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Brandon M. CasperMichele B. HalvorsenThomas J. CarlsonArthur N. Popper

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Onset of barotrauma injuries related to number of pile driving strike exposures in hybrid striped bass

Brandon M. Casper[a)]
Department of Biology, University of Maryland, College Park, Maryland 20742, USA

Michele B. Halvorsen[b)]
CSA Ocean Sciences, Stuart, Florida 34997, USA

Thomas J. Carlson
ProBioSound LLC, Holmes Beach, Florida 34217, USA

Arthur N. Popper
Department of Biology, University of Maryland, College Park, Maryland 20742, USA

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Previous studies exploring injury response to pile driving in fishes presented exposure paradigms (>900 strikes) that emulated circumstances where fish would not leave an area being ensonified. Those studies did not, however, address the question of how many strikes are needed before injuries appear. Thus, the number of strikes paired with a constant single strike sound exposure level (SEL_{ss}) that can cause injuries is not yet clear. In order to examine this question, hybrid striped bass (white bass *Morone chrysops* × striped bass *Morone saxatilis*) were exposed to 8–384 strikes in three different SEL_{ss} treatments that generated different cumulative sound exposure level values. The treatment with the highest SEL_{ss} values caused swim bladder injuries in fish exposed to as few as eight pile strikes. These results have important implications for pile driving operations where SEL_{ss} values meet or exceed the exposure levels used in this study.

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I. INTRODUCTION

The use of natural resources found in aquatic environments (oil, natural gas, offshore wind platforms) is increasing worldwide. A growing byproduct of acquiring and/or utilizing these resources is an increase in human-generated (anthropogenic) sounds. These sounds not only raise the overall ambient noise in aquatic bodies, but at times can reach levels with the potential to impact aquatic animals, including fishes (Southall *et al.*, 2007; Popper and Hawkins, 2012; Popper *et al.*, 2014; Popper and Hawkins, 2016). One such noise producing activity is impact pile driving, used during the construction phase of oil rigs, offshore wind farms, and other structures.

The U.S. National Oceanographic Atmospheric Administration (NOAA) Fisheries, in response to cases of fish mortalities during driving of steel shell piles, established interim guidelines for safe exposure of fishes to impulsive sound generated by impact pile driving on the U.S. West Coast (Woodbury and Stadler, 2008; Stadler and Woodbury, 2009). These criteria include a cumulative sound exposure level (SEL_{cum}) of 187 dB re 1 μPa^2 s for fishes more than 2 g and 183 dB re 1 μPa^2 s for fishes less than 2 g, and a peak sound pressure level (SPL_{peak}) of 206 dB re 1 μPa for all fish sizes (Stadler and Woodbury, 2009). Because the interim criteria were based on limited data, some of which were of questionable value and not peer reviewed (Popper and Hastings, 2009), the expectation was that they would be adjusted as new, and better, data became available.

Over the past few years, in order to better quantify the effects of pile driving on fishes and develop data that could be used to update the interim criteria, a series of controlled studies were conducted to evaluate the onset of barotrauma injuries from pile driving sound exposures (for a detailed list of potential injuries, see Halvorsen *et al.*, 2011). Several species were tested during these studies including Chinook salmon *Oncorhynchus tshawytscha* (Casper *et al.*, 2012; Halvorsen *et al.*, 2012b), hybrid striped bass (white bass *Morone chrysops* × striped bass *Morone saxatilis*) (Casper *et al.*, 2013a), Mozambique tilapia *Oreochromis mossambicus*, Nile tilapia *Oreochromis niloticus*, lake sturgeon *Acipenser fulvescens*, hogchoker *Trinectes maculatus* (Halvorsen *et al.*, 2012a), and the common sole *Solea solea* (Bolle *et al.*, 2012; Bolle *et al.*, 2016).

In general, these experiments showed that injury responses differed between many of these species at higher SEL_{cum}, though as sound levels approached onset of injury thresholds, these onset levels were not that dissimilar between species. In the hybrid striped bass, it was also determined that fish around 17 g were more susceptible to injury than fish less than 2 g (Casper *et al.*, 2013a), contrary to the NOAA interim guideline predictions (Stadler and Woodbury, 2009) which were based on studies with explosives rather than impulse sounds generated by pile strikes

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[a)]Electronic mail: bcasper@umd.edu

[b)]Also at: Department of Biology, University of Maryland, College Park, MD 20742, USA.

(Yelverton *et al.*, 1975). Additionally, results for the hybrid striped bass and Mozambique tilapia showed that damage to sensory hair cells of the inner ears from exposure to sound pressure only occurred at the highest $\mathrm{SEL_{cum}}$ generated in those experiments, suggesting that, with exposure to sound pressure, damage to tissue and organs is likely to occur well before damage to the auditory system (Casper *et al.*, 2013b).

Each of these studies used a "worst-case scenario" where fish were exposed to 960 or 1920 pile strikes. Use of a large number of strikes was based on the assumption in the interim criteria that fishes remain in an area ensonified by pile driving, something that may not happen in the wild (Krebs *et al.*, 2016). However, even if fishes leave an area, they would likely receive some level of exposure before reaching a safe distance. Accordingly, data are needed to better understand potential effects on fishes when exposed to fewer pile strikes, and to approximate instances when fishes move from an ensonified area. In these cases, the focus is on single strike sound exposure level ($\mathrm{SEL_{ss}}$) and a lesser number of strikes than used in previous investigations.

To examine this question, this study was designed to determine which combination of $\mathrm{SEL_{ss}}$ and the minimum number of pile strikes that can elicit an injurious response in a fish. Hybrid striped bass were chosen for this study because the range of injuries in response to pile driving has been documented in previous experiments (Casper *et al.*, 2013a; Casper *et al.*, 2013b). To find an onset of injury threshold in terms of numbers of pile strikes, the High Intensity Controlled Impedance Fluid Filled Wave Tube (HICI-FT) was used following the same methodology as previous studies (Casper *et al.*, 2012; Halvorsen *et al.*, 2012a; Halvorsen *et al.*, 2012b; Casper *et al.*, 2013a; Casper *et al.*, 2013b). The fish were exposed to one of three different $\mathrm{SEL_{ss}}$ treatments with a decreasing number of pile strikes and investigation of the same injuries documented in earlier studies with the HICI-FT (Halvorsen *et al.*, 2011; Casper *et al.*, 2013a; Casper *et al.*, 2013b). For this study, onset of injury was defined as the probability of occurrence of at least one injury (i.e., 0.10). The severity and type of observed injury were not considered for this probability analysis, and thus the threshold for injury was independent of injury type.

II. METHODS

A. Fish species information

Hybrid striped bass [93.8 ± 11.3 mm standard length (SL) and weight 14.1 ± 5.0 g] were obtained in June and July 2011 from Keo Fish Farms, Inc. (Keo, Arkansas, USA). All fish used in the study were acclimated in the laboratory for a minimum of two weeks following transportation from the fish farm before their use in experiments. Fish had their caudal fins clipped in different patterns to enable identification of individuals and were maintained on a 14:10 light/dark cycle in 235 gal round tanks. Fish scheduled to be used in experiments were not fed prior to a treatment so the digestive system would be void of food during sound exposure. Experiments were conducted under the supervision and approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland (protocol #R-09-23).

B. Pile driving exposure equipment and signal presentation

Pile driving exposure was conducted using the HICI-FT, a water-filled cylindrical holding chamber that was 45 cm long with a 25 cm internal diameter and 3.81 cm thick stainless steel walls (volume = 0.02 $\mathrm{m^3}$). Fixed at each end of the tube was a shaker (Vibration Test Systems, VG-150 Vibration Generator, Model VTS 150, Aurora, OH) with a rigid faceplate mounted on the piston driven by a moving coil. The shakers were controlled independently in amplitude and phase to generate plane-wave pressure and velocity fields within the tube. The HICI-FT produced highly accurate simulation of impulsive signals generated by pile driving; and reproduced impulsive signals with propagating plane wave characteristics up to $\mathrm{SPL_{peak}}$ of 215 dB re 1 μPa. The overall system permitted control of all exposure variables including the number of impulsive signals and the details of individual impulsive signals such as their duration, amplitude characteristics, and their energy level. Control of these factors enabled control of the total energy in an exposure event. Sound presentation was controlled using LabVIEW (National Instruments Corporation, Austin, Texas). For a detailed description and photographs of the equipment and its development see Halvorsen *et al.*, 2011; Halvorsen *et al.*, 2012b. The pile driving signals used in this study were field recordings taken 10 m from a 76.2 cm steel shell pile (outer diameter) driven using a diesel impact hammer at the Eagle Harbor Maintenance Facility (MacGillivray and Racca, 2005). Eight different recordings of pile driving strikes were normalized to the same $\mathrm{SEL_{ss}}$. Examples of these signals can be found in previous studies (Halvorsen *et al.*, 2011; Halvorsen *et al.*, 2012b).

The eight strikes were randomized each day using MATLAB (The MathWorks, Inc., Natick, MA) and presented to the fish either once (eight total strikes) or multiples of this including 3 (24 strikes), 6 (48 strikes), 12 (96 strikes), 24 (192 strikes), or 48 (384 strikes) presentations. Impulsive sound levels in the tube were continuously monitored and recorded using a hydrophone mounted inside the HICI-FT (Brüel & Kjær Sound & Vibration Measurement A/S, Naerum, Denmark, model 8103). Particle motion measurements could not occur during exposures because of the required central placement of the accelerometer in the tube to accurately measure the particle motion field. It should be acknowledged that particle motion could potentially play a role in fishes' injury response to pile driving exposure, though currently there is no experimental evidence to support this hypothesis.

C. Fish exposure

Four fish, acclimated at atmospheric pressure, were placed into the HICI-FT for each exposure or control treatment. The fish were allowed to recover from handling in the acrylic chamber mounted around the opening of the HICI-FT exposure chamber for 20 min. The fish were then gently directed to enter the exposure chamber, following which the upper shaker/lid was sealed over the chamber opening. The acrylic chamber was drained and the HICI-FT rotated from the vertical to the horizontal position. Following the completion of each treatment, the HICI-FT was again rotated to the vertical position and the fish were removed and immediately necropsied for barotrauma assessment following standard procedures (Halvorsen *et al.*, 2011; Halvorsen *et al.*, 2012b). Buoyancy was documented in all fishes as done in previous studies (Halvorsen *et al.*, 2011; Halvorsen *et al.*, 2012b), though the bass, having a closed swim bladder, always displayed neutral buoyancy indicating that there was no change in the amount of gas in their swim bladders during the handling required to move them from holding tanks to the experimental chamber.

These experiments used 320 experimental and 64 control fish. Control fish were subject to the identical process as exposed fish but without the pile driving sound. Fish were presented with exposures of from 8 to 384 pile strikes (discussed above) while keeping the $\mathrm{SEL_{ss}}$ constant at one of three sound levels: 183 dB re 1 $\mu\mathrm{Pa}^2$ s (treatment 1), 180 dB re 1 $\mu\mathrm{Pa}^2$ s (treatment 2), or 177 dB re 1 $\mu\mathrm{Pa}^2$ s (treatment 3). Each change in the number of strikes generated a different $\mathrm{SEL_{cum}}$ value. From here forward, the study will refer to treatments 1, 2, or 3 to simplify the reference to the exposure paradigms (Table I).

Following each treatment, fish were euthanized in a buffered MS-222 solution, necropsied, and examined for external and internal signs of barotrauma (e.g., damage to eyes, fins, gills) utilizing methodology from our previous studies (Halvorsen *et al.*, 2011; Halvorsen *et al.*, 2012b; Casper *et al.*, 2013a). Each potential injury was noted as present or not.

D. Statistical analysis

A generalized linear model (GLM), specifically a Poisson regression, was used to evaluate the effect of $\mathrm{SEL_{ss}}$ and number of pile strikes on the number of injuries observed (MATLAB). The predictor variables were $\mathrm{SEL_{ss}}$ and number of strikes, and the response variable was number of injuries observed. This GLM resulted in the following equation:

$$\log(\#\text{of injuries}) = \beta0 + \beta1 * (\mathrm{SEL_{ss}}) + \beta2 * (\#\text{of strikes}),$$

where $\beta0 = -38$; $\beta1 = 0.2$; $\beta2 = 0.003$.

TABLE I. List of treatment metrics in terms of single strike sound exposure level ($\mathrm{SEL_{ss}}$), number of pile strikes, and cumulative sound exposure level ($\mathrm{SEL_{cum}}$). HSB = hybrid striped bass; E = exposed; and C = control.

Treatment	$\mathrm{SEL_{ss}}$ (dB re 1 $\mu\mathrm{Pa}^2$ s)	Number of pile strikes	$\mathrm{SEL_{cum}}$ (dB re 1 $\mu\mathrm{Pa}^2$ s)	No. HSB
1	183	384	209	20 E/ 4 C
		192	206	20 E/ 4 C
		96	203	20 E/ 4 C
		48	200	20 E/ 4 C
		24	197	20 E/ 4 C
		8	192	20 E/ 4 C
2	180	384	206	20 E/ 4 C
		192	203	20 E/ 4 C
		96	200	20 E/ 4 C
		48	197	20 E/ 4 C
		24	194	20 E/ 4 C
3	177	384	203	20 E/ 4 C
		192	200	20 E/ 4 C
		96	197	20 E/ 4 C
		48	194	20 E/ 4 C
		24	191	20 E/ 4 C

A Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks (MATLAB) was used to evaluate the difference between the number of injuries observed within different numbers of pile strikes that yielded equivalent $\mathrm{SEL_{cum}}$. The response variable was the number of injuries observed. The grouping variable was number of pile strikes. This test was repeated for each of the five $\mathrm{SEL_{cum}}$ conditions.

An α of 0.05 was used for all statistical tests. The results of these tests are presented in Tables II and III.

The probability of at least one injury was estimated for each treatment group by the ratio of injured fish to the total number of fish in a treatment group (Table IV). A two parameter logistic function $P(Y \geq 1) = 1/(1 + e^{(-a(X-b))})$ was fit to the data (JMP 12, SAS Institute, Inc.), where probability of at least one injury was the dependent variable and $\mathrm{SEL_{cum}}$ was the independent variable (Fig. 3). Summary statistics for the fit of the model to the data are given in Table IV.

III. RESULTS

A. General results

No exposed or control animals died during a treatment in the HICI-FT. There were four different types of injuries

TABLE II. Kruskal-Wallis One-Way ANOVA on $\mathrm{SEL_{cum}}$ ranks for the number of injuries as a function of number of pile strikes.

Source of Variation	DF	SS	MS	Chi-sq	*P*
206 $\mathrm{SEL_{cum}}$					
# of Pile Strikes	1	90	90	3.162	0.075
Residual	38	1020	26.842		
Total	39	1110			
203 $\mathrm{SEL_{cum}}$					
# of Pile Strikes	2	3630	1815	26.240	<0.001
Residual	57	4532	79.509		
Total	59	8162			
200 $\mathrm{SEL_{cum}}$					
# of Pile Strikes	2	1908.175	954.088	8.655	0.013
Residual	57	11099.33	194.725		
Total	59	13007.5			
197 $\mathrm{SEL_{cum}}$					
# of Pile Strikes	2	1691.623	954.088	26.240	0.003
Residual	57	6735.377	120.275		
Total	59	8427			
194 $\mathrm{SEL_{cum}}$					
# of Pile Strikes	1	42.025	42.025	1.136	0.287
Residual	38	1400.975	36.868		
Total	39	1443			

TABLE III. Treatment, SEL_{ss}, number of strikes, SEL_{cum}, and probability of at least one injury.

Treatment	SEL_{ss} (dB re 1 $\mu\mathrm{Pa}^2$ s)	Number of Pile Strikes	SEL_{cum} (dB re 1 $\mu\mathrm{Pa}^2$ s)	Probability $\geq$1 injury
1	183	384	209	1.00
		192	206	1.00
		96	203	1.00
		48	200	0.90
		24	197	0.45
		8	192	0.15
2	180	384	206	1.00
		192	203	1.00
		96	200	0.95
		48	197	0.10
		24	194	0.05
3	177	384	203	0.65
		192	200	0.45
		96	197	0.05
		48	194	0.15
		24	191	0.00

observed across these studies in the exposed fish and no injuries in any control fish. The injuries were ruptured swim bladder, kidney hemorrhage, swim bladder hematoma, and herniated swim bladder. The first two are defined as *mortal* injuries that would likely result in death, and the other two are defined as *moderate* injuries from which recovery is likely to take place (Halvorsen *et al.*, 2011; Halvorsen *et al.*, 2012b), respectively. These injuries have been observed in our previous studies examining pile driving exposure in hybrid striped bass (for photographs of injuries see Casper *et al.*, 2013a). There were no external injuries observed in any of the fish.

B. SEL_{ss} injury analysis

The Poisson GLM analysis found that both treatment group ($p = 4\,e^{-17}$) and number of strikes ($p = 3\,e^{-15}$) had a significant effect on number of injuries observed. In each of the treatment groups, there was a general trend of decreasing frequency of occurrence of injuries as the number of strikes decreased [Figs. 1(A)–1(C)]. In addition, there was a decrease in the number and severity of injuries with a decrease in SEL_{ss} value as seen in treatment 1 as compared with 2 and with treatment 2 as compared with 3. Kidney hemorrhaging was observed in treatment 1 only. Ruptured swim bladders occurred in at least 70% of fish in treatment group 1 with 384, 192, and 96 strikes as well as for treatment 2 at 384 strikes, followed by a shift in swim bladder injuries towards herniated swim bladders becoming a more common injury as the number of pile strikes decreased. In addition, swim bladders hematomas were almost always present in association with the swim bladder ruptures and hernias, and the hematomas were present during the lower numbers of pile strike exposures when other injuries were no longer observed.

TABLE IV. Summary of the fit of a two-parameter logistic function and statistics for model parameter estimates.

R-Square	0.8539	
Parameter	Estimate	Standard error
Growth rate (a)	0.709	0.207
Inflection point (b)	198.430	0.493

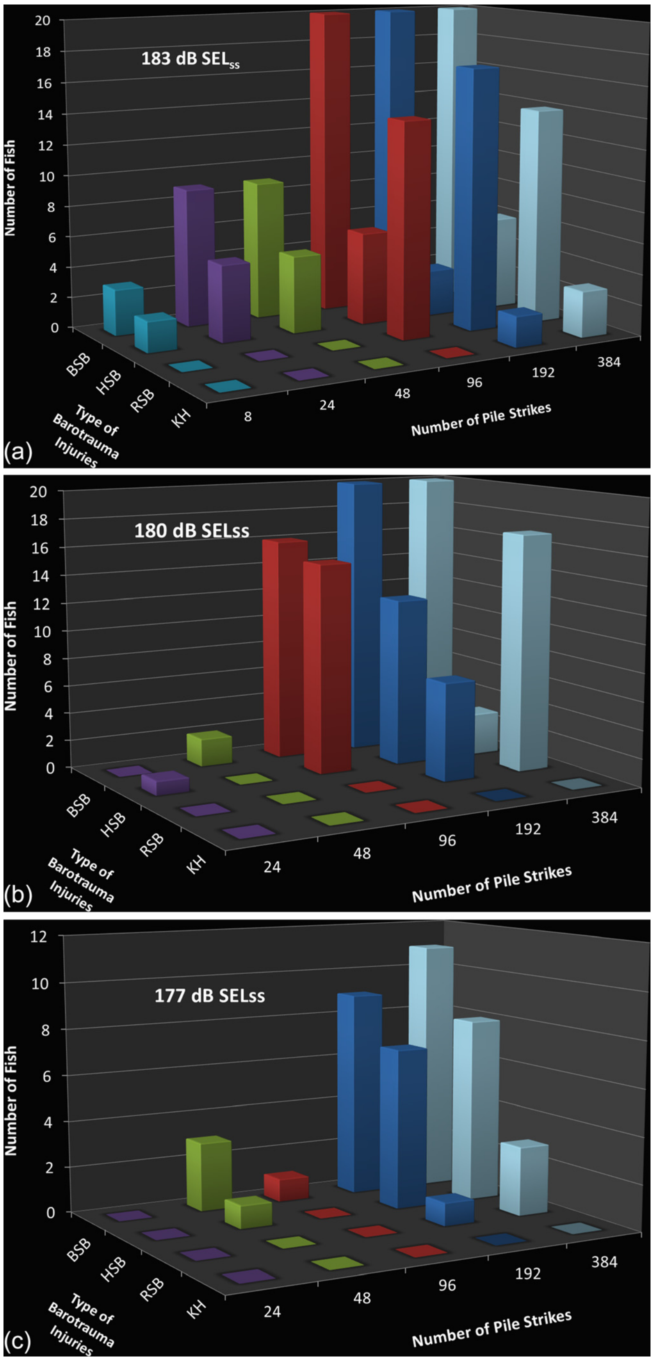

FIG. 1. (Color online) Frequency of occurrence of each injury type as a function of the number of pile strike exposures for (A) treatment 1 (183 dB SEL_{ss}); (B) treatment 2 (180 dB SEL_{ss}); and (C) treatment 3 (177 dB SEL_{ss}). BSB = swim bladder hematoma; HSB = herniated swim bladder; RSB = ruptured swim bladder; and KH = kidney hemorrhage.

Within treatment 1, injuries were found in 100% of the fish exposed to 96 pile strikes and higher, with ruptured swim bladders in approximately 75% of the animals [Fig. 1(A)]. Swim bladder hernias and swim bladder hematomas were found in 35% of the fish at 48 and 24 pile strikes, and a few of these injuries appeared with as few as 8 pile strikes.

Within treatment 2, all four types of injuries were found in all of the fish exposed to 384 and 192 pile strikes, and swim bladder hernias and swim bladder hematomas occurred at 80% and 75%, respectively, during 96 pile strikes [Fig. 1(B)]. However, only 10% and 5% of these two injury types were found in fish from the 48 and 24 strike exposures, respectively.

Within treatment 3, 4% of fish had ruptured swim bladder and kidney hemorrhage (*mortal*) injuries, while swim bladder hernias and swim bladder hematoma (*moderate*) injuries were observed in 55% and 45% of fish at the 384 and 192 pile strike exposures, respectively [Fig. 1(C)]. As the number of pile strikes decreased, fewer injuries were observed, and no injuries were found at 24 pile strikes.

C. $\mathrm{SEL}_{\mathrm{cum}}$ injury analysis

Injuries were also evaluated by looking at equivalent $\mathrm{SEL}_{\mathrm{cum}}$ levels reached with different numbers of strikes at different $\mathrm{SEL}_{\mathrm{ss}}$ values (e.g., 203 dB re 1 $\mu\mathrm{Pa}^2$ s $\mathrm{SEL}_{\mathrm{cum}}$ was obtained with 96, 192, and 384 pile strikes for treatments 1, 2, and 3, respectively). Shorthand for $\mathrm{SEL}_{\mathrm{cum}}$ and $\mathrm{SEL}_{\mathrm{ss}}$ levels will be reported as XX dB $\mathrm{SEL}_{\mathrm{cum}}$ or XX dB $\mathrm{SEL}_{\mathrm{ss}}$ and equates to XX dB re 1 $\mu\mathrm{Pa}^2$ s. The highest cumulative level, 209 dB $\mathrm{SEL}_{\mathrm{cum}}$, could only be obtained with 384 pile strikes in treatment 1 with an $\mathrm{SEL}_{\mathrm{ss}}$ of 183 dB [Fig. 2(A)]. The next level down, 206 dB $\mathrm{SEL}_{\mathrm{cum}}$, was obtained with 192 and 384 pile strikes in treatments 1 and 2, respectively [Fig. 2(B)]. The numbers and types of injuries were the same between these two treatment levels with the exception of one kidney hemorrhage that appeared in treatment 1 (Table II). The remaining $\mathrm{SEL}_{\mathrm{cum}}$ values of 203, 200, 197, and 194 dB [Figs. 2(C)–2(F)], were obtained in all three of the treatments, or two treatments in the case of 194 dB $\mathrm{SEL}_{\mathrm{cum}}$, and are discussed in detail in the following paragraph. The exceptions were 192 and 191 dB $\mathrm{SEL}_{\mathrm{cum}}$ which could only be obtained by 8 pile strikes with an $\mathrm{SEL}_{\mathrm{ss}}$ of 183 dB and by 24 pile strikes with an $\mathrm{SEL}_{\mathrm{ss}}$ of 177 dB, respectively [Fig. 2(G)]. The 191 dB $\mathrm{SEL}_{\mathrm{cum}}$ was the only exposure regiment which yielded no injuries eliminating any need for further consideration [Fig. 2(G)].

At 203 dB $\mathrm{SEL}_{\mathrm{cum}}$, the differences in injuries became apparent [Fig. 2(C)]. Ruptured swim bladders occurred more frequently during treatment 1 and the overall number of injuries was higher for treatments 1 and 2 compared with treatment 3 (Table II). A similar pattern was observed at 200 dB $\mathrm{SEL}_{\mathrm{cum}}$ [Fig. 2(D)] with swim bladder hernias and swim bladder hematomas occurring more frequently for treatments 1 and 2 compared with treatment 3 (Table II). At 197 dB $\mathrm{SEL}_{\mathrm{cum}}$, very few injuries were observed [Fig. 2(E)], however, and of these, most were observed during treatment 1 (Table II). There were few injuries observed at 194 dB $\mathrm{SEL}_{\mathrm{cum}}$ [Fig. 2(F)] resulting in no difference between the various $\mathrm{SEL}_{\mathrm{ss}}$ treatments (Table II).

IV. DISCUSSION

Previous studies (Halvorsen *et al.*, 2012a; Halvorsen *et al.*, 2012b; Casper *et al.*, 2013a; Casper *et al.*, 2013b) have exposed fishes to 960 or 1920 pile strikes with the assumption that this represents a worst case scenario in which the fishes would remain in an area being ensonified by pile strikes. The behavioral reactions of fishes to pile driving sounds in the wild are not known beyond initial alarm responses. Regardless of the behavior of fishes in the wild, the earlier studies are valuable since often a pile must be struck 1000 times or more to complete installation.

This current study approached the onset of injury from the perspective of *when* do injuries start to appear in the fish relative to the number of strikes; does it occur after 10 strikes, 400 strikes, or somewhere in between? The answer to this question could help regulatory agencies determine mitigation measures to the number of strikes correlated with $\mathrm{SEL}_{\mathrm{ss}}$ and $\mathrm{SEL}_{\mathrm{cum}}$ within a certain time-frame (Hawkins and Popper, 2017). Therefore, this current study is important to investigate how many pile strikes it might take for injuries to appear.

In this study, fish were exposed to three different treatments consisting of combinations of one of three $\mathrm{SEL}_{\mathrm{ss}}$ treatments and simulated pile strikes ranging from eight up to 392 strikes which generated a range of different $\mathrm{SEL}_{\mathrm{cum}}$ values. The number and characteristics of injuries observed in hybrid striped bass were analyzed to describe the patterns in injuries, by severity of exposure, in terms of $\mathrm{SEL}_{\mathrm{ss}}$ and number of strikes, which were altered.

In treatment 1, $\mathrm{SEL}_{\mathrm{ss}}$ was 183 dB re 1 $\mu\mathrm{Pa}^2$ s and *mortal* injuries were observed in fishes exposed to as few as 96 pile strikes. *Moderate* injuries were found after exposure to as few as eight pile strikes. Fish exposed to 48 pile strikes in both treatments 2 and 3 ($\mathrm{SEL}_{\mathrm{ss}}$ levels of 180 dB re 1 $\mu\mathrm{Pa}^2$ s and 177 dB re 1 $\mu\mathrm{Pa}^2$ s) had observed injuries and one fish had injuries from 24 strikes at treatment 2.

The results of this study make it clear that physical injuries sustained by fish upon exposure to impulsive sounds increase in number and severity as exposure becomes more severe. Two factors, the energy in each impulse ($\mathrm{SEL}_{\mathrm{ss}}$) and the number of impulses, determine exposure severity ($\mathrm{SEL}_{\mathrm{cum}}$). If the probability of at least one injury is considered, a general pattern of increasing injury probability with increasing exposure severity is evident in the data (Fig. 3). The logistic model that fits the data is asymptotic as injury probability approaches zero (no injury) and one (certainty of injury).

The condition of no injury is observed when $\mathrm{SEL}_{\mathrm{cum}}$ is near 192 dB re 1 $\mu\mathrm{Pa}^2$ s and there is certainty of injury (probability of one) when the $\mathrm{SEL}_{\mathrm{cum}}$ approaches 203 dB re 1 $\mu\mathrm{Pa}^2$ s. Examination of the study results finds that there are six combinations of $\mathrm{SEL}_{\mathrm{ss}}$ and number of strikes where the probability of at least one injury is ≥ 0.95. Similarly, there are three combinations of $\mathrm{SEL}_{\mathrm{ss}}$ and number of strikes where the probability of at least one injury is ≤ 0.05.

In addition, the high slope middle region of Fig. 3 shows that any particular $\mathrm{SEL}_{\mathrm{cum}}$ value can result in a wide range of probabilities for at least one injury. In all cases, the lowest

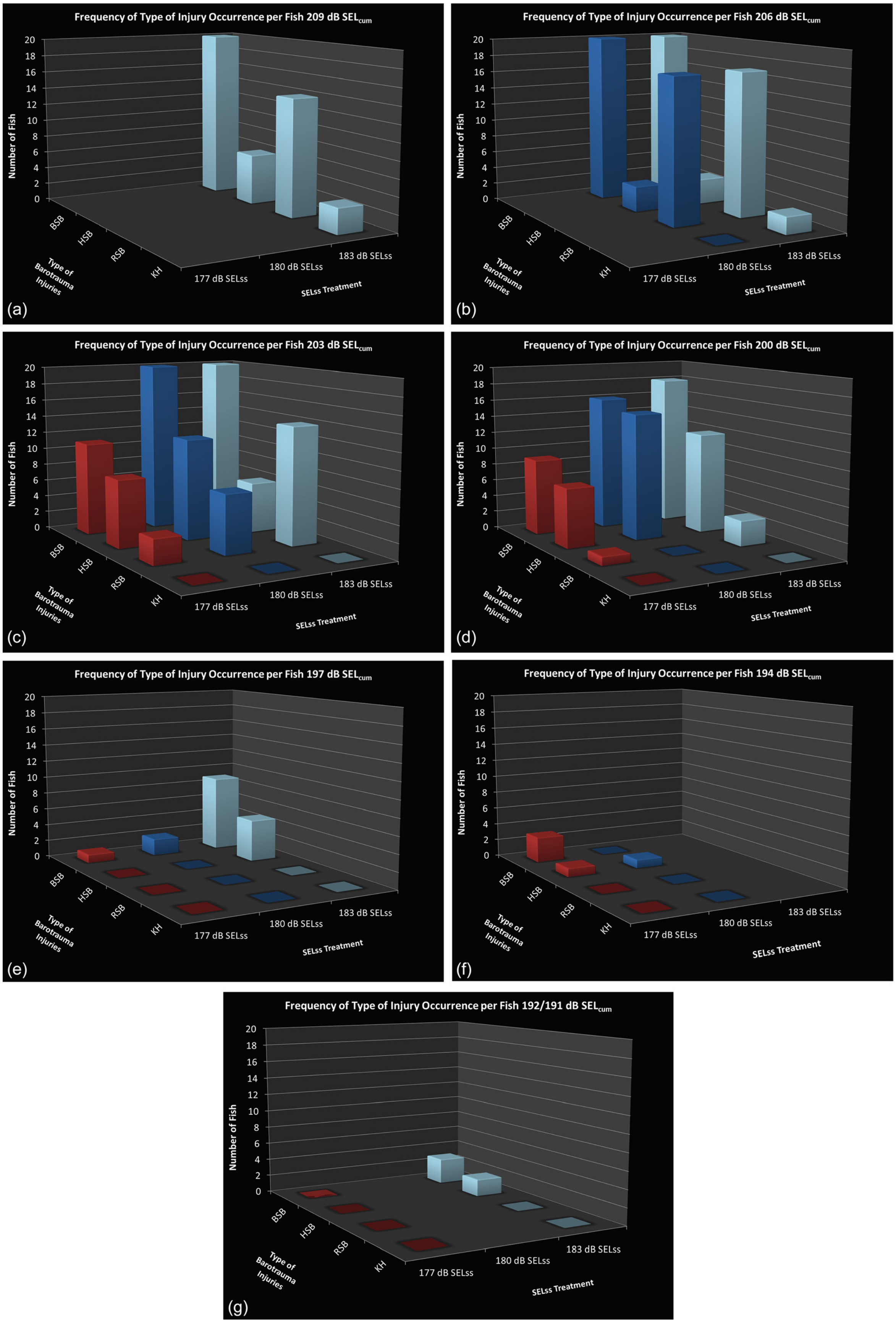

FIG. 2. (Color online) Frequency of occurrence of each injury type as a function of single strike exposure levels (SEL$_{ss}$) that yield cumulative sound exposure levels (SEL$_{cum}$). (A) 209 dB SEL$_{cum}$; (B) 206 dB SEL$_{cum}$; (C) 203 dB SEL$_{cum}$; (D) 200 dB SEL$_{cum}$; (E) 197 dB SEL$_{cum}$; (F) 194 dB SEL$_{cum}$; and (G) combination of 192 and 191 dB SEL$_{cum}$. It should be noted that the 192 dB SEL$_{cum}$ is derived from eight pile strikes at 183 dB SEL$_{ss}$ and the 191 dB SEL$_{cum}$ is derived from 24 pile strikes at 177 dB SEL$_{ss}$. See Table I for details on which SEL$_{ss}$ treatment yielded each SEL$_{cum}$. BSB = swim bladder hematoma; HSB = herniated swim bladder; RSB = ruptured swim bladder; and KH = kidney hemorrhage.

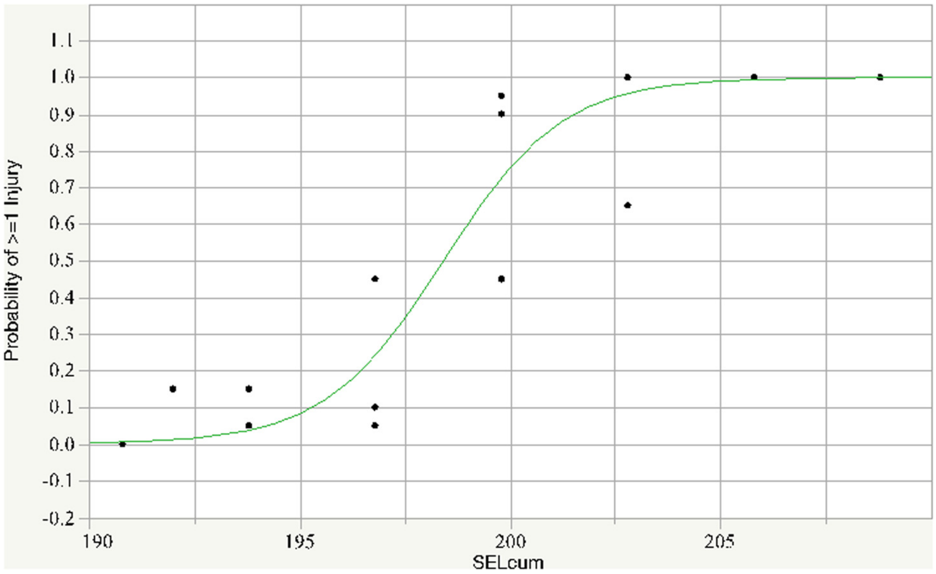

FIG. 3. (Color online) Probability of at least one injury as a function of SEL_{cum}.

injury probability is associated with the lowest SEL_{ss} and lowest number of strikes and the highest injury probability with the highest SEL_{ss} and the highest number of strikes. Between these extremes, the combinations of SEL_{ss} and number of strikes tested were limited. If other values had been tested that would have resulted in more SEL_{cum} values between the curve's inflection points, it is likely that much of the space between the probability bounds of 0 and 1 would have been filled with points. The best description of the probability of more than one injury within the range of SEL_{cum} values tested in this study would likely be a surface rather than a line if all of the SEL_{ss} and number of strike combinations that would produce SEL_{cum} values within the range of the experiment were tested. The resulting surface would taper to 0 for small SEL_{cum} values and to 1 for large SEL_{cum} values and would bulge at intermediate values. A curve similar to that shown in Fig. 3 would run along the "ridge" of the surface. This surface would most likely differ to one extent or another between species and size groups within species.

This analysis indicates that there is likely an "onset of injury" for each SEL_{cum} value where the number of strikes to onset of injury decreases as SEL_{ss} increases and vice versa. Similarly, the probability of one injury would likely increase, even for single strike exposures as SEL_{ss} increases. There exist SEL_{ss} values where even a single injury is unlikely for a large number of strikes. Likewise, there are SEL_{ss} values that would pose a high probability of a single injury to a fish with very few, perhaps only one strike.

For any particular group of fish, it appears that the onset of injury, defined as a particular probability of a single injury, would be an isopleth along the response surface describing the probability of injury as a function of SEL_{cum}.

Definition of injury onset is further complicated when injury severity is considered. Studies to date have shown that fish have a high recovery rate from minor and moderate injuries in a laboratory setting (Casper *et al.*, 2012; Casper *et al.*, 2013a). If fish can recover from minor and moderate injuries, then should injury onset for protection of fish populations be set in terms of probability of *mortal* injury without consideration of the occurrence of minor and moderate injuries?

An additional consideration is how well these studies with hybrid striped bass might translate to other species and/or sizes of fishes. Based on previous pile driving experiments with hybrid striped bass (Casper *et al.*, 2013a; Casper *et al.*, 2013b) and other species (Casper *et al.*, 2012; Halvorsen *et al.*, 2012a; Halvorsen *et al.*, 2012b), the injuries to the hybrid striped bass represent a worst case scenario. These previous studies showed that fishes, including hybrid striped bass, with physoclistous swim bladders are generally more susceptible to barotrauma injury and yield more severe injuries than fishes with physostomous swim bladders (e.g., lake sturgeon and Chinook salmon). An additional study also revealed that hybrid striped bass that were smaller than those used in this study were less susceptible to injuries than fish of comparable size (Casper *et al.*, 2013a). It is unknown how these results might translate to significantly larger hybrid striped bass.

One final, yet important, outcome of this study also relates back to the previous study with hybrid striped bass (Casper *et al.*, 2013a). The previous study showed that injuries occurred in the two different sizes of fish at 203 dB SEL_{cum} with a likely occurrence of injury in some fish at an exposure of 200 dB SEL_{cum}. Exposures of both sizes of fish consisted of 960 pile strikes with SEL_{ss} values of 174 dB re 1 μPa^2 s for the smaller and 171 dB re 1 μPa^2 s for the larger fish. In this current study, however, injuries (including ruptured swim bladders) occurred at SEL_{cum} exposure levels as low as 194 dB re 1 μPa^2 s SEL_{cum} with SEL_{ss} 180 dB re 1 μPa^2 s (number of strikes 24 and injury probability 0.05) and 177 dB re 1 μPa^2 s (number of strikes 48 and injury probability 0.15).

These results represent another example showing that the equal energy hypothesis does not apply to pile driving signals (Halvorsen *et al.*, 2011; Halvorsen *et al.*, 2012b). It is clear that fewer pile strikes at higher SEL_{ss} values will yield more severe injuries than an equivalent SEL_{cum} value derived from a larger numbers of pile strikes at lower SEL_{ss}.

From the series of pile driving exposure studies utilizing the HICI-FT, sufficient data have been presented to warrant that $\mathrm{SEL_{cum}}$ metric alone is not sufficient for predicting injury in fishes from exposure to impulsive sounds.

The results of this and previous studies further demonstrate the need to specify both $\mathrm{SEL_{ss}}$ and number of strikes to obtain a unique estimate of injury probability. Future assessments of injury to fish for purposes of identifying exposure thresholds for regulatory purposes should consider $\mathrm{SEL_{ss}}$ and number of strikes with an evaluation of onset of effect based on definitions that consider injury probability and injury severity.

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Bolle, L. J., de Jong, C. A., Bierman, S. M., van Beek, P. J., van Keeken, O. A., Wessels, P. W., van Damme, C. J., Winter, H. V., de Haan, D., and Dekeling, R. P. (**2012**). “Common sole larvae survive high levels of pile-driving sound in controlled exposure experiments,” PLoS One **7**, e33052.

Bolle, L. J., de Jong, C. A., Bierman, S. M., van Beek, P. J., Wessels, P. W., Blom, E., van Damme, C. J., Winter, H. V., and Dekeling, R. P. (**2016**). *Effect of Pile-Driving Sounds on the Survival of Larval Fish* (Springer, New York).

Casper, B. M., Halvorsen, M. B., Matthews, F., Carlson, T. J., and Popper, A. N. (**2013a**). “Recovery of barotrauma injuries resulting from exposure to pile driving sound in two sizes of hybrid striped bass,” PLoS One **8**, e73844.

Casper, B. M., Popper, A. N., Matthews, F., Carlson, T. J., and Halvorsen, M. B. (**2012**). “Recovery of barotrauma injuries in Chinook salmon, *Oncorhynchus tshawytscha* from exposure to pile driving sound,” PLoS One **7**, e39593.

Casper, B. M., Smith, M. E., Halvorsen, M. B., Sun, H., Carlson, T. J., and Popper, A. N. (**2013b**). “Effects of exposure to pile driving sounds on fish inner ear tissues,” Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. **166**, 352–360.

Halvorsen, M. B., Casper, B. M., Matthews, F., Carlson, T. J., and Popper, A. N. (**2012a**). “Effects of exposure to pile-driving sounds on the lake sturgeon, Nile tilapia and hogchoker,” Proc. R. Soc. B: Biol. Sci. B **279**, 4705–4714.

Halvorsen, M. B., Casper, B. M., Woodley, C. M., Carlson, T. J., and Popper, A. N. (**2011**). “Predicting and mitigating hydroacoustic impacts on fish from pile installations,” National Cooperative Highway Research Program Research Results Digest 363.

Halvorsen, M. B., Casper, B. M., Woodley, C. M., Carlson, T. J., and Popper, A. N. (**2012b**). “Threshold for onset of injury in Chinook salmon from exposure to impulsive pile driving sounds,” PLoS One **7**, e38968.

Hawkins, A. D., and Popper, A. N. (**2017**). “A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates,” ICES J. Marine Sci.: J. Cons. **74**, 635–671.

Krebs, J., Jacobs, F., and Popper, A. N. (**2016**). “Avoidance of pile-driving noise by Hudson river sturgeon during construction of the new NY Bridge at Tappan Zee,” in *The Effects of Noise on Aquatic Life II*, edited by A. N. Popper and A. D. Hawkins (Springer, Berlin), pp. 555–563.

MacGillivray, A., and Racca, R. (**2005**). *Sound Pressure and Particle Velocity Measurements from Marine Pile Driving at Eagle Harbor Maintenance Facility*, Bainbridge Island, WA (Jasco Research, Halifax, NS, Canada).

Popper, A. N., and Hastings, M. C. (**2009**). “The effects of anthropogenic sources of sound on fishes,” J. Fish Biol. **75**, 455–489.

Popper, A. N., and Hawkins, A. D. (**2012**). *The Effects of Noise on Aquatic Life* (Springer Science+Business Media, New York).

Popper, A. N., and Hawkins, A. D. (**2016**). *The Effects of Noise on Aquatic Life II* (Springer Science+Business Media, New York).

Popper, A. N., Hawkins, A. D., Fay, R. R., Mann, D. A., Bartol, S., Carlson, T. J., Coombs, S., Ellison, W. T., Gentry, R. L., and Halvorsen, M. B. (**2014**). “Sound exposure guidelines,” in ASA S3/SC1. 4 TR-2014: *Sound Exposure Guidelines for Fishes and Sea Turtles: A Technical Report*, prepared by ANSI-Accredited Standards Committee S3/SC1 and registered with ANSI (Springer, Berlin), pp. 33–51.

Southall, B. L., Bowles, A. E., Ellison, W. T., Finneran, J. J., Gentry, R. L., Greene, C. R., Jr., Kastak, D., Ketten, D. R., Miller, J. H., Nachtigall, P. E., Richardson, W. J., Thomas, J. A., and Tyack, P. L. (**2007**). “Marine mammal noise exposure criteria: Initial scientific recommendations,” Aquatic Mammals **33**, 411–521.

Stadler, J. H., and Woodbury, D. P. (**2009**). “Assessing the effects to fishes from pile driving: Application of new hydroacoustic criteria,” in *Inter-noise 2009 Innovations in Practical Noise Control*.

Woodbury, D., and Stadler, J. (**2008**). “A proposed method to assess physical Injury to fishes from underwater sound produced during pile driving,” Bioacoustics **17**, 289–297.

Yelverton, J. T., Richmond, D. R., Hicks, W., Saunders, H., and Fletcher, E. R. (**1975**). “The relationship between fish size and their response to underwater blast,” Report No. DNA 3677T, Director, Defense Nuclear Agency, Washington, DC.