6	4	4	1	1	0	0	0	
			% remaining	100%	38%	25%	25%	6%	6%	0%	0%	0%	
Lake Whitefish	N	November 2013	Number	6	3	2	1	1	0	0	0	0	0
			% remaining	100%	50%	33%	17%	17%	0%	0%	0%	0%	0%
Deepwater Sculpin	N	November 2013	Number	605	461	425	385	366	348	341	333	327	305
			% remaining	100%	76%	70%	64%	60%	58%	56%	55%	54%	50%
Burbot	N	November 2013	Number	20	20	20	20	20	20	20	20	20	18
			% remaining	100%	100%	100%	100%	100%	100%	100%	100%	100%	90%
Siscowet	N	November 2013	Number	5	5	5	5	5	5	5	5	5	5
			% remaining	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Deepwater Sculpin	N	June-July 2015	Number	498	301	252	235	235	233	231	229	215	
			% remaining	100%	60%	51%	47%	47%	47%	46%	46%	43%	
Siscowet	N	June-July 2015	Number	3	3	3	3	3	3	3	3	3	3
			% remaining	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Deepwater Sculpin	N	Oct-Nov 2015	Number	41	40	37	36	35	33	33	33	30	30
			% remaining	100%	98%	90%	88%	85%	80%	80%	80%	73%	73%
Deepwater Sculpin	D	Oct-Nov 2015	Number	176	132	81	65	60	55	54	50	45	40
			% remaining	100%	75%	46%	37%	34%	31%	31%	28%	26%	23%
Ninespine Stickleback	D	Oct-Nov 2015	Number	112	53	31	22	21	20	19	19	11	11
			% remaining	100%	47%	28%	20%	19%	18%	17%	17%	10%	10%
Siscowet	D	Oct-Nov 2015	Number	8	8	8	6	6	6	6	6	6	6
			% remaining	100%	100%	100%	75%	75%	75%	75%	75%	75%	75%
Burbot	D	Oct-Nov 2015	Number	5	3	3	3	3	3	3	3	3	3
			% remaining	100%	60%	60%	60%	60%	60%	60%	60%	60%	60%
Deepwater ciscoes	D	Oct-Nov 2015	Number	11	0	0	0	0	0	0	0	0	0
			% remaining	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Lake Whitefish	D	Oct-Nov 2015	Number	7	7	4	3	2	0	0	0	0	0
			% remaining	100%	100%	57%	43%	29%	0%	0%	0%	0%	0%

Ninespine Stickleback showed low survival; only 10% survived more than two weeks after capture in 2015 collections. Coregonids (Bloater, Shortjaw Cisco, Kiyi, and Lake Whitefish) showed poor survival; only a few survived more than a day and all were dead within five days of capture. The first two field trials of the *HAfF* in 2015 did not demonstrate that RRCD improved the survival of deep-caught fishes (Table 1; Figures 4, 5). However, most fish showed reduced visible symptoms of barotrauma upon removal from the *HAfF* vessels compared to untreated (not decompressed) fish; typically < 25% of eyes, fins, and body surfaces showed signs of hemorrhaging. In particular, Siscowet Lake Trout treated with RRCD showed improved condition and rapid recovery vs. untreated fish (Figures 3, 6, 7). Within 48 hours of capture, surficial hemorrhaging and hematomas were greatly reduced in Lake Trout treated with RRCD while these symptoms persisted for weeks in untreated fish (Figure 6). After 6 days of recovery, visible symptoms of barotrauma were greatly reduced in most Lake Trout treated with RRCD (Figure 7). After 3-7 days all treated Siscowet Lake Trout actively took live food compared to ~30 days for untreated fish.

Fish treated with RRCD were relatively active vs. untreated fish which were relatively inactive. Treated coregonids were very active, swimming incessantly around the perimeter of holding tanks. The hyperactivity of treated coregonids in the holding tanks continued until a few hours before death. We suspect that cessation of administering MS-222 anesthetic resulted in increased stress and activity levels which may have contributed to exhaustion and death. Upon initial introduction into holding tanks, treated Lake Trout swam around the tank several times and settled in the middle of the tank suspended in the water column, slowly moving fins and easily maintaining equilibrium while surveying the environs. Untreated Lake Trout remained motionless floating at the surface or resting of the bottom. Within a few days, treated Lake Trout began to actively pursue prey (golden shiners or small white suckers) when introduced into holding tanks, while untreated Lake Trout showed no interest in prey items for 30 days or more. Treated Burbot showed a similar pattern of behavior as Lake Trout, except that they assumed a resting position on the bottom. Differences in behavior of treated vs. untreated Deepwater Sculpin were more difficult to assess as fish in both groups were relatively inactive and rest on the bottom.

In the first *HAfF* field trial in October 2015, we encountered two problems that affected the RRCD sequence. First, a faulty back pressure regulator began leaking sufficiently so that maintaining target pressure was not possible. As a result, the decompression sequence was shortened from 8 hours to 5 hours. Second, the vessel was pressurized by infusion of oxygen, which resulted in very high dissolved oxygen levels (~45 ppm) for 2 to 3 hours of the decompression sequence. The combination of these two stressors may have negated any benefit of decompression.

In the second *HAfF* field trial in November 2015, the decompression sequence was shortened from 8 hours to 7 hours by shortening the last two decompression stops. This was done to allow off-loading of fish upon arrival at the UMD laboratory. This resulted in some fish having slightly bloated swim bladders upon removal from the *HAfF*, indicating that decompression was not complete. The shortened decompression sequence may have compromised the benefit of RRCD.

3. Develop recuperative treatment protocols to promote recovery and long-term survival of captive deepwater fishes.

Our protocols for recovery and care of captive deepwater fishes (Appendix C) proved effective in realizing long-term survival (> 30 days) of Lake Trout and Burbot. For example, of five Siscowet Lake Trout that were captured in October and November 2013 (Table 1), all were still alive in July 2015 when they were transferred to the Duluth Aquarium. A Siscowet Lake Trout captured on May 19, 2015 survived till September 15, 2015 when it succumbed to *Columnaris* infection. A similar fate resulted in the loss of two Siscowets captured on July 31, 2015; one died October 15 and the other November 15. Of the 20 Burbot captured in October 29, 2013, 18 survived through February 12, 2014 when they were euthanized due to space limitations. The two Burbot mortalities represented smaller individuals (< 300 mm TL). Of 8 Siscowet Lake Trout captured in October and November 2015, the two smaller individuals (< 300 mm TL) died after two days, but the 6 remaining Lake Trout (> 300 mm TL) were still alive and well after 4 months; during this period they performed well in a series of feeding behavior experiments at UMD. Unfortunately, we have not been able to extend the life of captive

coregonids beyond 3-5 days.

In October and November 2015, Deepwater Sculpin were split into a RRCD treatment group of 176 fish housed at the UMD laboratory and a control group of 41 untreated fish housed at the LSBS facility. After 24 days, 30 (73%) of the LSBS fish were still alive while only 40 (23%) of the UMD fish were alive (Table 1; Figure 5A). Previous collections of Deepwater Sculpin in 2013 (not decompressed) showed 50% survival after 24 days. These results suggest handling and other stress factors may have overridden barotrauma in determining survival of sculpins in our trials. We are concerned that over-crowding in the few tanks available for recovery of fish at the UMD lab may be a contributing factor.

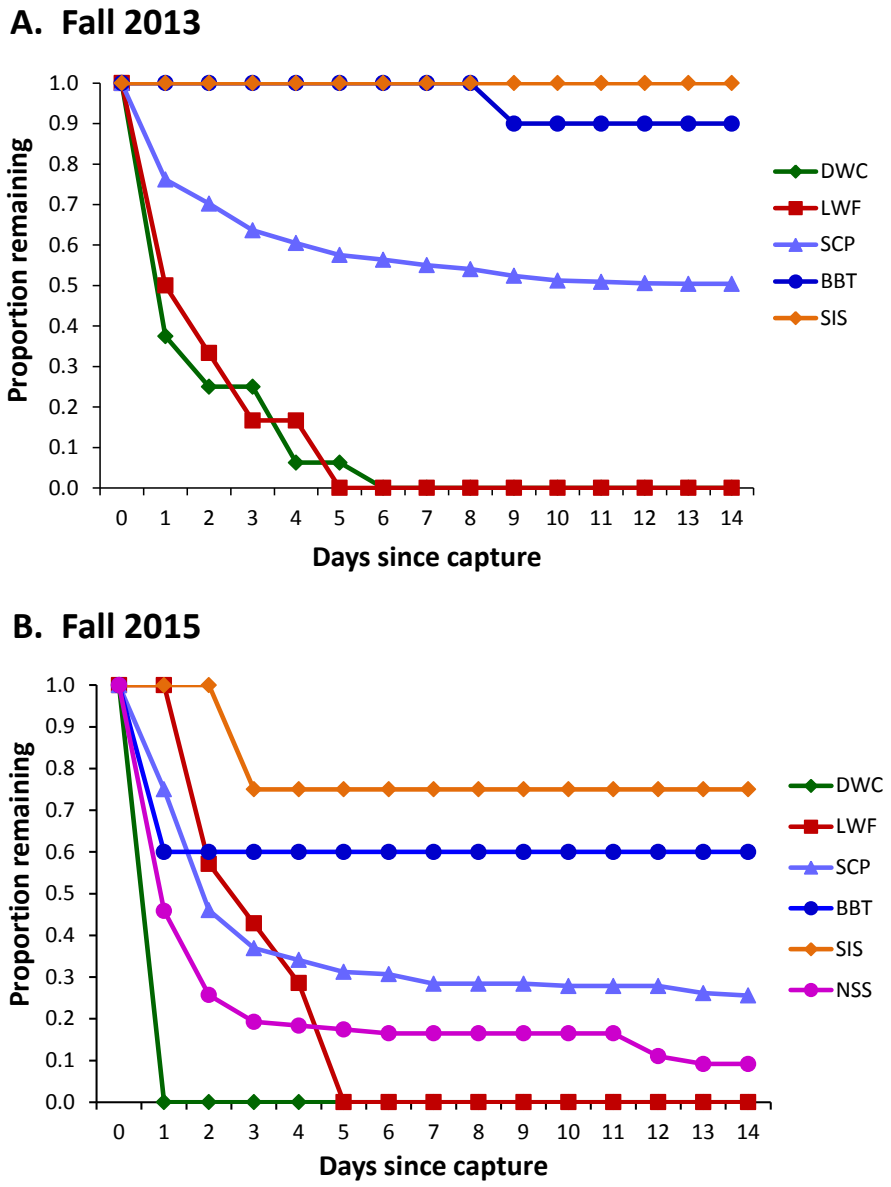


Figure 4. Survival plots of deepwater fishes captured in Lake Superior in 2013 and 2015. A. Fish from 2013 were not decompressed but swim bladders were vented, and treated with oxygen and 0.5% salt solution for 8 days. B. Fish from 2015 were treated similarly and recompressed and decompressed (RRCD) in the *HAfF*. DWC = deepwater ciscoes (Bloater, Kiyi, Shortjaw Cisco); LWF = Lake Whitefish; SCP = sculpins (mostly Deepwater, some Slimy and Spoonhead); BBT = Burbot; SIS= Siscowet Lake Trout; NSS = Ninespine Stickleback.

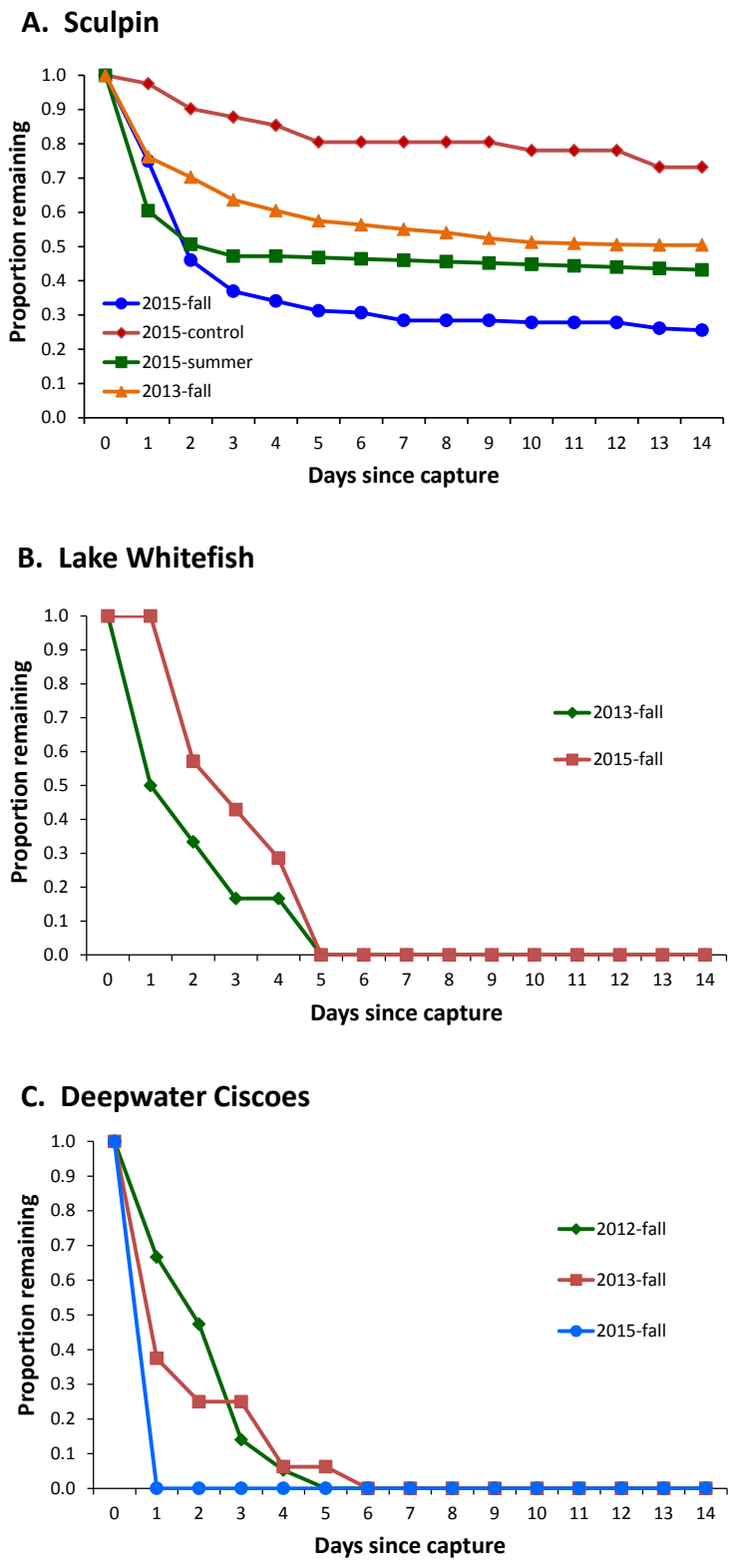


Figure 5. Survival plots of fish captured in Lake Superior in 2013 and 2015. A. sculpins (mostly Deepwater, some Slimy and Spoonhead). B. Lake Whitefish. C. deepwater ciscoes (Bloater, Kiyi, Shortjaw Cisco). Fish from 2013 and from the 2015 summer and 2015 control were not decompressed but were treated with oxygen and 0.5% salt solution for 8 days. Fish from 2015 fall were treated similarly and recompressed and decompressed (RRCD) in the *HAfF*.

4. Develop recommendations for live collection of deepwater fishes for use by the Great Lakes fishery community.

The protocols for collection, recuperation, and care of deep-caught fish provided in this report (Appendix C) serve as recommendations for live collection of deepwater fishes by Great Lakes fishery scientists and managers. Here we provide additional considerations for collection of live deepwater fishes in the Great Lakes.

Collection gear

We used bottom trawls to collect live deepwater fishes. Shorter trawl times decrease the physical injury to fish and increase the likelihood of survival. Sampling at depths deeper than needed to capture target species decreases the likelihood of survival because of longer trawl retrieval times and greater degree of barotrauma. We note that extending the retrieval time does not reduce the degree of barotrauma (Rummer and Bennett 2005) and may increase physical damage and stress to fish. Gillnets are not ideal collection gear in that fish are usually dead upon retrieval because gilling and tangling of fish in gillnets is effective in killing fish before retrieval.

Season

Spring and fall are the best seasons to collect fish as the lake is typically not stratified and surface temperatures are < 10°C. Summer collections when surface waters are warm (> 10°C) pose increased thermal stress for fish collected in demersal stratum where temperatures range 2-5°C. When retrieving trawls through warm epilimnetic waters, most deepwater ciscoes were usually dead before removal from the trawl and most sculpins died shortly after transfer to holding tanks.

Addressing trauma and shock

Deepwater fish captured with bottom trawls suffer from acute barotrauma that must be addressed quickly. We recommend venting swim bladders, oxygen aeration, addition of 0.5% NaCl to water in holding tanks, and chilling water to 2-6°C to avoid initial mortality of the entire catch. We observed that unvented coregonids died within 5 minutes of landing. The addition of 25 mg/*l* MS-222 as a sedative and 0.26 ml/*l* Stresscoat® improves survival over the next 20-30 minutes and continuation of low levels of sedation over 3-7 days may be essential for survival of coregonids. Continued application of cold temperature (4-6°C), oxygen aeration, and 0.5% NaCl for the first 7 days will improve the likelihood of long-term survival for all species.

Adequate space for recovery and long-term care

Ideally, wild fish should be housed in a facility with tanks of adequate volume and isolated recirculation and filtration systems. We had limited space for recovery and long-term care of deep-caught fishes at the UMD lab, so fish were crowded in the four 150-gallon (568-liter) tanks available for use. An outbreak of *Columnaris* and subsequent mortality of Lake Trout in September 2015 is evidence of overcrowding. Adequate space in recovery tanks is essential to avoid stress and deterioration of water quality. We recommend circular tanks stocked with a maximum of 5 Lake Trout (450 mm TL or less) or 20 ciscoes (250 mm TL or less) per 300 gallons (1136 liters) with filtration rates of 350 gallons (1325 liters) per hour. We recommend that smaller fish (< 250 mm TL) be kept in circular tanks no smaller than 250 gallons (946 liters) and larger fish (> 300 mm TL) be kept in circular tanks of at least 500 gallons (1893 liters). Sculpins are easier to maintain in small, shallow tanks; stocking rates should not exceed 20 fish per 30 gallons (114 liters) with filtration rates of at least 150 gallons (568 liters) per hour. The facility should be darkened (illuminated with red light) and maintained at 4-6°C.

Decompression vs. no decompression

Thus far, our first trials of RRCD did not improve the survival of deepwater fish. However, our first trials showed that symptoms of barotrauma were alleviated in Lake Trout treated with RRCD within 48 hours of capture and recovery progressed rapidly (Figures 6, 7). This rapid improvement was not observed in Lake Trout that were not decompressed in earlier collections of live fish. Additional research and trials is needed to determine whether RRCD improves survival of deepwater fishes, especially sensitive species, e.g., ciscoes and whitefish.

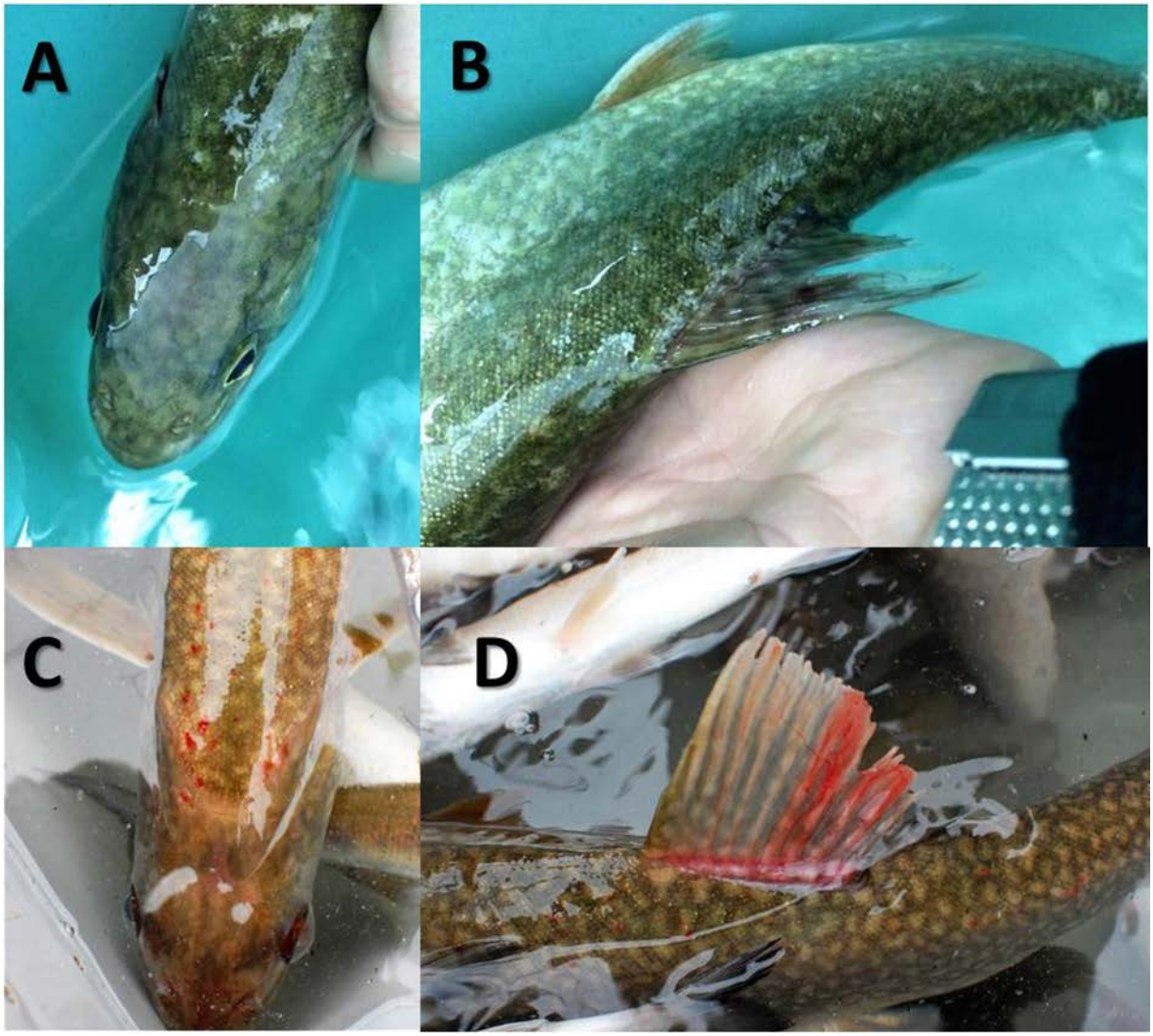


Figure 6. Symptoms of barotrauma in Siscowet Lake Trout after capture from 120 m depth. A, B. Siscowet Lake Trout given rapid recompression and decompression (RRCD) in the *HAfF* (photo taken 48 hours after capture on 18 November 2015). C, D. Siscowet Lake Trout not decompressed (photo taken 6 hours after capture on 29 October 2013). Both fish had swim bladders vented and were treated with 0.5% NaCl and oxygen aeration. The fish treated with RRCD was very active and showed visible resolution of hemorrhaging and hematomas after 48 hours. The fish not decompressed was sluggish and showed extensive hemorrhaging and hematomas. Fish treated with RRCD began feeding within 7 days while fish not decompressed began feeding after 30 days, at which time the symptoms of barotrauma were resolved. Both fish were long-term survivors. The condition of the fish treated with RRCD after 6 days is shown in Figure 7 (Siscowet 1). Photo credit: Trevor Keyler.

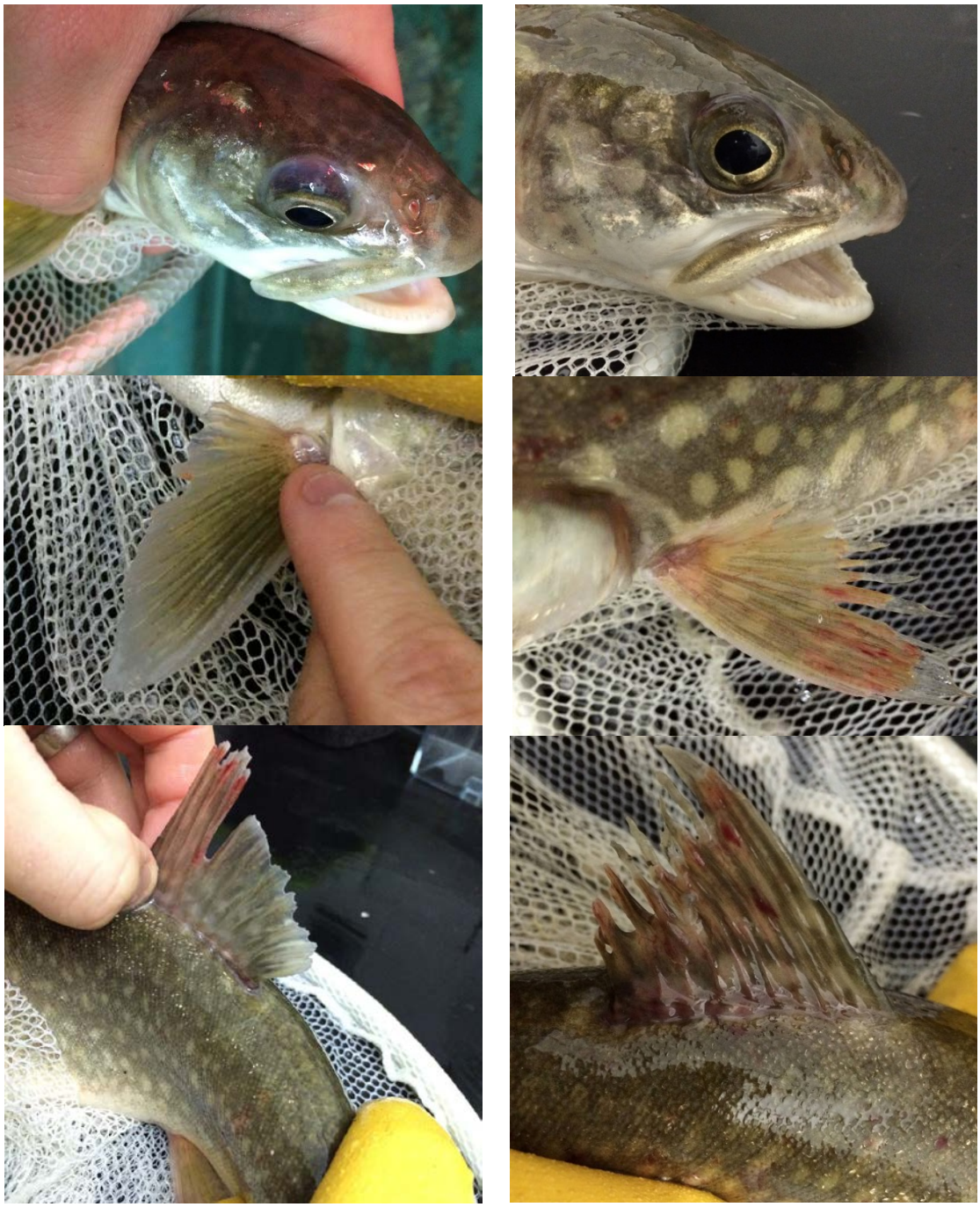
DISCUSSION:

Our barotrauma research grew out of a need to supply live deepwater fish for a Sea Grant-funded study to T. Hrabik, A. Messinger, B. Roth, and OTG titled "Foraging mechanisms and interactions between Siscowet, Kiyi and Deepwater Sculpin: forecasting change in the Lake Superior food web" (Hrabik et al. 2012). Collections of live deepwater fishes were to begin in the summer of 2012, but record-breaking warm epilimnetic temperatures (> 20°C) delayed collections until November 2012 when Lake Superior cooled and became nearly isothermic.

Siscowet 1. decomp. 42 cm

Siscowet 2. decomp. 45 cm

Figure 7. Condition of Siscowet Lake Trout treated with rapid recompression and decompression (RRCD) in the *HAfF* 6 days after capture. All fish were captured on 18 November 2015; photos taken 25 November 2015. Siscowet 1 is shown 48 hours after capture in Figure 6, panels A, B. Photo credit: Trevor Keyler.



Siscowet 3. decomp. 39 cm

Siscowet 4. decomp. 42 cm

Figure 7. (continued)

During the first collections in 2012, protocols for collecting, recuperation, and care of deepwater fishes without decompression were developed but could not be evaluated because many of the fish were sacrificed for research purposes. Application of revised protocols in 2013, with an emphasis on recovery and care of deepwater fish, resulted in improved long-term survival of Siscowet and Burbot. As evidence of our success, Siscowets caught in 2013 were used as test subjects in the UMD laboratory for more than 1.5 years before transferring them to the Duluth Aquarium in July 2015. From 5 deepwater collections made in 2012-2013, fish were supplied to complete two research thesis projects, now published (Harrington et al. 2015, Keyler et al. 2015).

Despite our success in achieving long-term survival (> 1.5 years) in Siscowets collected from the wild without decompression, we were less successful with achieving long-term survival in Deepwater Sculpin (6 months maximum) and were unsuccessful with ciscoes (5 days maximum). Moreover, surviving Siscowets showed a range of symptoms of barotrauma, the most visible being hemorrhaging in surface tissues, fins and eyes and hematomas in musculature (Figure 6C, D), and these fish required prolonged recovery to resolve these symptoms (> 30 days). The progression from initial hemorrhaging to extensive bleeding and formation of hematomas in Lake Trout is evident in comparing of the condition of Lake Trout shortly after capture (Figure 3A) with that 48 hours later (Figure 6C, D).

Inspired by the work of Jarvis and Lowe (2008) and Pribyl et al (2009, 2011, 2012a), suggesting that recompression of deep-caught rock fish (*Sebastes*) can increase survival from barotrauma, and by the work of Drazen et al. (2005) and Smiley and Drawbridge (2007, 2008) who constructed devices for rapid recompression and controlled decompression (RRCD) of deep-caught fishes for laboratory studies, we set out to build the *HAfF* system that would address the need to supply healthy fish for research and broodstock development of native Great Lakes fishes. We succeeded in designing and building a unique apparatus for RRCD of deep-caught fishes. The *HAfF* represents a significant improvement over previous designs by being both a transport and RRCD vessel and by its remote monitoring and logging of physico-chemical conditions and fish behavior inside the pressure vessels.

Funding for construction of the *HAfF* became available in mid-2013 and design and construction of the pressure vessels and transport frame was completed in early 2014, and design and construction of the HCS was completed in mid-2015. The complexity of the *HAfF* posed a number of engineering challenges and contributed to a prolonged design and construction schedule. Nevertheless, we had anticipated conducting field trials with the *HAfF* in fall 2014. Scheduling those trials was tight as our research ship, the *R/V Kiyi*, was scheduled to be overhauled in September and October. As it turned out, mechanical problems extended the overhaul to three months, which effectively eliminated the 2014 fall research schedule and led to our request for an extension of the project. A crowded 2015 field schedule and the need to avoid sampling during warm summer months left fall 2015 as our only option for conducting the first field trials with the *HAfF*. But once again, the *R/V Kiyi* had mechanical problems and was in the shipyard for 6 weeks, leaving few opportunities for field work in fall 2015. Two field trials with the *HAfF* were conducted during the last days of the *R/V Kiyi* field season, October 27 and November 18, 2015. Additional field trials with the *HAfF* in the spring and fall of 2016 are planned.

In the two field trials conducted with the *HAfF* in fall 2015, we found that fish treated with RRCD were more active and had reduced visible symptoms of barotrauma compared to fish not decompressed but did not have improved rates of survival. However, the improved condition and rapid recovery of RRCD-treated Siscowets showed marked improvement compared to fish not decompressed (Figures 6, 7). This result suggests that RRCD is beneficial, but other stressors may be masking that benefit in more sensitive species, e.g., coregonids. For example, ciscoes treated with RRCD appeared healthy but hyperactive and all died in 1-4 days. This suggests that the reduced level of anesthetic (2 mg/*l* MS-222) in the HAfF vessels and recovery tanks was insufficient to reduce stress and subsequent mortality in these sensitive species (Carmichael et al. 1984). We anticipate that increased and prolonged dosage of anesthetic in recovery tanks will improve health and survival of deep-caught fishes, especially coregonids.

The additional field research planned in 2016 with the *HAfF* will be critical in improving the decompression table and refining RRCD protocols for fishes. Examples from the literature, e.g., Smiley and Drawbridge (2007, 2008)

apply U.S. Navy diving decompression tables to fish even though these tables were developed for humans. We question this practice as decompression of air-breathing humans is likely to be very different than decompression of water-breathing fishes; in particular, we expect that fish can be decompressed in much shorter periods than the typical 24 hours used for human subjects. That being said, our shortened decompression periods of 5-7 hours used during our trials was likely too short, or improperly designed. We expect that like for human subjects, the first and last decompression stops for fish should be of longer duration. We anticipate that refinement of decompression tables will improve the health and survival of deep-caught fishes. A revised decompression table will be tested in 2016 (Appendix B).

The original impetus for this project was to provide live deepwater fishes for ongoing research projects and for future initiatives aimed at recovery of deepwater ciscoes in the Great Lakes. The *HAfF* system was designed and constructed to alleviate barotrauma in deep-caught fishes by RRCD and thereby improve the health and survival of deepwater fishes for research and management applications. While we have succeeded in constructing a fully operational *HAfF* system, we have not yet demonstrated the efficacy of the *HAfF* in improving long-term survival of deep-caught fishes. Given the limitations of available opportunities to collect fish and the vagaries of the size of the catches, our priority has always been to provide live fish for research. Moreover, the small size of the UMD laboratory facility limited us to four 150-gallon (568-liter) tanks to house live fish, which limited our ability to examine survival under different collection and treatment scenarios. We suspect that the limited capacity of the tanks at UMD created additional crowding stress on captive fish. Another possible source of stress was the use of inefficient oxygen aerators in our UMD recovery tanks that prevented us from consistently achieving full saturation. We suspect that the combination of these sources of stress with insufficient levels of anesthetic and incomplete decompression likely contributed to the poor survival of coregonids. Together, these factors inhibited our ability to conduct research on barotrauma and the effect of various collection, handling, recovery, and care treatments on the survival of deepwater fishes.

The solution to the problem of inadequate follow-up research on barotrauma is to assemble an adequate holding facility to evaluate various treatments on long-term survival of deepwater fishes affected by barotrauma. That facility would ideally be located near the research vessel dock and thus be accessible to researchers on a 24-hr basis to monitor fish, tanks, filtration systems, etc. Ideally, that facility would be located near Ashland, Wisconsin, and would be seasonal in nature, i.e., it would operate in the fall and winter months in an unheated building, and would be easily dismantled for storage. An ideal capacity would be four 250-gallon (946 liter) and four 500-gallon (1893 liter) round tanks with refrigeration, recirculation and filtration systems. Potential nearby recovery facilities include the University of Wisconsin-Stevens Point Northern Aquaculture Demonstration facility in Bayfield, Wisconsin, and the U.S. Fish and Wildlife Service Iron River National Fish Hatchery in Iron River, Wisconsin. Both these facilities can be reached within 40 minutes by car. Unknown however, is whether either of these facilities have available space for an isolated facility, as wild fish would need to be quarantined.

An inherent problem in collection of deepwater fishes is that temperatures in the deep hypolimnion where they are collected are much colder than epilimnetic temperatures during warmer months of the year. During summer 2012, hypolimnetic temperatures were in the 2-5°C range while epilimnetic temperatures were 18-22°C. Retrieval of trawls through a warm epilimnion resulted in dead catches. During cooler summers, such as 2013 and 2015, epilimnetic temperatures were typically 10-12°C but still posed a significant thermal stress on deepwater fishes. Even during spring or fall months, surface water temperatures can range upwards to 8-9°C. Compounding this problem is that holding and transport tanks and the *HAfF* vessels are filled with warmer surface lake water and chilling volumes of water of more than 100 liters with ice is not practical. The solution is to use refrigeration units to chill water in the tanks or to use a 15-20-m suction line to pump water from below the epilimnion.

To summarize, the goal of this pilot project was to design and construct a *HAfF* for RRCD of fishes and develop protocols that would improve survival and health of deep-caught fishes for the purpose of providing live deepwater fishes for research and broodstock development. We were successful in building a *HAfF* and have demonstrated its functionality and utility. Thus far, the funding for this project has been entirely dedicated to building the *HAfF*, but no funding or resources have been available to refine its use as a tool for management and research. We recommend that a research program be advanced that addresses development of improved

protocols for operation of the *HAfF* and for handling of fish to improve long-term survival and health of deep-caught fishes with barotrauma, with a special emphasis on deepwater ciscoes. That research program will require a dedicated holding facility to evaluate various treatments for fish with barotrauma. Improved survival of deepwater ciscoes, even if for only a matter of weeks, would greatly facilitate the collection of gametes for establishing captive broodstocks for native fish recovery programs.

Relevance of Barotrauma in Great Lakes Fishes to Research and Management

In the Great Lakes, we identified three topics where understanding the physiological impacts of barotrauma in fishes can inform management agencies in developing management strategies to improve health of sportfish stocks and lead to greater understanding of how depth-limited habitat use in fishes affects food webs in the context of lake ecosystems.

First, we recognize that nearly all released Lake Trout captured by sport fishers from depths > 30 m suffer from barotrauma and high mortality is a likely outcome, similar to the example of Pacific rockfish. Although fishery data on the impacts of catch-and-release mortality on Lake Trout stocks have not been released, S. Sitar, Michigan DNR, is preparing a report on such a study, which shows high mortality in catch-and-release Lake Trout. Research on barotrauma in Lake Trout under controlled conditions would identify possible measures to reduce mortality in deep-caught fish and thereby reduce this source of fishing mortality. Specifically, research is needed to assess the utility of venting swim bladders and deep-release devices in reducing mortality of deep-caught Lake Trout.

Second, efforts to establish broodstocks of deepwater ciscoes for restoration in the lower Great Lakes can be facilitated by reducing barotrauma in deep-caught fishes intended for propagation. As these fish typically inhabit waters deeper than 60 m, they suffer acute barotrauma when brought to the surface and typically die within a few minutes. Additional research is needed to find ways to alleviate the effects of barotrauma in deepwater ciscoes and thereby increase survival. Presently, the severe barotrauma exhibited by deepwater ciscoes is an impediment to successful harvest of gametes for captive broodstock development.

A third area of interest focuses on the effects of barotrauma on deepwater fishes of the Great Lakes as they undergo large daily vertical migrations (DVM). Species that undergo > 50 m of DVM in Lake Superior include deepwater ciscoes and Siscowet Lake Trout (Hrabik et al. 2006, Ahrenstorff et al. 2011). Approximately 80% of the offshore fish biomass in Lake Superior expresses DVM (Gorman et al. 2012a, b). Feeding behaviors in predators and prey drive DVM and fish serve as vectors linking deep benthic-demersal habitats to upper pelagic habitats through exchange of nutrients and energy (Stockwell et al 2010; Gorman et al. 2012a, b; Stockwell et al. 2014). Unknown is the potential for the physiological effects of barotrauma to limit the extent of DVM in fishes, either by species or size class. For example, we have little understanding of how Siscowet Lake Trout are able to undergo rapid DVM from depths > 200 m to surface strata without suffering the effects of barotrauma. A related topic of interest focuses on how deepwater fishes interact and find food in the profundal depths of Lake Superior. Keyler et al. (2015) showed that the threshold for visual detection of prey fish for Siscowet Lake Trout occurs at 150 m depth under mid-day light levels. Research on physiology and behavior of deepwater fishes in a laboratory setting requires development of methods of collecting, handling, and recovery of deep-caught fish subjected to barotrauma. An essential tool for collection and studies of deepwater fishes is a hyperbaric apparatus that recompresses and decompresses fish subjected to barotrauma.

REFERENCES:

Ahrenstorff, T.D., Hrabik, T.R., Stockwell, J.D., Yule, D.L., and Sass, G.G. 2011. Seasonally dynamic diel vertical migrations of *Mysis diluviana*, coregonines fishes, and Siscowet in the pelagia of Western Lake Superior. Trans. Amer. Fish. Soc. 140:1504–1520.

Brown, I.W., W.D. Sumpton, M. McLennan, D. J. Welch, J. Kirkwood, A. Butcher, A. Mapleston, D. Mayer, G. Begg, M. Campbell, I. Halliday, and W. Sawynok. 2008. National strategy for the survival of released line-caught fish: tropical reef species. Project Report PR07-3313, Queensland Department of Primary Industries and Fisheries, Brisbane, Australia.

Bruesewitz, R.E., Coble, D.W., and Copes, F. 1993. Effects of deflating the expanded swim bladder on survival of Burbot. N. Amer. J. Fish. Manage. 13:346-348.

Carmichael, G.J., Tomasso, J.R, Simco, B.A., and Davis, K.B. 1984. Characterization and alleviation of stress associated with hauling largemouth bass. Trans. Amer. Fish. Soc. 113:778-785.

Casillas, E,, S. E. Miller, L. S. Smith, and B. G. D'Aoust. 1975. Changes in hemostatic parameters in fish following rapid decompression. Undersea Biomedical Research 2:267-276.

Drazen, J.C., Bird, L.E., and Barry, J.P. 2015. Development of a hyperbaric trap-respirometer for the capture and maintenance of live deep-sea organisms. Limnol. Oceanogr.: Methods 3:488-498.

Feathers, M.G., and Knable, A.E. 1983. Effects of depressurization upon largemouth bass. N. Am. J. Fish. Manag. 3: 86–90.

Gorman, O. T., Yule, D. L., and Stockwell, J. D. 2012a. Habitat use by fishes of Lake Superior. I. Diel patterns of habitat use in nearshore and offshore waters of the Apostle Islands region. Aquat. Ecosys. Health & Mngt. 15: 333-354.

Gorman, O. T., Yule, D. L., and Stockwell, J. D. 2012b. Habitat use by fishes of Lake Superior. II. Consequences of diel habitat use for habitat linkages, habitat coupling and energy and nutrient transfers in nearshore and offshore waters. Aquat. Ecosys. Health & Mngt. 15: 355-368.

Hannah, R. W., S. J. Parker, and E. M. Matteson. 2008. Escaping the surface: the effect of capture depth on submergence success of surface-released Pacific rockfish. N. Am. J. Fish. Manag. 28:694-700.

Harrington, K.A., Hrabik, T.R., Mensinger, A.F., 2015. Visual sensitivity of deepwater fishes in Lake Superior. PLoS One 10.

Heath, A. G. 1973. Ventilatory responses of teleost fish to exercise and thermal stress. American Zoologist 13: 491-503.

Heinicke, E.A., and Houston, A. H. 1965. Effect of thermal acclimation and sub-lethal heat shock upon ionic regulation in the goldfish, *Carassius auratus* L. J. Fish. Res. Bd. Canada 2: 1455-1476.

Hrabik, T.R., Jensen,O.P., Martell, S.J. D.,Walters, C.J., and Kitchell, J.F. 2006. Diel vertical migration in the Lake Superior pelagic community. I. Changes in vertical migration of coregonids in response to varying predation risk. Can. J. Fish. Aquat. Sci. 63, 2286–2295.

Hrabik, T.R., Mensinger, A., Roth, B.M., and Gorman, O.T. 2012. Foraging mechanisms and interactions between Siscowet, Kiyi and Deepwater Sculpin: forecasting change in the Lake Superior food web. Minnesota Sea Grant Project, 2012-2014, $102,203.

Jarvis, E.T., and Lowe, C.G. 2008. The effects of barotrauma on the catch-and release survival of southern California nearshore and shelf rockfish (Scorpaenidae, *Sebastes* spp.). Can. J. Fish. Aquat. Sci. 65: 1286–1296

Keniry, M.J., Brofka, W.A., Horns, W.H., and Marsden, J.E. 1996. Effects of decompression and puncturing the gas bladder on survival of tagged yellow perch. N. Amer. J. Fish. Manage. 16:201-206.

Keyler, T.D, Hrabik, T.R., Austin, C.L., Gorman, O.T., Mensinger, A.F. 2015. Foraging mechanisms of Siscowet Lake Trout (*Salvelinus namaycush Siscowet*) on pelagic prey. J. Great Lakes Res. 41: 1162-1171.

Mazeaud, M.M., Mazeaud, F., Donaldson, E.M. 1977. Primary and secondary effects of stress in fish: Some new data with a general review, Trans. Amer. Fish. Soc. 106: 201-212.

Morrissey, Ì. B., C. D. Suski, K. R. Esseltine, and B. L. Tufts. 2005. Incidence and physiological consequences of

decompression in smallmouth bass after live-release angling tournaments. Trans. Amer. Fish. Soc. 134:1038-1047.

Neiffer, D.L., and Stamper, M.A. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: Considerations, methods, and types of drugs. ILAR Journal 50:343-360.

Pribyl, A.L. 2010. A Macroscopic to Microscopic Study of the Effects of Barotrauma and the Potential for Long-term Survival in Pacific Rockfish. Doctoral Dissertation submitted to Oregon State University, Eugene, OR. 172p.

Pribyl, A.L., Schreck, C.B., Kent, M.L., and Parker, S.J. 2009. The differential response to decompression in three species of nearshore Pacific rockfish. N. Am. J. Fish. Manag. 29: 1479-1486.

Pribyl A.L., Kent M.L., Parker S.J. & Schreck C.B. 2011. The response to forced decompression in six species of Pacific rockfish. Trans. Am. Fish. Soc. 140: 374–383.

Pribyl, A.L., Schreck, C.B., Kent, M.L., Kelley, K.M., and Parker, S.J. 2012a. Recovery potential of black rockfish, *Sebastes melanops* Girard, recompressed following barotrauma. Journal of Fish Diseases 35:275-286.

Pribyl, A.L., Schreck, C.B., Parker S.J., and Weis, V.M. 2012b. Identification of biomarkers indicative of barotrauma and recovery in black rockfish *Sebastes melanops*. Journal of Fish Biology 81:181–196.

Rogers, S. G., Ç. T. Langston, and Ô. E. Targett. 1986. Anatomical trauma to sponge-coral reef fishes captured by trawling and angling. Fishery Bulletin 84:697-703.

Rummer, J.L., and Bennett, W.A. 2005. Physiological effects of swim bladder overexpansion and catastrophic decompression on red snapper. Trans. Am. Fish. Soc. 134:1457-1470.

Smiley, J.E., and Drawbridge, M.A. 2007. Techniques for live capture of deepwater fishes with special emphasis on the design and application of a low-cost hyperbaric chamber. Journal of Fish Biology 70: 867–878.

Smiley, J.E., and Drawbridge, M.A. 2008. A simple apparatus for maintaining gas-supersaturated seawater in the laboratory for experimental purposes. N. Am. J. Aquaculture 70:61-67.

St. John, J., and C. J. Syers. 2005. Mortality of the demersal West Australian dhufish, (Richardson 1845) following catch and release: the influence of capture depth, venting and hook type. Fisheries Research 76:106-116.

Stockwell, J.D., Hrabik, T.R., Jensen, O.P., Yule, D.L., and Balge, M., 2010. Empirical evaluation of predator-driven diel vertical migration in Lake Superior. Can. J. Fish. Aquat. Sci. 67: 473-485.

Stockwell, J.D., Yule, D.L., Hrabik, T.R., Sierszen, M.E., and Isaac, E.J. 2014. Habitat coupling in a large lake system: delivery of an energy subsidy by an offshore planktivore to the nearshore zone of Lake Superior. Freshwater Biology 59: 1197–1212

Wilde, G.R. 2009. Does Venting Promote Survival of Released Fish? Fisheries, 34: 20-28.

ACKNOWLEDGMENTS:

Many people contributed to the success of this complex field, laboratory, and engineering project. During the early phases of this project, USGS technician Gary Cholwek, a former Navy Seal, contributed his knowledge and experience of diving to the initial concept of the *HAfF*. Alloy Products Corp. engineer Ray Woo guided the final design and construction of the *HAfF* pressure vessels and frame. USGS technician Lori Evrard provided essential logistical support for field work and ensured that field data were properly recorded when most of the attention was focused on attending to live or dying fish. Many students gave support for the field effort. From Northland College, Taylor Stewart, Taylor Lepak, Tyler Sikora, Allison DeRose, Ryan Menebroeker, Joseph Mrnak, and Kara Winter assisted with field collections. From UMD, Kelly Harrington, Matthew Santo, Emily Wack, Graham

Hanson, Lee Austin, Marc Sizer, Brooke Vetter, Ian Harding, Loranzie Rogers, and Jamie Dobosenski provided assistance with field collections and care of captive fish. USGS vessel crew Joe Walters, Keith Peterson, and Chuck Carrier ensured that field operations went smoothly and safely guided the transfer of heavy tanks and other equipment on and off the ship without a hitch. USGS Great Lakes Science center provided support for field work and funding for the construction of *HAfF* pressure vessels and frame. The GLFC provided most of the funding for the *HAfF* hyperbaric control system. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

DELIVERABLES:

The hyperbaric system (*HAfF*) was completed in August 2015 and tested for functionality in October and November 2015. A final written report summarizing results of field collections with recommendations for live collection of deepwater fishes and a description of design and operation of the *HAfF* was submitted to GLFC by January 31, 2016.

Other products:

Progress report to GLFC, December 2014

Presentations:
- Lake Superior Technical Committee Meeting, Red Cliff, Wisconsin, July 29, 2014
- Ashland Rotary Club Luncheon, Ashland, Wisconsin, September 9, 2014
- Ashland Medical Society, Ashland, Wisconsin, September 11, 2014
- Lake Superior Technical Committee Meeting, Marquette, Michigan, January 12, 2016
- Ashland Science on Tap, Ashland, Wisconsin, January 19, 2016
- USGS Great Lakes Science Center, All-Hands Meeting, Erie, PA, February 23-25, 2016 (poster)

Publications:
Harrington, K.A., Hrabik, T.R., Mensinger, A.F., 2015. Visual sensitivity of deepwater fishes in Lake Superior. PLoS One 10.

Keyler, T.D, Hrabik, T.R., Austin, C.L., Gorman, O.T., Mensinger, A.F. 2015. Foraging mechanisms of Siscowet Lake Trout (Salvelinus namaycush Siscowet) on pelagic prey. J. Great Lakes Res. 41: 1162-1171.

PRESS RELEASE:

In late 2015, the U.S. Geological Survey tested a novel hyperbaric apparatus for fish (*HAfF*) that decompresses fish collected in deep waters of the Great Lakes. Like humans, fish brought quickly to the surface from deep water suffer from barotrauma, commonly known as the “bends”, and if not quickly recompressed and slowly decompressed, will suffer severe physiological damage or death. The *HAfF* consists of two 50-gallon stainless steel pressure vessels and a control system mounted in a sturdy frame and is transportable by truck from laboratory to ship. The *HAfF* is capable of recompressing fish to 7 atmospheres (equivalent depth of 70 m, or 230 feet). Video cameras and instrumentation inside the pressure vessels record fish behavior and physical and chemical conditions and are monitored on remote displays. Initial field tests of the *HAfF* in October and November 2015 demonstrated its functionality and decompressed Lake Trout caught at depths of 120 m were nearly free of visible symptoms of barotrauma. Additional research is planned in 2016 to develop and refine operation of the new equipment. The *HAfF* system is intended to improve the survival of deep-caught fish for research programs and establishment of captive broodstocks for Great Lakes native fish recovery programs. The *HAfF* will also be a useful research tool for studying physiological adaptations of fish that undergo daily vertical migrations (DVM) and for studying the effects of barotrauma on survival of deepwater Lake Trout caught and released by anglers. The development and construction of the *HAfF* was funded jointly by the U.S. Geological Survey and the Great Lakes Fishery Commission.

APPENDIX A.

Conceptual design and engineering drawings for the Hyperbaric Apparatus for Fish (*HAfF*)

Figure A.1 Hyperbaric Pressure Vessel Conceptual Design, Hyperbaric Control System. Concept Drawing, v4b, June 20, 2013.

Figure A.2. Hyperbaric Pressure Vessel Conceptual Design, Vessel Top Lay-out. Concept Drawing, v2, July 12, 2013.

Figure A.3. Hyperbaric Pressure Vessel Conceptual Design, Vessels Mounted in Frame, Top View. Concept Drawing, v2, July 12, 2013.

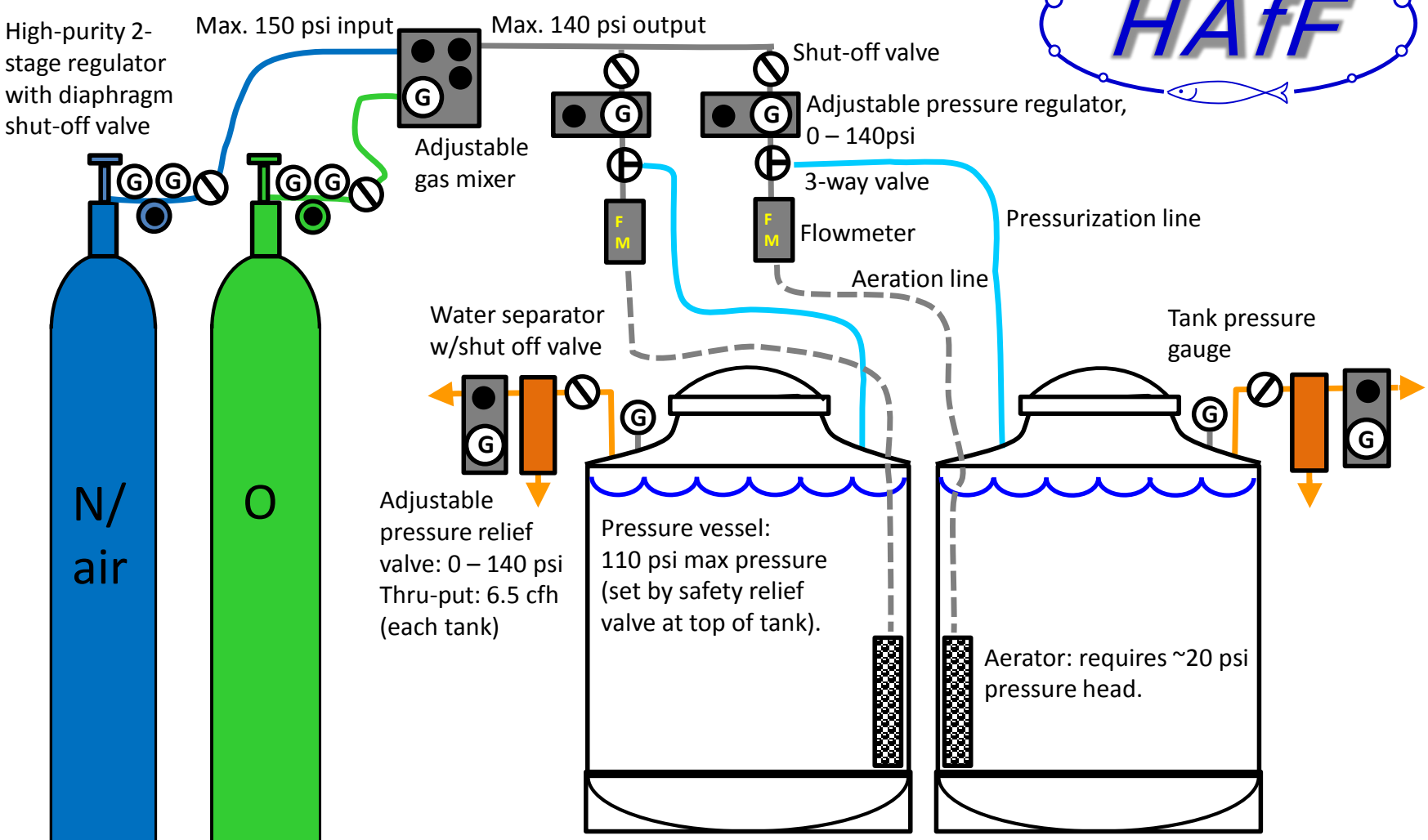
Figure A.4. Hyperbaric Pressure Vessel Conceptual Design, Vessels Mounted in Frame, Side and Front/Rear Views. Concept Drawing v2, July 12, 2013

Figure A.5. Engineering Drawing, Pressure Vessel. Revision B, 9/5/2013.

Figure A.6. Engineering Drawing, Frame. Revision B, 9/5/2013.

Figure A.1. Hyperbaric Pressure Vessel Conceptual Design

Hyperbaric Control System, Concept Drawing v4b, June 20, 2013



Hyperbaric vessels with mixed oxygen and nitrogen/air regulated gas pressure. Vessel pressure is regulated by adjustable precision pressure regulator in tandem with relief valve.

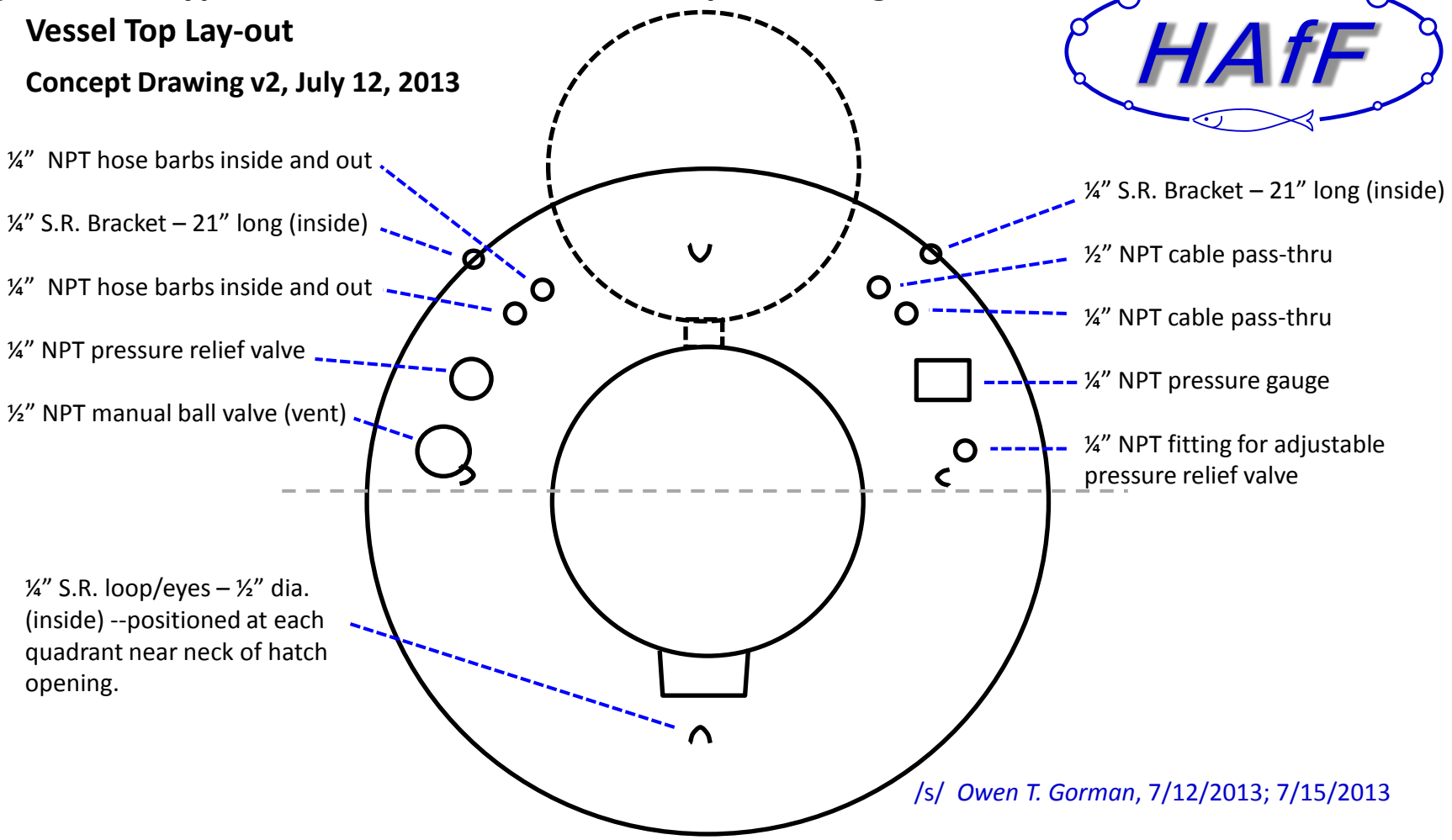
/s/ Owen T. Gorman, 6-20-14

USGS science for a changing world

Figure A.2. Hyperbaric Pressure Vessel Conceptual Design

Vessel Top Lay-out

Concept Drawing v2, July 12, 2013



/s/ Owen T. Gorman, 7/12/2013; 7/15/2013

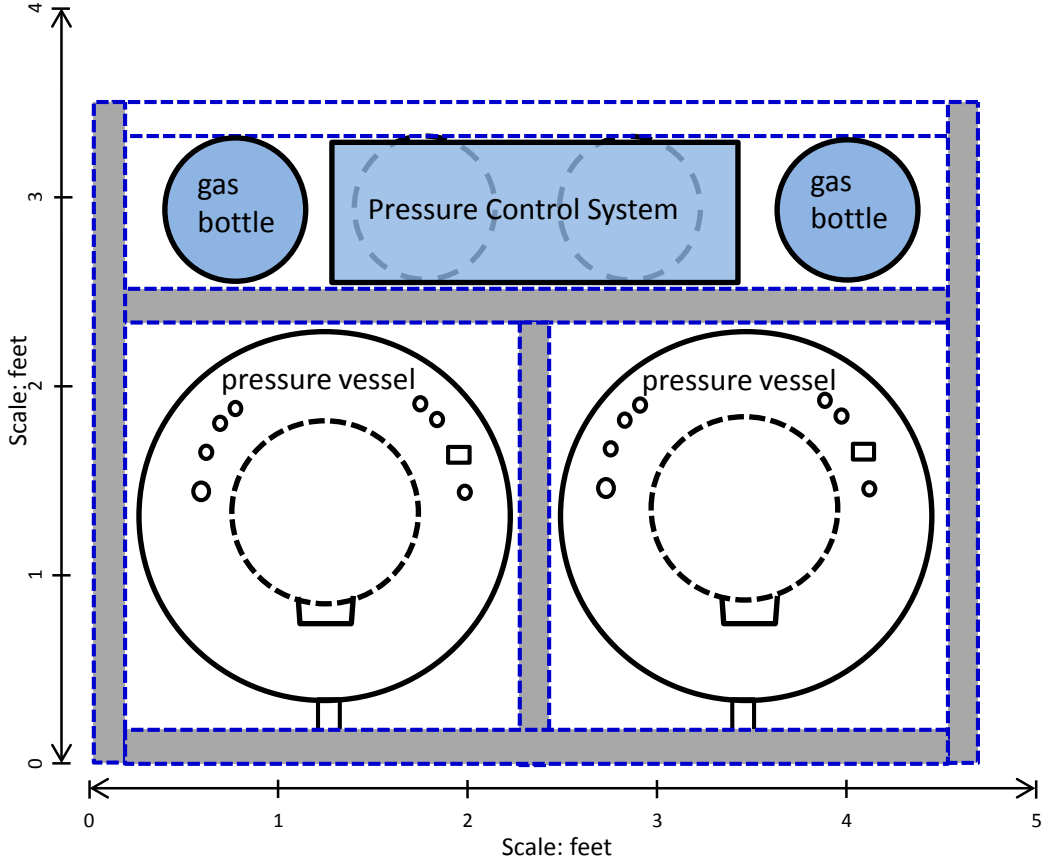
Vessel top: suggested lay-out of connections, pass-thrus, hatch hinge and lift handle

- Connections, etc. are placed toward the rear of the tank as shown to provide access to the hatch and protection.
- Second ¼" NPT for hose connection should be plugged; reserved for future use.
- Additional ¼" NPT fitting for pressure relief valve to be added.
- ¼" S.R. loop/eyes – ½" dia. (inside) –positioned inside at each quadrant near neck of hatch opening;
- ¼" S.R. Bracket – 21" long (inside) shown for relative placement near hose barbs and cable pass-thrus.
- Brackets and loop/eyes provide attachments for various instruments inside the vessel.

Figure A.3. Hyperbaric Pressure Vessel Conceptual Design

Vessels Mounted in Frame, Top View

Concept Drawing v2, July 12, 2013



- <u>Frame design considerations:</u>
- Frame dimensions (depth, width) adjusted to contain and protect pressure vessels (no gas bottles inside frame). One dimension of the footprint cannot exceed 48”
- Frame height set just below hatches to protect connections and gauges.
- Gas bottle rack –back of frame. Gas bottles are mounted in a rack outside vessel frame to facilitate mounting and securing up to four bottles and the Pressure Control System. Horizontal bars at top and bottom of back of frame serve as anchors to strap four bottles to rack.
- Cross members stiffen and strengthen the frame.
- Lift lugs/eyes at top corners of vessel frame for attachment of crane harness.
- Vessels are to be bolted securely in frame at four points at top and four points at the bottom of the frame.
- Frame base designed to accept fork lift, front or rear.
- <u>Vessel installation design considerations</u>
- Vessels are mounted so the hatches lift from front; gauges, valves, lines, pass-thrus located on back half of tank top.
- Vessel drain valves mounted toward front so that frame cross members do not interfere with access (can be angled L or R).

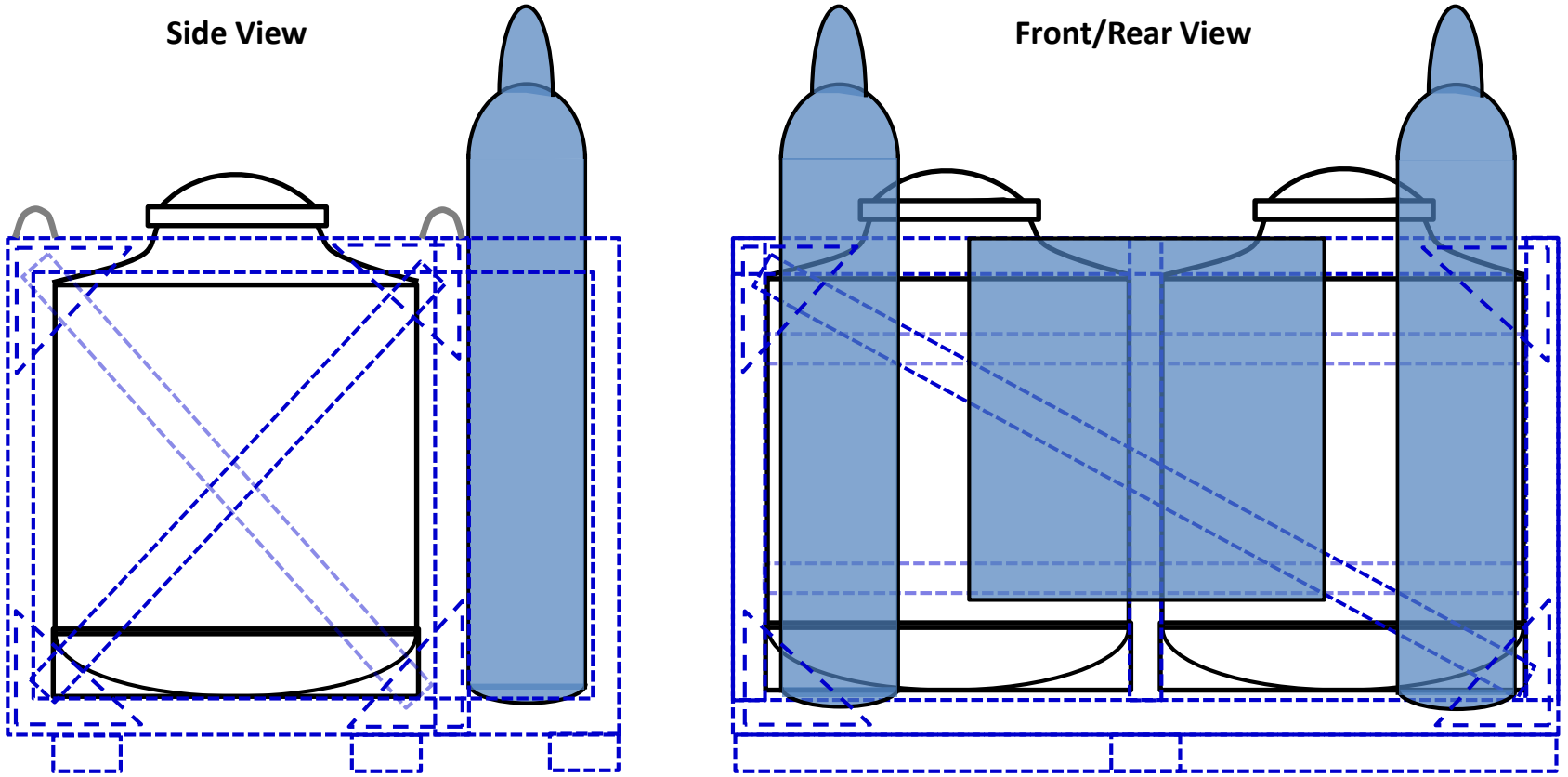
/s/ Owen T. Gorman, 7/12/2013

Figure A.4. Hyperbaric Pressure Vessel Conceptual Design

Vessels Mounted in Frame, Side and Front/Rear Views

Concept Drawing v2, July 12, 2013

HAfF



USGS
science for a changing world

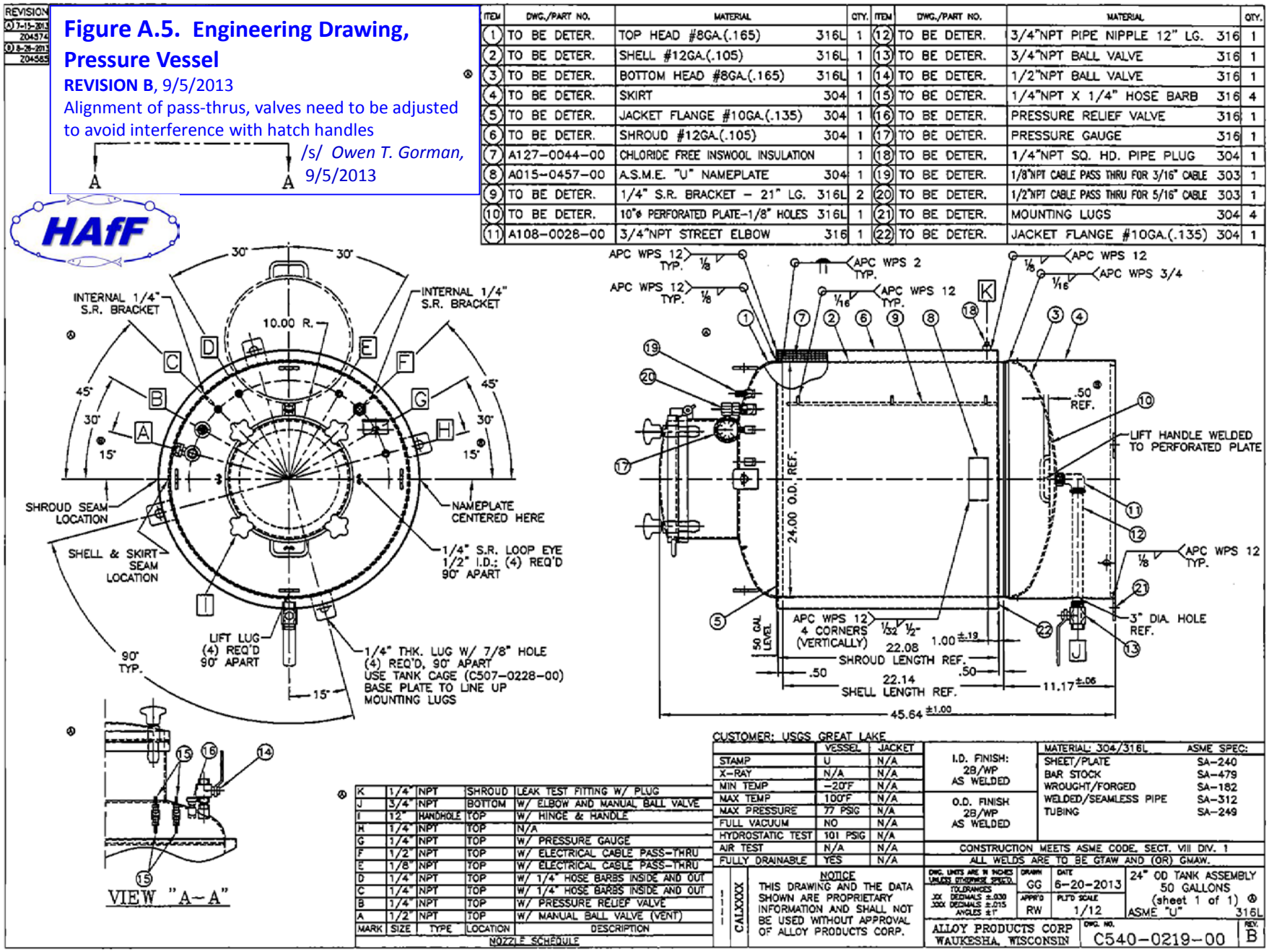
/s/ Owen T. Gorman, 7-12-13

Figure A.5. Engineering Drawing, Pressure Vessel

REVISION B, 9/5/2013

Alignment of pass-thrus, valves need to be adjusted to avoid interference with hatch handles

/s/ Owen T. Gorman, 9/5/2013



ITEM	DWG./PART NO.	MATERIAL	QTY.	ITEM	DWG./PART NO.	MATERIAL	QTY.
1	TO BE DETER.	TOP HEAD #8GA.(.165) 316L	1	12	TO BE DETER.	3/4"NPT PIPE NIPPLE 12" LG. 316	1
2	TO BE DETER.	SHELL #12GA.(.105) 316L	1	13	TO BE DETER.	3/4"NPT BALL VALVE 316	1
3	TO BE DETER.	BOTTOM HEAD #8GA.(.165) 316L	1	14	TO BE DETER.	1/2"NPT BALL VALVE 316	1
4	TO BE DETER.	SKIRT 304	1	15	TO BE DETER.	1/4"NPT X 1/4" HOSE BARB 316	4
5	TO BE DETER.	JACKET FLANGE #10GA.(.135) 304	1	16	TO BE DETER.	PRESSURE RELIEF VALVE 316	1
6	TO BE DETER.	SHROUD #12GA.(.105) 304	1	17	TO BE DETER.	PRESSURE GAUGE 316	1
7	A127-0044-00	CHLORIDE FREE INSWOOL INSULATION	1	18	TO BE DETER.	1/4"NPT SQ. HD. PIPE PLUG 304	1
8	A015-0457-00	A.S.M.E. "U" NAMEPLATE 304	1	19	TO BE DETER.	1/8"NPT CABLE PASS THRU FOR 3/16" CABLE 303	1
9	TO BE DETER.	1/4" S.R. BRACKET - 21" LG. 316L	2	20	TO BE DETER.	1/2"NPT CABLE PASS THRU FOR 5/16" CABLE 303	1
10	TO BE DETER.	10"ø PERFORATED PLATE-1/8" HOLES 316L	1	21	TO BE DETER.	MOUNTING LUGS 304	4
11	A108-0028-00	3/4"NPT STREET ELBOW 316	1	22	TO BE DETER.	JACKET FLANGE #10GA.(.135) 304	1

MARK	SIZE	TYPE	LOCATION	DESCRIPTION
K	1/4"	NPT	SHROUD	LEAK TEST FITTING W/ PLUG
J	3/4"	NPT	BOTTOM	W/ ELBOW AND MANUAL BALL VALVE
I	12"	HANDHOLE	TOP	W/ HINGE & HANDLE
H	1/4"	NPT	TOP	N/A
G	1/4"	NPT	TOP	W/ PRESSURE GAUGE
F	1/2"	NPT	TOP	W/ ELECTRICAL CABLE PASS-THRU
E	1/8"	NPT	TOP	W/ ELECTRICAL CABLE PASS-THRU
D	1/4"	NPT	TOP	W/ 1/4" HOSE BARBS INSIDE AND OUT
C	1/4"	NPT	TOP	W/ 1/4" HOSE BARBS INSIDE AND OUT
B	1/4"	NPT	TOP	W/ PRESSURE RELIEF VALVE
A	1/2"	NPT	TOP	W/ MANUAL BALL VALVE (VENT)

NOZZLE SCHEDULE

CUSTOMER: USGS GREAT LAKE

	VESSEL	JACKET
STAMP	U	N/A
X-RAY	N/A	N/A
MIN TEMP	-20°F	N/A
MAX TEMP	100°F	N/A
MAX PRESSURE	77 PSIG	N/A
FULL VACUUM	NO	N/A
HYDROSTATIC TEST	101 PSIG	N/A
AIR TEST	N/A	N/A
FULLY DRAINABLE	YES	N/A

I.D. FINISH: 2B/WP AS WELDED

O.D. FINISH 2B/WP AS WELDED

MATERIAL: 304/316L	ASME SPEC:
SHEET/PLATE	SA-240
BAR STOCK	SA-479
WROUGHT/FORGED	SA-182
WELDED/SEAMLESS PIPE	SA-312
TUBING	SA-249

CONSTRUCTION MEETS ASME CODE, SECT. VIII DIV. 1

ALL WELDS ARE TO BE GTAW AND (OR) GMAW.

CAL XXXX

NOTICE

THIS DRAWING AND THE DATA SHOWN ARE PROPRIETARY INFORMATION AND SHALL NOT BE USED WITHOUT APPROVAL OF ALLOY PRODUCTS CORP.

DWG. UNITS ARE IN INCHES UNLESS OTHERWISE SPECIFIED. TOLERANCES: XX DECIMALS ±.030, XXX DECIMALS ±.015, ANGLES ±1°

DRAWN GG | DATE 6-20-2013

APP'D RW | PLT'D SCALE 1/12

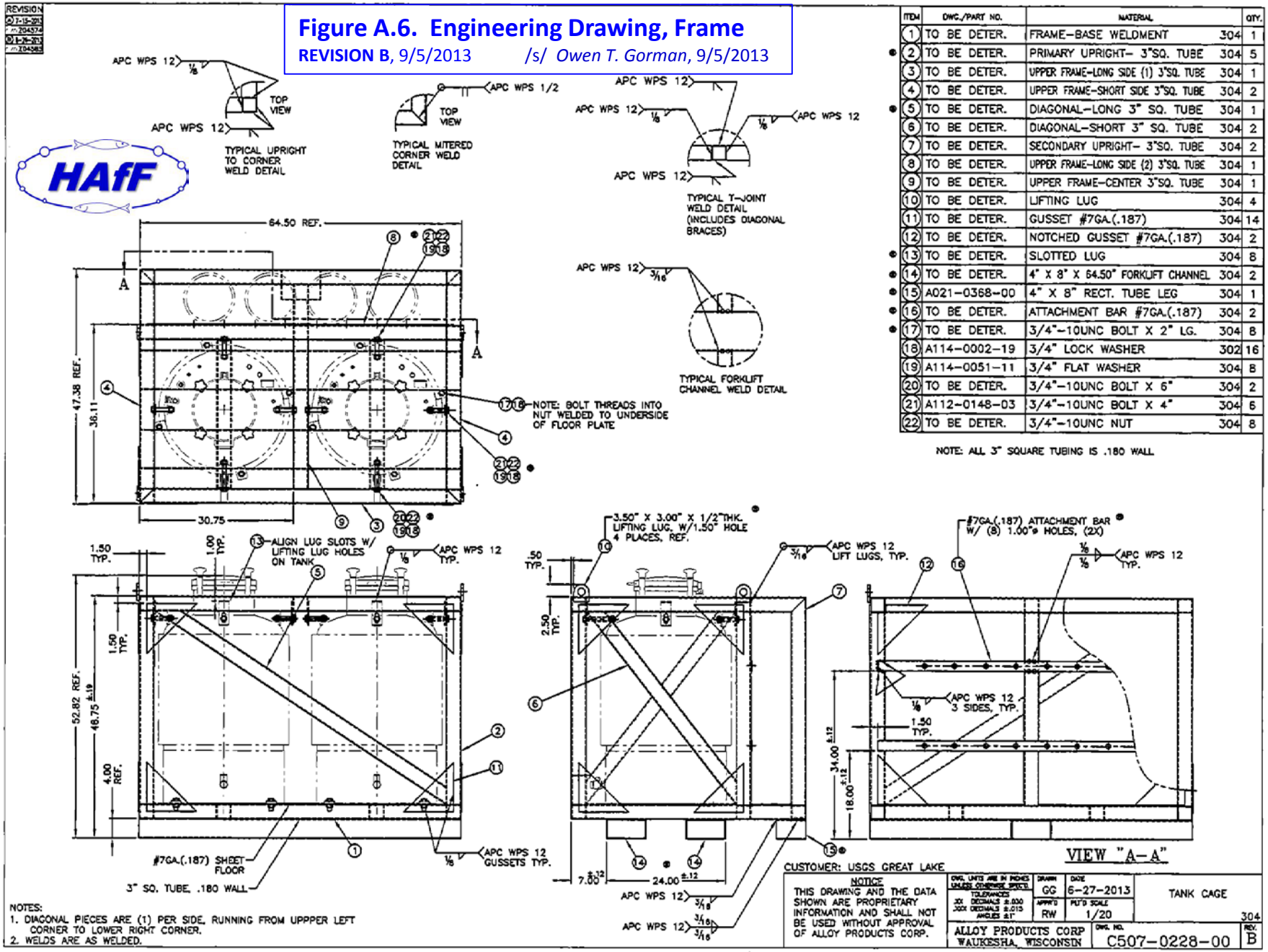
24" OD TANK ASSEMBLY 50 GALLONS (sheet 1 of 1) ⊗ ASME "U" 316L

ALLOY PRODUCTS CORP WAUKESHA, WISCONSIN

DWG. NO. C540-0219-00 REV. B

Figure A.6. Engineering Drawing, Frame

REVISION B, 9/5/2013 */s/ Owen T. Gorman*, 9/5/2013



ITEM	DWG./PART NO.	MATERIAL		QTY.
1	TO BE DETER.	FRAME-BASE WELDMENT	304	1
2	TO BE DETER.	PRIMARY UPRIGHT- 3"SQ. TUBE	304	5
3	TO BE DETER.	UPPER FRAME-LONG SIDE (1) 3"SQ. TUBE	304	1
4	TO BE DETER.	UPPER FRAME-SHORT SIDE 3"SQ. TUBE	304	2
5	TO BE DETER.	DIAGONAL-LONG 3" SQ. TUBE	304	1
6	TO BE DETER.	DIAGONAL-SHORT 3" SQ. TUBE	304	2
7	TO BE DETER.	SECONDARY UPRIGHT- 3"SQ. TUBE	304	2
8	TO BE DETER.	UPPER FRAME-LONG SIDE (2) 3"SQ. TUBE	304	1
9	TO BE DETER.	UPPER FRAME-CENTER 3"SQ. TUBE	304	1
10	TO BE DETER.	LIFTING LUG	304	4
11	TO BE DETER.	GUSSET #7GA.(.187)	304	14
12	TO BE DETER.	NOTCHED GUSSET #7GA.(.187)	304	2
13	TO BE DETER.	SLOTTED LUG	304	8
14	TO BE DETER.	4" X 8" X 64.50" FORKLIFT CHANNEL	304	2
15	A021-0368-00	4" X 8" RECT. TUBE LEG	304	1
16	TO BE DETER.	ATTACHMENT BAR #7GA.(.187)	304	2
17	TO BE DETER.	3/4"-10UNC BOLT X 2" LG.	304	8
18	A114-0002-19	3/4" LOCK WASHER	302	16
19	A114-0051-11	3/4" FLAT WASHER	304	8
20	TO BE DETER.	3/4"-10UNC BOLT X 6"	304	2
21	A112-0148-03	3/4"-10UNC BOLT X 4"	304	6
22	TO BE DETER.	3/4"-10UNC NUT	304	8

NOTE: ALL 3" SQUARE TUBING IS .180 WALL

NOTES:
1. DIAGONAL PIECES ARE (1) PER SIDE, RUNNING FROM UPPPER LEFT CORNER TO LOWER RIGHT CORNER.
2. WELDS ARE AS WELDED.

DRAWN GG | DATE 6-27-2013 | APP'D RW | PLT'D SCALE 1/20 | 304 | REV. B

APPENDIX B.

Hyperbaric Apparatus for Fish (*HAfF*): Hyperbaric Control System. Overview, Principle of Operation, and Decompression Procedure

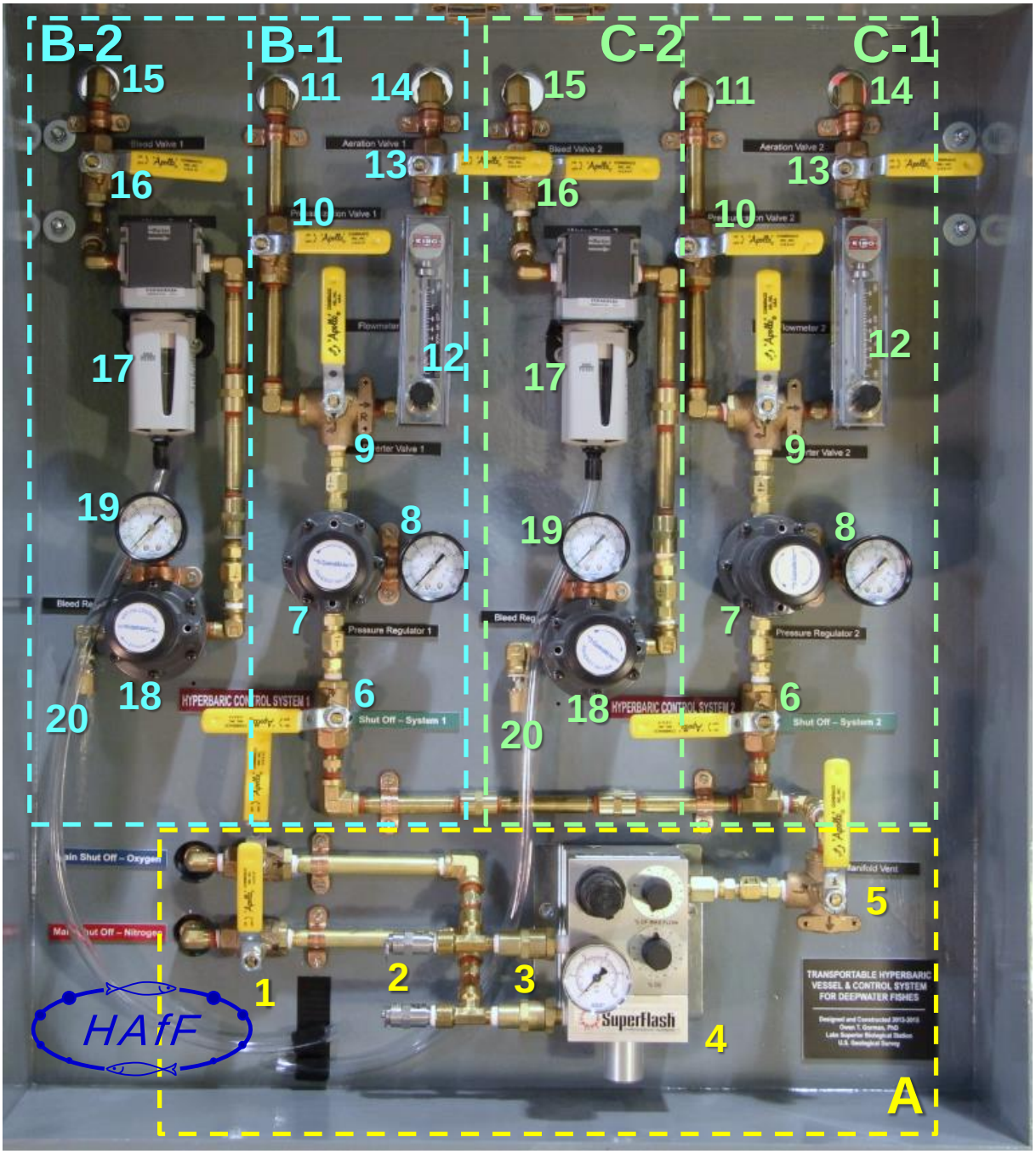


Figure B.1. *HAfF* Hyperbaric Control System (HCS). A. Input gas control subsystem: shutoff valves (1), relief valves (2), check valves (3), SuperFlash® gas mixer (4), diverter/vent valve (5). HCS pressurization subsystems (B-1, C-1): shutoff valve (6), precision regulator (7), pressure gauge (8), diverter valve (9), shutoff valve (10), check valve and vessel input hose connection (11), flowmeter (12), shutoff valve (13), check valve and vessel input hose connection (14). HCS venting subsystem (B-2, C-2): vessel outlet hose connection (15), shutoff valve (16), water trap/filter (17), backpressure regulator (18), pressure gauge (19), exhaust muffler (20). Photo credit: Owen Gorman.

HAfF Hyperbaric Control System (HCS): Overview and Principle of Operation

Gas Supply

High-pressure bottled gas provides compressed oxygen and nitrogen or air to the HCS via 2-stage pressure regulators mounted on the top of each gas cylinder. Each pressure regulator has a shut-off valve to control output gas flow. Regulators are set at 145 psi output pressure.

Input gas control subsystem

The input gas control subsystem (A) provides pressure- and flow-regulated mixed gas to the HCS pressurization subsystems (B1, C1). Inline shutoff valves (1) just downstream of the gas supply connections allow isolation of the system. Downstream of the shutoff valves, relief valves (2) with soft seats vent input pressure in excess of 150 psi (begins venting > 140 psi). One-way check valves (3) at the input of the gas mixer (4) prevent reverse flow of gas and retain downstream pressure should the upstream gas supply pressure be disrupted. The Superflash Gas Mixer® (4) regulates gas pressure, mixture, and flow. The mixer is designed for a maximum input pressure of 145 psi and a minimum 123 psi output. Exceedances of 145 psi will do no harm but will reduce the accuracy of the gas mixture slightly. Approximately 22 psi pressure head is needed to ensure precise mixing of gases. In practice, the output of the mixer is 135 psi @ 145 psi input. A diverter valve (5) immediately downstream of the gas mixer allows venting of the mixer and isolates HCS pressurization subsystems (B1, C1). The diverter valve prevents depressurization of the HCS pressurization subsystems when venting the gas mixer.

HCS pressurization subsystems

Input gas pressure to the vessels is regulated by the HCS pressurization subsystems (B1, C1). Shutoff valves (6) isolate each pressurization subsystem, allowing independent pressure regulation of each vessel. Precision regulators (7) regulate input gas pressure to the vessels and a pressure gauge (8) indicates the regulated pressure. A diverter valve (9) downstream of each precision regulator allows the option of pressurization or aeration of a vessel. The vessel is isolated from the pressurization lines by a shutoff valve (10) and a one-way check valve (11) to prevent reverse flow of gas or fluid. Similarly, the vessel is isolated from the aeration lines by a shutoff valve (13) and a one-way check valve (14) to prevent reverse flow of gas or fluid. A precision flow meter (12) in the aeration line downstream of the diverter valve indicates and controls the rate of gas flow to the aerators inside each vessel. Target flow rates are ~1-2 liters/minute or ~2-4 cubic feet/hour.

HCS venting subsystem

Gas is vented from the vessels through the HCS venting subsystem (B2, C2) and works in tandem with the HCS pressurization subsystems (B1, C1) to regulate vessel pressure, decrease vessel pressure, and allow aeration of pressurized vessels. Gas exits the vessel via a sintered bronze breather vent inside the tank just under the hatch; this location prevents large amounts of water from entering the line. Immediately downstream of the vessel outlet line (15) is a shutoff valve (16) to isolate the vessel. When open, gas passes through a downstream filter (17), preventing water and debris from entering the downstream backpressure regulator (18). The backpressure regulator controls and vents gas via a sintered bronze muffler (20). A pressure gauge (19) on the backpressure regulator indicates upstream pressure in the vent line (which should equal the vessel pressure). When closed, the shutoff valve at the head of the venting line (16) isolates the pressure vessel and prevents venting when the filter bowl needs servicing or for other reasons when gas flow must be stopped.

HAfF Hyperbaric Control System (HCS): Initial Settings

1. Fill vessel with water to just above the shoulder (just above weld line where straight sidewalls meet curved neck below the hatch). This is approximately 50 gallons. Close hatch and tighten the four dogs in a cross-way pattern. Hand-tighten compression nuts on sealing glands for instrument cable pass-throughs on the top of the vessels.

2. Set up gas supply; attach regulators to gas bottles, open bottle valves and adjust regulator pressure to 145 psi. Keep outlet valve on regulator closed until the gas hoses are connected and the HCS is set for initial operation.

3. Input gas control subsystem (A), initial settings: Close the two input shutoff valves (1) (handles up). Set gas mixer (4) dials to 0% flow and 0% mixture. Pressure setting is not critical at this time. Move diverter valve (5) from vent (handle up) to downstream pressurization (handle right).

4. HCS pressurization subsystems (B1, C1), initial settings: Close input shutoff valves (6) (handles left). Close output shutoff valves (10, 13) (handles right). Diverter valves (9) are set to pressurization (handles up). Precision regulators (7): these should be preset for ~40-50 psi. If the setting is unknown, decrease the pressure regulation (rotate control knobs 4-6 revolutions counter-clockwise) to set the initial pressure regulation low.

5. HCS venting subsystems (B2, C2), initial settings: Close outlet shutoff valves (16) (handles right). Set backpressure regulators (18) at the same or higher pressure as the precision regulators (7).

***HAfF* Hyperbaric Control System (HCS): Operation**

1. Gas supply: open the shutoff valves on the regulators.

2. Input gas control subsystem (A): Open the two shutoff valves (1) upstream of the gas mixer (handles left). Readjust regulated pressure from cylinders as needed to avoid safety valves (2) from relieving (~145 psi). Gas mixer (4): Readjust the pressure regulator on the mixer to the desired output pressure (maximum = 135 psi). Set flow rate at 100% and set gas mixture at 0 to 100% oxygen (the balance being nitrogen or air).

4. HCS pressurization subsystems (B1, C1): Open the inline shutoff valve (6) upstream of precision regulators (handle down). Set desired vessel pressure on the precision regulator (7). Open the shutoff valve (10) (handle up). Gas pressure will drop until the vessel is fully pressurized. Maximum internal vessel pressure should not exceed 103 psi; 7 bar/atm and is indicated on the backpressure regulator gauge, the pressure gauge located on the neck of each vessel, and on the iPad® display running the In-Situ SmarTroll® I-Situ® application. This initial pressurization recompresses fish to an effective depth of 70 m.

5. HCS venting subsystems (B2, C2): After vessels reach desired vessel pressure, open the shutoff valve (16) (handle up) and turn knob on the backpressure regulator (18) clock-wise to stop the venting of gas. The venting pressure (19) should be less than or equal to pressure indicated on the precision pressure regulator (8).

6. Adjusting vessel pressure: To <u>increase</u> vessel pressure, first increase the setting on the backpressure regulator (18) by turning the knob clockwise, and then increase the setting on the precision regulator (7) by turning the knob clockwise. To <u>decrease</u> vessel pressure, first decrease the setting on the precision pressure regulator (7) by turning the knob counter-clockwise, and then decrease the setting on the backpressure regulator (18) by turning the knob counter-clockwise. Pressure reductions are made in small steps (< 10 psi) to avoid overshooting target pressures listed on the decompression table.

7. Aeration under pressure: Close the shutoff valve (10) on pressurization line (handle right). Move the diverter valve (9) to the aeration setting (handle left). Venting will stop. Open valve (knob) on the flowmeter (12) by turning counter-clockwise one full revolution. Turn knob on the pressure regulator (7) clockwise until the line pressure (8) is 25 psi higher than vessel pressure indicated on the backpressure regulator (19). Open the shutoff valve (13) (handle up). Aeration starts when line pressure is 25 psi higher than vessel pressure (e.g., 128 psi at vessel pressure of 103 psi) and takes up to 2-3 minutes for gas to purge the air stone. Visually check for aeration inside the vessel on the Sea-View® video monitor. At no time should the pressure difference between the precision regulator (7) and internal vessel pressure reading exceed 50 psi to avoid over-pressurizing air stones. Once aeration is evident, adjust the flow rate on the flow meter (12) to 1-2 liters/minute (2-4 cu ft/hr). If no flow is indicated, increase pressure on the precision regulator (7) or decrease pressure on the backpressure regulator (18). To cease pressurized aeration and resume normal tank pressurization, close the shutoff valve (13) (handle right), and then decrease pressure setting on the precision pressure regulator (7) to match that of the backpressure regulator (18). Shift the diverter valve (9) to pressurization setting (handle up). Open the shutoff valve (10)

(handle up). Readjust pressure regulators to achieve desired vessel pressure.

8. Unpressurized aeration: Vessel hatches are to be open. Close shutoff valves to pressurization line (10) and aeration line (13) (handle right). Set diverter valve (9) to aeration (handle left). Open valve (knob) on the flowmeter (12) by turning counter-clockwise one full revolution. Adjust pressure on the regulator (7) to 25-35 psi. Open shutoff valve (13) on aeration line. Gas flow will begin after a few minutes when the air stone is purged and will be indicated on the flow meter (12). Adjust the flow rate on the flow meter (12) to 1-2 liters/minute (2-4 cu ft/hr). Maximum input pressure to air stones should not exceed 50 psi. To cease aeration, turn the knob on the flow meter (12) clockwise until stopped and close shutoff valve (13) (handle right).

***HAfF* Hyperbaric Control System (HCS): Recompression and Controlled Decompression Procedure**

1. The decompression sequence begins when the *HAfF* pressure vessel is sealed and pressurization starts. Pressurize the vessel to 7 atm (103 psi) with oxygen. Do not pressurize by aeration as this will cause supersaturation of gases. This initial pressurization recompresses the fish to an effective depth of 70 m and represents the first decompression stop. Proceed with the decompression sequence by following the provisional decompression table below. This decompression table was developed in December 2015 following two field trials in October and November 2015.

DECOMPRESSION TABLE

Stop	Psi	Atmospheres	Depth, m	Depth, ft	Long Time, hrs	Short Time, hrs
1	103	7	70	230	0.5	0.33
2	96	6.5	65	213	0.5	0.33
3	88	6	60	197	0.5	0.33
4	81	5.5	55	180	0.5	0.33
5	73	5	50	164	0.5	0.33
6	66	4.5	45	148	0.5	0.33
7	59	4	40	131	0.5	0.33
8	51	3.5	35	115	0.5	0.33
9	44	3	30	98	0.5	0.33
10	37	2.5	25	82	0.5	0.33
11	29	2	20	66	0.5	0.33
12	22	1.5	15	49	0.5	0.33
13	15	1	10	33	0.75	0.67
14	7	0.5	5	16	0.75	0.67
15	0	0	0	0	0	0
Total time, hrs					7.5	5.33

2. For collection depths > 70 m, add 0.25 hours for each additional 10 m depth to the first decompression stop. For collection depths > 80 meters, add 0.25 hours to the second decompression stop. Example: decompression time for collection depths of 120 meters is 9 hours. Extended first stops for collection depths > 70 m is intended to equilibrate nitrogen levels in the fish to the 70 m level before proceeding to shallower depths. As holding fish in the decompression vessel longer than needed adds stress, shorter stops may be adequate, e.g., 20 minute stops will reduce the duration of decompression at 70 m to 5 hours, 20 minutes.

3. Decompression stops can be made while transporting the *HAfF* by making short vehicle stops; the HCS is accessible from a standing position at the side of the truck bed.

4. The In-Situ SmarTroll® multi-probe instrument will record the entire decompression sequence on the iPad® I-Situ® application which can be emailed as a .csv file for analysis.

APPENDIX C.

Protocols for Collection, Handling, Recovery and Care of Lake Superior Deepwater Fishes

I. Collection and Handling of Lake Superior Deepwater Fishes

Target species, Kiyi, Deepwater Sculpin, and Siscowet, are collected with bottom trawls from waters exceeding 100 m. These fish are acutely stressed by the ascent from cold-dark to warmer and illuminated surface waters. Quick action and several preparations will make their survival much more likely upon their arrival on deck.

1. Prepare primary holding tanks to receive fish from bottom trawl as follows: Fill large coolers (~100 liter size) with chilled water (2-4°C) that contains 0.5% NaCl, 0.26 ml/*l* Stresscoat® and 25 mg/*l* MS-222 anesthetic (tricaine methanesulfonate). The higher dosage of anesthetic in the primary holding tanks is intended to reduce the effect of acute stress. Aerate with pure oxygen and chill water should to 4°C or colder with bagged ice (to avoid contamination with chlorine). If possible, holding tanks should be filled with hypolimnetic water (≤ 4°C). This may be achieved by pumping water from below the thermocline (5-15 m below the surface). Oxygen aeration of holding tanks should be maintained as continuously as possible. Secondary holding tanks are prepared the same way except for a lower dose of MS-222 (15 mg/*l*). These stress mitigation protocols follow Carmichael et al. (1984).

2. Remove fish from the trawl and transfer to primary holding tanks. As quickly as possible, teams of two persons deflate swim bladders of bloated fish. One person deflates the swim bladder with a 14- or 18-gauge syringe needle while the other sterilizes needles in 70% ethanol and swabs the needle wound with Betadine® iodine tincture. Transfer vented fish to secondary holding tanks. First fish to be treated should be deepwater ciscoes as their condition is more critical. Siscowets, Burbot and Lake Whitefish are less time-critical. Sculpins are not considered here as they have no swim bladders. Swim bladders in Ninespine Sticklebacks and other small species are not vented. To deflate swim bladders in ciscoes, insert the needle at the mid-body just above the lateral line while gently pressing the body cavity to expel the swim bladder fully. For Siscowets with bloated swim bladders, insert the needle at an upward angle above and just forward of the insertion of the pelvic fin while gently pressing the underbelly of the fish. Burbot with bloated swim bladders may be vented by inserting the needle through the side of the body wall. If the bloated swim bladder has caused the stomach to evert from the mouth, attempt venting through the body wall first. If that fails, venting through the everted stomach may be possible. If the stomach is not reverted into the body cavity, the everted stomach will block the flow of water over the gills and the fish will die.

3. Transfer live sculpins, sticklebacks and other small species (not vented) to the secondary holding tanks. Vented ciscoes, white fish, Burbot and Lake Trout are already in secondary holding tanks. Active fish in upright position in the secondary holding tanks are transferred to *HAfF* pressure vessels for recompression and decompression. Each vessel can hold approximately 4-6 large-sized fish (300-400 mm TL), 20 medium-sized fish (150-250 mm TL) and 50 small-sized fish (< 100 mm TL). *HAfF* pressure vessels are to be filled with chilled water (2-4°C) containing 0.5% NaCl, 0.26 ml/*l* Stresscoat® and 15mg/*l* MS-222 (anesthetic). Prior to transfer of fish, aerate the water in the pressure vessels with pure oxygen for 15-20 minutes; stop when DO levels reach 15-16 ppm. The low dosage of anesthetic in transport tanks and *HAfF* vessels is intended to sedate fish and reduce stress. *Anesthetic level may need to be adjusted to maintain sedation but not over-sedate.* Stress mitigation protocols follow Carmichael et al. (1984).

4. As soon as the quota fish is reached, aeration is halted, the *HAfF* pressure vessel is sealed, and the recompression-decompression sequence commences. Follow decompression procedure in Appendix B. Hyperbaric Apparatus for Fish (*HAfF*).

5. Fish are transported in the *HAfF* from the ship to a recovery facility by truck. Adjust work schedules to allow for 6-8 hours of decompression time before fish are removed from pressure vessels.

6. Upon arriving at the recovery facility, net fish out of the *HAfF* pressure vessels, place in large coolers, and then transfer to receiving tanks inside the facility. Water in transfer coolers and receiving tanks should be chilled (2-6°C) and treated with 0.5% NaCl, 0.26 ml/*l* Stresscoat®, and 15 mg/*l* MS-222 (same as *HAfF* pressure vessels). *Anesthetic level may need to be adjusted to maintain sedation but not over-sedate*. Circulate water in tanks with power filters at a minimum rate of 185 gal/hr (700 *l*/hr) and containing biological media (e.g., Cascade® Biofloss or Biosponge) but without carbon filtration (to avoid removal of anesthetic). Zeolite® filter bags may be used in the power filters to remove excess ammonia in tanks. Power filters should not aerate the water as this will counter the effect of using oxygen aeration to saturate the water. Follow procedures for *Recovery and Care of Lake Superior Deepwater Fishes*, below.

Additional suggestions:

Water treatments can be measured out into dosages for specific holding, transfer, and receiving tanks and *HAfF* vessels and packaged in Ziplock® bags for salt, vials or Eppendorf® tubes for MS-222, and small bottles for Stresscoat®. Calculations used to determine dosages should be checked by another person, and all weights for aliquots should also be checked by another person.

Stress on deepwater fish can be reduced by minimizing light exposure. Immediately after capture and transfer of fish to coolers, close lids or cover to block light.

II. Recovery and Care of Lake Superior Deepwater Fishes

First 24 hours

Upon delivery, fish are netted out of transport tanks or *HAfF* vessels and transferred via large coolers to receiving tanks in wet lab. Receiving tanks contain chilled water treated with 0.5% NaCl, 0.26 ml/*l* Stresscoat®, and 15 mg/*l* MS-222 (same as transport tanks and *HAfF* pressure vessels). Pure oxygen aeration is to be continued to maintain saturation. The intent of oxygen aeration is to lower the partial pressure of nitrogen in the water and create a gradient to draw nitrogen from fish tissues and promote recovery from decompression trauma. Oxygen aeration is also therapeutic by increasing oxygen availability to fish that have impaired respiratory and circulatory systems. Maintain water circulation without aeration and carbon filtration for first 24 hours. The cessation of carbon filtration is to avoid removing anesthetic from the water for the first 24 hours. Maintain water temperature between 3-6°C. Maintain dark conditions or red or infrared illumination. Take initial water quality readings (dissolved oxygen (DO), temperature, pH, salinity) and continue at 6 hour intervals over the first 24 hours. Check tanks and take water quality readings at 6-hour intervals, if possible. Remove dead fish, place in plastic bags with labels noting date, time, and tank identification and store in freezer.

24 hours to 7 days

Oxygen aeration and water circulation continues. At 12-hour intervals check tanks and take water quality readings. Measure ammonia, nitrate, and nitrite levels at 24-hour intervals. Remove dead fish, place in plastic bags with labels noting date, time, and tank identification and store in freezer. Retreat with MS-222 to maintain sedation as needed for coregonids. If anesthetic is required, try doses of 5 mg/*l* MS-222 to achieve adequate sedation. Water may be re-treated with Stresscoat® on day four @ 5 ml/10 gal (0.13 ml/*l*). After 2-3 days, carbon filtration may be resumed in tanks containing decompressed Lake Trout and Burbot showing nearly complete recovery from barotrauma as anesthetic is no longer needed. Siscowet and Burbot that have been decompressed, show minimal signs of barotrauma (*barotrauma indicator scale*, ≤ 1, below), and are active, may be offered live food after day 3.

8-14 days

Normal aeration can resume. Check condition of fish at 6-hr intervals over next 24 hours. If fish show signs of stress or lack of equilibrium, resume oxygen aeration for 48 hours. Treatment with anesthetic may be required to alleviate stress in coregonids. After resuming normal aeration check again for signs of stress. Repeat oxygen aeration if necessary for another 48 hours. If fish appear healthy and active on day 8, food may be offered; if none is consumed, attempt again at 48-hr intervals. Water quality is monitored at 24 hour intervals. If oxygen aeration is be continued during this period, reduce level to just maintain saturation.

Notes

Stocking levels of fish in recovery tanks
Circular or oval tanks are recommended holding fish. We recommend smaller fish (< 200 mm TL) be kept in tanks of at least 100 gallons (379 liters) capacity and larger fish (> 300 mm TL) be kept in circular tanks of at least 200 gallons (757 liters) capacity. Sculpins are easier to maintain in small, shallow tanks; stocking rates should not exceed 7-10 fish per 10 gallons (38 liters) with filtration rates of at least 150 gallons (568 liters) per hour. For larger fish (>300 mm TL) we recommend using tanks of at least 300 gallon capacity with stocking rates of 0.3-0.7 fish per 10 gallons with filtration rates of 350 gallons (1325 liters) per hour.

Aerators and water circulation
Ceramic aerators such as Pentair P4® Micro Bubble Oxygen Diffuser are recommended as these emit a wall of very fine bubbles which enhances gas exchange and uses less oxygen to maintain saturation. Check and record dissolved oxygen (DO) levels daily in the tanks to ensure that sufficient aeration is occurring to maintain saturation. During periods when tanks are aerated with oxygen, other sources of ambient air aeration should be stopped. Water circulation and filtration should be continued in tanks but without any ambient air aeration as this counters the effect of oxygen aeration.

Alleviating stress in captive fish
Upon arrival at the recovery facility, fish are in a physiologically stressed state because of handling, barotrauma, and exposure to increased temperature. Low temperature, oxygen aeration, and the addition of salt, Stresscoat®, and anesthetic (MS-222) are measures intended to reduce stress. Failure to apply these measures will result in increased stress and mortality of fish. Coregonids appear especially sensitive to stress and require prolonged sedation with anesthetic. Hyperactivity indicated by incessant swimming and rapid gilling are signs of stress that can be monitored and reduced by application of appropriate dosages of anesthetic (5-15 mg/*l* MS-222).

Handling and intervention of fish
Fish should be anesthetized with MS-222 (tricaine methanesulfonate) prior to handling or intervention, e.g., weighing and measuring, equilibrating air bladder, surgical operations, etc. Adequate anesthesia for handling should be achieved within 5 minutes. At temperatures of 4-7°C, anesthesia can be achieved within 5 minutes by immersion in an anesthetizing holding tank treated with 50 mg/*l* MS-222. After achieving anesthesia, fish are transferred to an anesthetic-free holding tank as they await intervention. If fish recover before intervention is completed, they can be returned to the anesthetizing tank until adequate anesthesia is re-achieved. Fish should recover rapidly when returned to untreated water (20 minutes or less). Water in all holding tanks should be the same temperature. If fish were held in oxygen-saturated water, water in the anesthetizing tank should also be oxygen-saturated. Likewise, if fish were held in water containing 0.5% NaCl, water in the anesthetizing tank should also contain 0.5% NaCl. Rubber-coated mesh dip-nets are recommended for handling fish to reduce damage to fish skin and scales. For dosages of 50 mg/*l* MS-222, water temperatures should be < 8°C. We note that lower dosages of MS-222 may be effective at higher temperatures, e.g., 25 mg/*l* MS-222 may be an effective dosage for temperatures of 10-12°C. The key is to monitor the dosage, how fast anesthesia is achieved, and limit exposure to anesthetic to no more than 5 minutes on a continuous basis. At a dosage of 50 mg/*l* MS-222 at 6-7°C, Slimy, Spoonhead, and Deepwater Sculpins took 4-5 minutes to achieve sufficient anesthesia for handling while Burbot, Ninespine Stickleback, Ruffe, Lake Whitefish, and Lake Trout took 3-4 minutes. Neiffer and Stamper (2009) provide additional guidance on anesthesia and sedation of fish.

Cohabitation of different fishes
Large Siscowet will eat any fish that fits into their mouths. For the larger Siscowets, this would be any fish < 250 mm TL (this includes small Siscowets). Both the Siscowets and Burbot are predators and sculpins are highly vulnerable in smooth-bottom holding tanks. Large Siscowets and Burbot should be kept in tanks separate from smaller fish. Ciscoes and sculpins can peacefully co-habit the same tanks. Supplying short sections of PVC pipe and couplings of appropriate diameters in tanks provides hiding places for sculpins and Burbot.

Wet lab environment for fish tanks
Wet lab should be quiet, limited access to minimize disturbance, dark, and water temperatures maintained at 3-

7°C. Place screens over tanks to prevent escape. Maintain a diel photo period of 14 hr light and 10 hr dark to simulate a circadian rhythm; reduce daytime light levels to that found mid-day at a depth of 45 m in Lake Superior (0.0005 lux). To avoid light disturbance on fish, caretakers worked in infrared or red light illumination.

Feeding
Offer food for the first time after 7 days, and again in 48 hours. Once fish have acclimated, food can be offered every 24 hrs. Recommended foods include live or frozen *Mysis relicta*, brine shrimp, and blood worms. Note and remove uneaten food. Develop a feeding schedule; captive fish readily cue into this schedule, which affects their behavior, and thus can affect behavioral experiments. Food may be offered sooner than 7 days for fish that were decompressed, are active, and show nearly complete resolution of signs of barotrauma (*barotrauma indicator score*, ≤ 1, below), e.g., decompressed Siscowet Lake Trout.

Records
A log for each tank should be maintained to record dissolved oxygen (DO), temperature, pH, salinity, nitrogen content (ammonia, nitrate), filter media changes, fish health, and mortalities. A feeding record should include time of feeding, food offered, food preference, and consumption.

Disposition of dead fish
Fish that die or are sacrificed can be worked up or frozen whole for later transfer to the LSBS for workup. Record the following information for each fish: species, total length, weight, tank identifier, dates and time of death. Additional information (sex, maturity, etc.) is useful. If possible, collect aging structures (scales, otoliths) and store in scale envelopes or vials provided by LSBS.

Preparation of laboratory tanks
Food (prey) fish (e.g. golden shiners, white suckers) may be stocked in laboratory tanks for 2-4 weeks prior to stocking with wild deepwater fish to biologically prime the power filters. Stress-zyme® may be added to the tanks to speed up the development of biological filtration.

Barotrauma indicator score (BIS)
The barotrauma indicator score (BIS) provides a qualitative estimate of barotrauma in fish. The score reflects an areal estimate (percent) of the extent of barotrauma (hemorrhaging, hematomas) in fins, skin, and eyes. Separate scores are recorded for fins, skin, and eyes at 12-hr intervals beginning with delivery to the recovery facility. BIS scores are 0 = < 1%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = > 75%.

Dosage of Stresscoat®
As recommended by the manufacturer, the dosage of Stresscoat® is doubled when fins and skin are damaged; this is the case for all fish collected with trawls. The standard dosage of Stresscoat® is 0.13 ml/*l* (5 ml/10 gallons) and double dosage is 0.26 ml/*l* (10 ml/10 gallons).

Salt used for treatments
Morton® All Natural Canning and Pickling Salt is a good choice for salt treatment of holding vessels. This is pulverized natural salt and dissolves rapidly. Cost is ~$1.50 for 4 pounds. Kosher salt can be substituted, but costs twice as much. For treating the wet lab tanks, salt is added directly to the sump. Suitable kinds of salt include Diamond Crystal® Solar Naturals® salt crystals for water softeners. This salt is the consistency of coarse rock salt and is additive-free. Morton® sells a similar product (Morton® White Crystal® Solar Salt). Cost runs $4-5 for 40 pounds.