

## Trauma to Juvenile Pinfish and Spot Inflicted by Submarine Detonations

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**Abstract.**—Juvenile pinfish *Lagodon rhomboides* and spot *Leiostomus xanthurus* exposed to pressure waves emanating from experimental submarine detonations exhibited both sublethal and probable antemortem trauma. Hyperemia within the swim bladder and liver, hematuria, coagulative liver necrosis, and rupture of the pancreas were the most recurrent and significant traumas evident from histopathological examination and were directly attributed to exposure to pressure waves. These traumas were probably caused by the rapid compression and expansion of the swim bladder as the impulse passed. Of these traumas, hyperemia within visceral organs and hematuria are probably sublethal. Rupture of the pancreas and coagulative liver necrosis are typically irreversible and hence probably antemortem.

Comprehensive assessment of the effects of submarine detonations on fish have been limited by the size of the fish assessed and the morphological level of assessment. The fish exposed to pressure waves emanating from detonations—either experimental or related to in situ engineering (i.e., blasting and dredging) or military (e.g., ordinance testing) projects—have been larger than 54 mm in length (Wiley et al. 1981) or 0.02 g in body weight (Yelverton et al. 1975). The effects of exposure have typically been assessed by mortality estimates and the trauma evident at the gross anatomical level. Extrapolation of trauma to fish smaller than 54 mm is tenuous because larval and small juvenile fishes appear to be more sensitive than adults to insults of any kind, including increases in pressure (Bishai 1961) and exposure to pressure waves (Fitch and Young 1948). Moreover, the swim bladder, an internal gas-filled vessel that reacts instantaneously to changes in ambient pressure, is thinly lined and more distensible in larval and juvenile fish than in adults (Govoni and Hoss 2001). No histopathological assessment of the trauma potentially caused by pressure waves is available for fish of any size, while assessment at this level is the only accurate method for larvae and small juveniles.

The traumas currently recognized as resulting from submarine detonations are typically contusions of external anatomy or visceral organs, rupture of visceral organs, and external or internal hemorrhages. Hubbs and Rechnitzer (1952) and

Linton et al. (1985) diagnosed rupture of the peritoneum, liver, kidneys, spleen, gallbladder, alimentary canal, and swim bladder. Hemorrhage has been observed within the coelomic cavity and within or about the liver, kidneys, spleen, and swim bladder (Hubbs and Rechnitzer 1952; Linton et al. 1985).

The principal cause of trauma evidenced in the external anatomy is the impact of the pressure wave (particle displacement within the shock front); the cause of trauma to the viscera is rapid compression and expansion of the swim bladder as the pressure wave passes (Wiley et al. 1981). Maximum pressure (above the ambient level), and thus swim bladder compression, occurs initially when the shock front passes; minimum pressure, and thus swim bladder expansion, occurs at rarefaction after the passage of the shock front and typically results from reflection from the air–water interface (Yelverton et al. 1975).

Juvenile pinfish *Lagodon rhomboides* and spot *Leiostomus xanthurus* are potentially at risk in the field owing to exposure to pressure waves produced by ongoing engineering projects (Settle et al. 2002). Controlled, experimental submarine detonations that produced pressure waves commensurate with those produced by current blasting and dredging operations (Settle et al. 2002) exposed young juvenile pinfish and spot to pressure waves and provided specimens for histopathological assessment of injury. In this article we report sublethal and antemortem traumas resulting from these experimental detonations.

### Methods

Methods for the experimental detonations followed Wiley et al. (1981), as modified by Settle

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Received August 28, 2002; accepted February 28, 2003

et al. (2002). A week prior to the field experiment, several thousand juvenile pinfish and spot were collected from a tidal passage off the northwestern end of Pivers Island, Beaufort, North Carolina, with a 2-m  $\times$  2-m plankton net equipped with an 8-m-long net with 0.947-mm mesh. The attachment of a live-box to the cod end of the net allowed the removal of fish with minimal stress (Hettler 1979). These fish were transferred to a 2,000-L holding tank with flowing seawater at ambient temperature and salinity ( $\sim 14^\circ\text{C}$  and 28–30‰) with a 12 h light : 12 h dark cycle and fed pelletized fish feed (Corey Hi-Pro Starter and Fry Feed [fish meal, fish oil, wheat, krill, salt, vitamins, minerals, pigments, and methionine]). The constitution of visceral organs indicate that spot begin transformation from larvae to juveniles at about 7 mm standard length (SL) and complete transformation at 14 mm (Govoni 1980; Govoni and Hoss 2001); we assumed the same for pinfish. Subject fish were young juveniles, that is, 13.8–21.3-mm pinfish and 15.1–25.3-mm spot, at the time of the experiment.

Twenty-four hours before the experimental detonations, several hundred fish were removed from holding tanks, introduced into twenty-four 2-mil polyethylene bags in 5 L of seawater under constant aeration, and held overnight. Between 20 and 30 pinfish were placed in each of 12 bags and between 20 and 30 spot in the remaining 12 bags. The following morning, dead or moribund fish were removed from the bags and the bags sealed. Bags with healthy fish were moved to the same channel from which fish were collected for exposure to pressure waves emanating from experimental detonations.

Bags with fish were submerged to a depth of 2.0 m at three distances from the detonation—3.6, 7.5, and 17.0 m—in a tidal passage adjacent to Pivers Island. The temperature and salinity at the 2.0-m depth were  $\sim 13$ – $14^\circ\text{C}$  and 27–31‰. Fish were not allowed to equilibrate swim bladder volume to the pressure encountered at 2.0 m because equilibration periods are on the order of hours (for spot  $> 2$  h; Govoni and Hoss 2001). The average submergence time of each bag was 12.8 min. Triplicate charges were detonated, one bag of each species being submerged at each distance for each detonation. Exposure was instantaneous for each of the three replicates. The remaining six bags, one for each species at each distance, were submerged subsequently for 7 min without detonation and were treated as control fish.

The form of the pressure wave generated by the experimental detonations and measured by sensors

TABLE 1.—Average pressure maximum ( $P_{\max}$ ) and minimum ( $P_{\min}$ ), energy flux density ( $E$ ), and impulse ( $I$ ) experienced by juvenile pinfish and spot exposed to submarine detonations.

Pressure wave	Distance from blast (m)		
	3.6	7.5	17.0
$P_{\max}$ (kPa)	636.92	230.86	109.93
$P_{\min}$ (kPa)	-92.07	-70.77	-60.46
$E$ ( $\text{J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ )	2.21	0.36	0.10
$I$ ( $\text{Pa}\cdot\text{s}$ )	8.67	3.95	2.18

placed inside and outside the polyethylene bags (Settle et al. 2002) was similar to those reported by Lynch and Revy (2002), with characteristic pressure maxima ( $P_{\max}$  [kPa]), energy flux densities ( $E$  [ $\text{J}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ]), and impulses ( $I$  [ $\text{Pa}\cdot\text{s}$ ]). According to Yelverton et al. (1975), an average value of  $I$  of 4.7 Pa·s is the threshold for a measurable impact on small fish; the average value of  $I$  generated by the experimental detonations herein ranged from  $\sim 2.0$  to 8.7 Pa·s (Table 1). The polyethylene bags were transparent to the pressure wave.

Following the detonations, the bags were retrieved and the number of dead fish and the behavioral condition of live fish (i.e., apparently healthy, stunned, or moribund) recorded in the field (Settle et al. 2002). In the laboratory, the bags were reopened and the number of dead fish and the behavioral condition of live fish again recorded. Fish showing no signs of gross anatomical trauma or aberrant behavior were held in aerated seawater at  $14^\circ\text{C}$  in the laboratory and reexamined 4 and 24 h later. Immediately after exposure and at the 4-h and 24-h junctures, dead, stunned, and moribund fish were examined under a stereo-microscope, measured (SL [mm]), and preserved in histological-grade neutral buffered (phosphate) formalin. Histological injury assessment was conducted on subsamples from all bags. Samples of living fish exposed and not exposed (controls) were examined, measured, and preserved at 24 h. Moribund and living fish were anesthetized with MS-222 (tricaine methanesulfonate) before examination and fixation.

The anesthetic effect of MS-222 is brought about by depression of the medulla of the brain, which in turn depresses respiration and causes blood hypoxia (Smith et al. 1999). Aside from some changes in erythrocyte morphology, there is no known histopathology associated with anesthesia produced by MS-222 (Smit et al. 1979). To

TABLE 2.—Observed trauma to juvenile pinfish and spot in submarine detonation experiments.

Trauma	Distance from blast (m)					
	3.6		7.5		17.0	
	Exposed	Control	Exposed	Control	Exposed	Control
<b>Pinfish</b>						
Number of fish	23	6	19	6	19	6
Coelomic hemorrhage <sup>a</sup>	2	0	1	1	1	1
Swim bladder hyperemia	5	0	0	0	0	0
Liver hyperemia	2	0	0	0	0	0
Hematuria	14	0	4	0	0	0
Alimentary canal necrosis <sup>a</sup>	4	4	5	2	2	2
Liquefactive liver necrosis <sup>a</sup>	0	0	0	0	0	0
Coagulative liver necrosis	2	0	0	0	0	0
Ruptured pancreas	2	0	0	0	0	0
<b>Spot</b>						
Number of fish	27	7	18	6	18	6
Coelomic hemorrhage <sup>a</sup>	0	0	0	0	0	0
Swim bladder hyperemia	8	0	0	0	0	0
Liver hyperemia	16	0	0	0	0	0
Hematuria	24	0	2	0	0	0
Alimentary canal necrosis <sup>a</sup>	11	5	6	6	6	3
Liquefactive liver necrosis <sup>a</sup>	0	0	0	2	0	1
Coagulative liver necrosis	11	0	0	0	0	0
Ruptured pancreas	4	0	0	0	0	0

<sup>a</sup> Observed trauma not due to shock wave exposure.

assess this further, two pinfish and two spot, none of which were exposed to pressure waves or treated as controls, were anesthetized with the same concentration of MS-222 and fixed for histopathology. None of these fish displayed any of the traumas reported herein.

For histopathology (Table 2), preserved fish were divided into two groups ( $\leq 20.4$  mm and  $>20.4$  mm). Small fish were decalcified in a 10% solution of formic acid for 1 d; large fish were subjected to the same treatment for 2 d. Fish were prepared by standard procedures for paraffin embedding and sectioning (Humason 1979). Ten to eighteen 5- $\mu$ m parasagittal sections (including the medial) were cut through each fish with five to six sections each at 50- $\mu$ m intervals. Sections were stained with Mayer's-Harris's hematoxylin and counterstained with eosin y-phloxine.

The swim bladder volumes of both pinfish and spot were estimated from the regression equations given in Govoni and Hoss (2001) for spot. Inasmuch as juvenile pinfish are morphologically similar to spot (Hildebrand and Cable 1938) and their close relatives, the grunts (family Haemulidae), and react similarly to submarine detonations (Hubbs and Rechnitzer 1952), we assumed that their swim bladder volumes were comparable.

Frequency analysis, either Fisher's exact test or chi-square, were used to determine differences in

the frequency of traumas observed in exposed and control fish.

## Results

Many of the fish exposed to the pressure waves that emanated from the experimental detonations were found to be dead, stunned (i.e., disoriented and lying on the bottom of the bag), or moribund when examined in the field (Settle et al. 2002). One pinfish and six spot were found to be partially eviscerated when examined in the laboratory. In addition, 2 exposed pinfish, 14 exposed spot, and 3 control spot exhibited autolysis of viscera. All fish with autolysis were dead before preservation, and as is typical of autolytic tissue, reacted poorly to histological staining. These partially eviscerated and autolytic fish were excluded from histopathological assessment.

No other external lesions were evident in exposed fish, but internal hemorrhaging was evident from gross examination of five pinfish exposed at 3.6 m from the detonations and one exposed at 17.0 m. Four spot exposed at 3.6 m showed internal hemorrhaging. Gross examination showed no internal hemorrhaging among control fish.

Both exposed and control pinfish had aggregations of erythrocytes in the caudad dorsal coelom that indicated hemorrhage, but these aggregations could not be related to the rupture of specific ar-

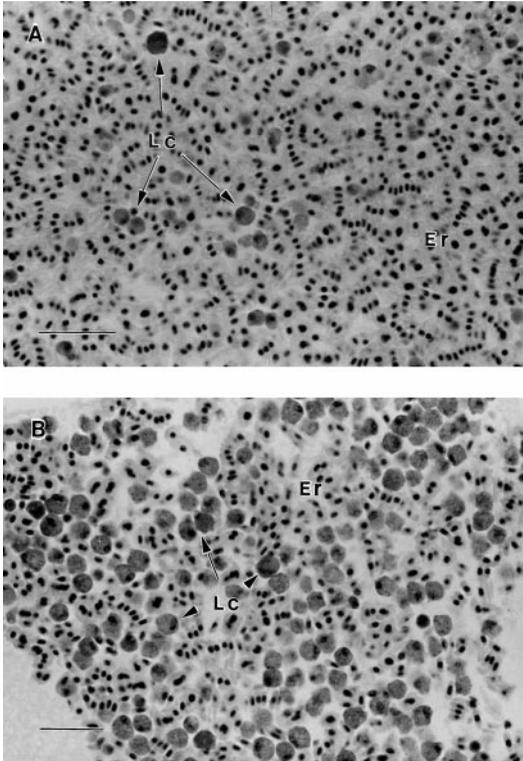


FIGURE 1.—Hemorrhage in the caudad dorsal coelom of (A) a 16.7-mm (standard length) pinfish exposed to a pressure wave from a submarine detonation (scale bar = 20  $\mu\text{m}$ ) and (B) a 19.2-mm pinfish not exposed to such a pressure wave (scale bar = 30  $\mu\text{m}$ ). Abbreviations are as follows: Er, erythrocytes; and Lc, leukocytes.

teries or veins. While this hemorrhage appeared more extensive in exposed fish than in control fish, the incidence of coelomic hemorrhage (Table 2) was independent of exposure at any distance from the detonation (replicates pooled; Fisher's exact test;  $P \leq 1.000$  for distance from detonation and  $P \leq 0.6081$  for exposure). Leukocytes were more prevalent in the regions exhibiting coelomic hemorrhage among control pinfish than among fish exposed to pressure waves (Figure 1A, B), but the ratios of erythrocytes to leukocytes were not significantly different ( $\chi^2 = 2.4050$ ;  $P \leq 0.1209$ ).

Exposed pinfish and spot had hyperemia within the swim bladder serosa (Figure 2A), the mucosa of the gas gland, the rete mirabile (Figure 2B), and within the liver (Figure 3). Only exposed fish (Table 2) had aggregations of erythrocytes in the interstices of these tissues. Hyperemia within the liver was evident in regions proximal to the swim bladder. Contusion of the liver in the region prox-

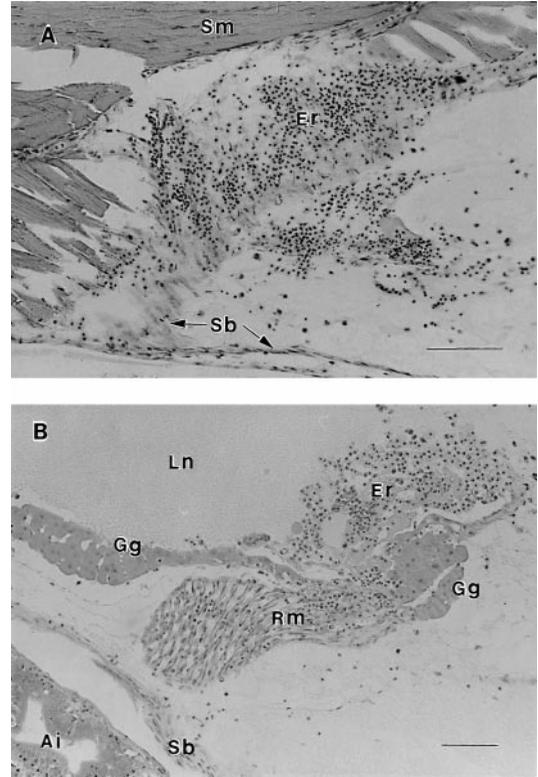


FIGURE 2.—(A) Hyperemia of the posterior swim bladder serosa of a 21.2-mm spot exposed to a pressure wave from a submarine detonation (scale bar = 75  $\mu\text{m}$ ); and (B) hyperemia of the swim bladder gas gland tissue and rete mirabile of a 17.7-mm spot exposed to such a pressure wave (scale bar = 45  $\mu\text{m}$ ). Abbreviations are as follows: Ai, anterior intestine; Er, erythrocytes; Gg, gas gland tissue; Ln, swim bladder lumen; Rm, rete mirabile; Sb, swim bladder serosa; and Sm, striated axial muscle.

imal to the swim bladder was also evident in one exposed spot.

The kidney tubules of exposed pinfish and spot (Table 2; Figure 4) exhibited hematuria. Erythrocytes appeared in the lumen of the proximal kidney tubules. No control fish evidenced hematuria.

Apparent liquefactive necrosis was evident in the mucosa of the dorsal region of the anterior intestine of exposed and control pinfish and spot (Table 2). No pinfish and only 8% of the spot that evidenced this intestinal necrosis were dead before fixation. The frequency of necrosis of the anterior intestine was dependent on exposure to pressure waves but with no significant differences among distances from detonation. The exact probability of observing a frequency distribution this extreme was 0.0254 for pinfish and 0.0182 for spot.

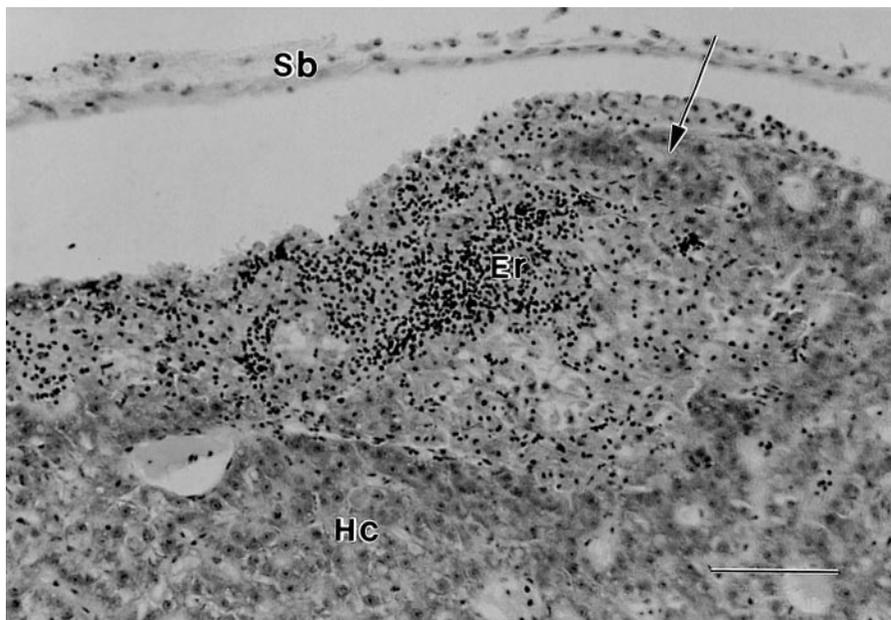


FIGURE 3.—Hyperemia of the liver of a 21.2-mm spot exposed to a pressure wave from a submarine detonation. The arrow indicates contusion (scale bar = 50  $\mu$ m). Abbreviations are as follows: Er, erythrocytes; Hc, hepatocytes; and Sb, swim bladder serosa.

Pinfish and spot exhibited two types of liver necrosis: apparent liquefactive and coagulative (Table 2). Only control spot exhibited liquefactive necrosis of the liver. Liquefactive necrotic liver tissue was not associated with hyperemia and was less eosinophilic than tissue that had coagulative necrosis. Coagulative liver necrosis was evident only in exposed pinfish and spot (Table 2) and occurred proximal to the swim bladder (Figure 5A). Coagulative necrotic infarcts were evident as areas of cellular disorganization where cell membranes were ill defined and always proximal to areas of hyperemia within the liver (Figure 5B). The overabundance of erythrocytes in one area created ischemia in the adjacent area, the necrotic zone.

Of the viscera, only the pancreas ruptured in exposed pinfish and spot (Table 2). The mesentery surrounding the pancreas was disrupted and pancreatic zymogen granules were present in the interstices surrounding the organ. Rupture was always evident in regions where the organ was proximal to the swim bladder (Figure 6). No control fish had a ruptured pancreas.

### Discussion

Most of the traumas revealed herein could not have been identified or adequately described by

gross anatomical examination. The small size of larval and juvenile fish, along with the attributes of the traumas observed, necessitates histopathology. Visceral hemorrhage is the single exception, but this trauma may relate to experimental procedures and not to exposure to pressure waves.

With our experimental design, visceral hemorrhage and liquefactive necrosis were not attributed to exposure to pressure waves. Visceral hemorrhage is a trauma often attributed to the exposure of large juvenile and adult fish to pressure waves emanating from submarine detonations (Hubbs and Rechnitzer 1952; Linton et al. 1985). The aggregations of erythrocytes within the coelom that were observed in this study may have resulted from the compression and subsequent expansion of the swim bladder when pinfish were lowered to and raised from the 2-m depth (Table 3). Of the two types of necrosis, only coagulative necrosis can be attributed to exposure to pressure waves. The apparent liquefactive necrosis of the mucosa of the alimentary canal and liver, which were observed in both exposed and control pinfish and spot, were possibly related to high food intake in that these lesions resembled those reported by Mobin et al. (2000, 2001). In our study, juvenile pinfish and spot were held in the laboratory for up to 1 week before exposure to detonations, during

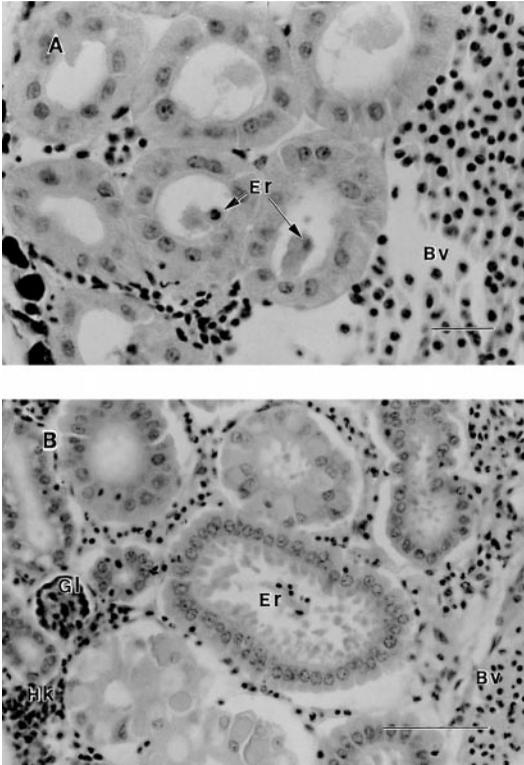


FIGURE 4.—(A) Hematuria of the kidney of a 16.7-mm pinfish exposed to a pressure wave from a submarine detonation (scale bar = 18  $\mu$ m); and (B) hematuria in the proximal nephric tubule of the kidney of a 20.2-mm spot exposed to such a pressure wave (scale bar = 43  $\mu$ m). Abbreviations are as follows: Bv, blood vessel; Er, erythrocytes; Gl, glomeruli; and Hk, head of the kidney.

which time they were fed pelletized fish feed ad libitum. While the actual quantity of food intake was not known, ad libitum feeding may have resulted in superfluous food intake. Liquefactive necrosis of the liver may be caused by the permeation of digestive enzymes released into proximal liver tissue by lesions of the alimentary canal (Mobin et al. 2001). Coagulative liver necrosis, which was evident only in exposed fish, differed from liquefactive necrosis in that the lesions had intratissue hyperemia and were always proximal to the swim bladder.

Hyperemia within the swim bladder and liver, hematuria, coagulative liver necrosis, and rupture of the pancreas were the most recurrent and pathologically significant traumas evident and the only ones directly attributable to exposure to submarine detonations. These traumas were probably caused by the rapid compression and expansion of the swim bladder as the pressure wave passed. This

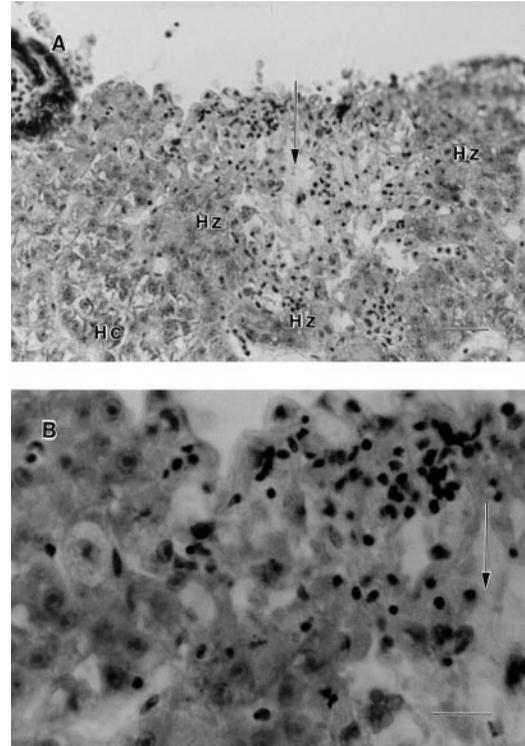


FIGURE 5.—(A) Coagulative necrosis of the liver of a 19.7-mm spot exposed to a pressure wave from a submarine detonation. The arrow indicates necrosis (scale bar = 23  $\mu$ m). Panel (B) shows a higher magnification of the infarct, the arrow again indicating necrosis (scale bar = 13  $\mu$ m). Abbreviations are as follows: Hc, normal hepatocytes; and Hz, hyperemic zone.

movement disrupts the serosa and surrounding mesentery of the pancreas and the endothelium of arterioles and venules and probably causes the leaking of erythrocytes into the lumen of kidney tubules (hematuria). Rapid compression of the liver can cause disruption of cell membranes, which, along with localized ischemia, causes coagulative liver necrosis.

Wiley et al. (1981) devised a dynamic model of injury to fish caused by submarine detonations that embodies swim bladder compression and expansion. In this model, injury results from the oscillations (repeated compression and expansion) of the swim bladder volume that lag the  $P_{max}$  of the pressure wave. Minimum volume is reached soon after the  $P_{max}$  and maximum volume is reached after rarefaction. This model assumes a discrete rather than a continuous decrease in pressure after the passage of the  $P_{max}$ , while oscillations in swim bladder volume result from the step function. Impact injury to

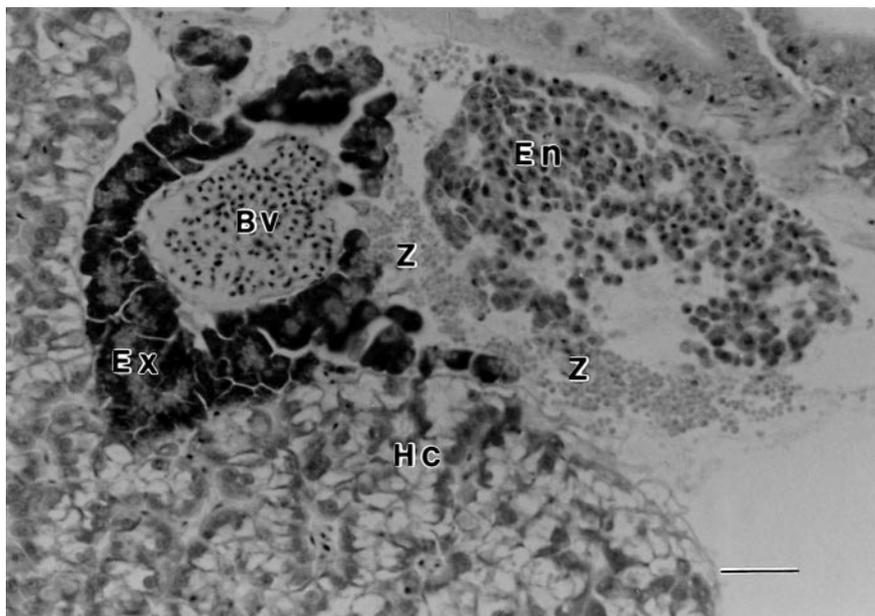


FIGURE 6.—Rupture of the pancreas of an 18.7-mm spot exposed to a pressure wave from a submarine detonation (scale bar = 30  $\mu\text{m}$ ). Abbreviations are as follows: Bv, blood vessel with erythrocytes; En, endocrine pancreatic tissue; Ex, exocrine pancreatic tissue; Hc, hepatocytes; and Z, zymogen granules inside and outside of the ruptured tissue.

the external anatomy might result from the passage of a singular wave form. Rapid compression and expansion of the swim bladder, whether static or oscillatory, can cause trauma to internal organs if the expansion and contraction is of sufficient volume. Our pressure wave data depict neither a discrete decrease in pressure following  $P_{\text{max}}$  (Settle et al. 2002) nor recurrent oscillation of swim bladder volume, but trauma to visceral organs owing to the disruption of viscera caused by single, static expansion and contraction was clearly evident. The pressure changes experienced by transforming larval and young juvenile pinfish and spot were within the range reported as the threshold for injury to small fish (Yelverton et al. 1975). These pressure changes were several orders of magnitude higher than those reported to have no injurious effect on fish (Traxler et al. 1993).

Given the ambient temperatures and pressures recorded in situ during the experiments, the  $P_{\text{max}}$  and  $P_{\text{min}}$  of the pressure waves encountered by these fish during exposure (Settle et al. 2002), and the regression equation for swim bladder volume and SL of larval spot at atmospheric pressure in Govoni and Hoss (2001), the swim bladders of juvenile spot probably would have contracted by an order of magnitude and expanded by approximately 2.4  $\mu\text{L}$  (averaged swim bladder volumes for the three distances from the detonation) relative to resting volumes (Table 3). The physical characteristics of pressure waves (Settle et al. 2002) indicate that the swim bladders of these juvenile fish were engulfed completely and instantaneously. The disruption that resulted from this compression and expansion was apparently sufficient to cause hyperemia within the swim bladder and

TABLE 3.—Estimated swim bladder volume (L) of a 19.0-mm juvenile spot before and during exposure to experimental submarine detonations (Govoni and Hoss 2001);  $P_{\text{max}}$  and  $P_{\text{min}}$  are the average pressure maximum and minimum.

Swim bladder volume	Distance from blast (m)		
	3.6	7.5	17.0
Estimated resting volume at surface	$1.58 \times 10^{-6}$	$1.58 \times 10^{-6}$	$1.58 \times 10^{-6}$
In situ estimated resting volume	$1.31 \times 10^{-6}$	$1.31 \times 10^{-6}$	$1.31 \times 10^{-6}$
In situ volume at $P_{\text{max}}$	$2.09 \times 10^{-7}$	$4.50 \times 10^{-7}$	$6.87 \times 10^{-7}$
In situ volume at $P_{\text{min}}$	$5.42 \times 10^{-6}$	$3.14 \times 10^{-6}$	$2.62 \times 10^{-6}$

liver, hematuria, coagulative liver necrosis, and rupture of the pancreas.

The presence of stunned fish or fish with aberrant behavior may indicate trauma or even the likelihood of death, as reported by Settle et al. (2002), but confirming the imminence of death requires postexperimental observation for more than 24 h. Moreover, the determination of the lethality of injury requires comprehensive assessment of the severity or extent of the injury, which cannot be inferred from histopathological examination. Of the traumas evident here, hyperemia and hematuria are probably sublethal, and juvenile pinfish and spot may recover from them. Rupture of the pancreas and coagulative liver necrosis are typically irreversible and possibly antemortem. Behavioral abnormalities owing to these sublethal and antemortem traumas could render fish more susceptible to predation in nature, but this source of mortality was not apprehensible with the design employed.

Species-specific differences in the susceptibility to injury from submarine detonations are evident for large juvenile and adult fish (Hubbs and Rechnitzer 1952; Wiley et al. 1981; Linton et al. 1985). Small juvenile spot are more susceptible to sublethal and antemortem trauma than are pinfish; 46% of the spot in our study exhibited such trauma, versus 31% of the pinfish.

### Acknowledgments

We acknowledge the U.S. Army Corps of Engineers for providing the support for this research (MIPR number W91LJ810383780). Histological sections were prepared by the North Carolina State University College of Veterinary Medicine under the direction of S. Horton. C. M. Horsch, U.S. Fish and Wildlife Service, provided an invaluable compact disk entitled "Fish Histology and Pathology." M. D. Greene of the National Oceanic and Atmospheric Administration, National Ocean Service, Center for Coastal Fisheries and Habitat Research (NOAA/NOS/CCFHR) assisted in the experimental detonations and in data management. J. A. Hare (NOAA/NOS/CCFHR) and E. Noga (North Carolina State University College of Veterinary Medicine) provided helpful reviews of the manuscript. The U.S. Government has the right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

### References

Bishai, H. M. 1961. The effect of pressure on the survival and distribution of larval and young fish. *Journal du Conseil International pour l'Exploration de la Mer* 26:292–311.

- Fitch, J. E., and P. H. Young. 1948. The effects of explosives in California coastal waters. *California Fish and Game* 34:53–67.
- Govoni, J. J. 1980. Morphological, histological, and functional aspects of alimentary canal and associated organ development in larval *Leiostomus xanthurus*. *Revue Canadienne de Biologie* 39:69–80.
- Govoni, J. J., and D. E. Hoss. 2001. Comparison of the development and function of the swimbladder of *Brevoortia tyrannus* (Clupeidae) and *Leiostomus xanthurus* (Sciaenidae). *Copeia* (2001):430–442.
- Hettler, W. F., Jr. 1979. Modified neuston net for collecting live larval and juvenile fish. *Progressive Fish-Culturist* 41:32–33.
- Hildebrand, S. F., and L. E. Cable. 1938. Further notes on the development and life history of some teleosts at Beaufort, North Carolina. *U.S. Bureau of Fisheries Bulletin* 24:505–642.
- Hubbs, C. L., and A. B. Rechnitzer. 1952. Report on experiments designed to determine effects of underwater explosions on fish life. *California Fish and Game* 38:333–366.
- Humason, G. L. 1979. *Animal tissue techniques*. Freeman, San Francisco.
- Linton, T. L., A. M. Landry, Jr., J. E. Buckner, Jr., and R. L. Berry. 1985. Effects upon selected marine organisms of explosives used for sound production in geophysical exploration. *Texas Journal of Science* 37:342–353.
- Lynch, R. T., and G. Revy. 2002. Investigation of impacts of underwater explosions on larval and early juvenile fishes, part III. U.S. Army Corps of Engineers, Instrumentation Report, Wilmington, North Carolina.
- Mobin, S. M., K. Kanai, and K. Yoshikoshi. 2000. Histopathological alterations in the digestive system of larval and juvenile Japanese flounder *Paralichthys olivaceus* reared on four feeding levels. *Journal of Aquatic Animal Health* 12:196–208.
- Mobin, S. M., K. Kanai, and K. Yoshikoshi. 2001. Effects of feeding levels on the pathological alterations in the digestive system and mortality of larvae and juveniles of *Pagrus major*. *Journal of Aquatic Animal Health* 13:202–213.
- Settle, L. R., J. J. Govoni, M. D. Greene, and M. A. West. 2002. The effects of underwater explosions on larval fish with implications for the Wilmington Harbor Project. U.S. Army Corps of Engineers, Wilmington, North Carolina.
- Smit, G. L., J. Hattingh, and A. P. Burger. 1979. Haematological assessment of the effects of the anesthetic MS-222 in natural and neutralized form in three freshwater fish species: hemoglobin electrophoresis, ATP levels, and corpuscular fragility curves. *Journal of Fish Biology* 15:655–663.
- Smith, D. A., S. A. Smith, and S. D. Holladay. 1999. Effects of previous exposure to tricaine methanesulfonate on time to anesthesia in hybrid tilapias. *Journal of Aquatic Animal Health* 11:183–186.

- Traxler, S. L., B. R. Murphy, and T. L. Linton. 1993. Subsediment seismic explosions do not injure caged fishes in a freshwater reservoir. *Journal of Freshwater Ecology* 8:73-74.
- Wiley, M. L., J. B. Gaspin, and J. F. Goertner. 1981. Effects of underwater explosions on fish with a dynamical model to predict fishkill. *Ocean Science and Engineering* 6:223-284.
- Yelverton, J. T., D. R. Richmond, W. Hicks, K. Saunders, and E. R. Fletcher. 1975. The relationship between fish size and their response to underwater blast. Defense Nuclear Agency, Washington, D.C.