APPENDIX D:

REVIEW OF THE EFFECTS OF AIRGUN SOUNDS ON MARINE INVERTEBRATES AND FISH

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1 This appendix provides a detailed summary of the limited data and literature available on the observed 2 effects (or lack of effects) of exposure to airgun sound on marine invertebrates and fish. Specific 3 conditions and results of the studies including SELs and sound thresholds of responses are discussed 4 when available. However, it is sometimes difficult to interpret studies on the effects of underwater sound 5 on marine animals because authors often do not provide enough information, including received sound 6 levels, source sound levels, and specific characteristics of the sound. Specific characteristics of the sound 7 include units and references, whether the sound is continuous or impulsive, and its frequency range. 8 Underwater sound pressure levels are typically reported as a number of dB referenced to a reference level, 9 usually 1 micro-Pascal (μ Pa). However, the sound pressure dB number can represent multiple types of 10 measurements, including "zero to peak", "peak to peak", or averaged ("rms"). SELs may also be reported 11 as dB. The SEL is the integration of all the acoustic energy contained within a single sound event. Unless 12 precise measurement types are reported, it can be impossible to directly compare results from two or more 13 independent studies.

Sound caused by underwater seismic survey equipment results in energy pulses with very high peak pressures (Richardson et al. 1995). This was especially true when chemical explosives were used for underwater surveys. Virtually all underwater seismic surveying conducted today uses airguns which typically have lower peak pressures and longer rise times than chemical explosives. However, sound levels from underwater airgun discharges might still be high enough to potentially injure or kill animals located close to the source. Also, there is a potential for disturbance of normal behavior upon exposure to airgun sound.

21 The following sections provide an overview of sound production and detection in marine invertebrates 22 and fish, and information on the effects of exposure to sound on marine invertebrates and fish, with an 23 emphasis on seismic survey sound. DFOC has published two internal documents that provide a literature 24 review of the effects of seismic and other underwater sound on invertebrates (Moriyasu et al. 2004; Payne 25 et al. 2008). The potential effect of seismic sounds on fish has been studied with a variety of taxa, 26 including marine, freshwater, and anadromous species (reviewed by Fay and Popper 2000; Ladich and 27 Popper 2004; Hastings and Popper 2005; Popper and Hastings 2009a, b). The available information as 28 reviewed in those documents and here includes results of studies of varying degrees of scientific rigor as

29 well as anecdotal information.

30 D.1 MARINE INVERTEBRATES

31 D.1.1 Acoustic Capabilities

Much of the available information on acoustic abilities of marine invertebrates pertains to crustaceans, specifically lobsters, crabs and shrimps. Other acoustic-related studies have been conducted on cephalopods. Many invertebrates are capable of producing sound, including barnacles, amphipods, shrimp, crabs, and lobsters (Au and Banks 1998; Tolstoganova 2002). Invertebrates typically produce sound by scraping or rubbing various parts of their bodies, although they also produce sound in other

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I ways. Sounds made by marine invertebrates may be associated with territorial behavior, mating,

- 2 courtship, and aggression. On the other hand, some of these sounds may be incidental and not have any
- 3 biological relevance. Sounds known to be produced by marine invertebrates have frequencies ranging
- 4 from 87 Hz to 200 kHz, depending on the species.
- 5 Both male and female American lobsters (*Homarus americanus*) produce a buzzing vibration with the 6 carapace when grasped (Pye and Watson 2004; Henninger and Watson 2005). Larger lobsters vibrate 7 more consistently than smaller lobsters, suggesting that sound production may be involved with mating 8 behavior. Sound production by other species of lobsters has also been studied. Among deep-sea lobsters, 9 sound level was more variable at night than during the day, with the highest levels occurring at the lowest
- 10 frequencies.
- 11 While feeding, king crab (*Paralithodes camtschaticus*) produce impulsive sounds that appear to stimulate
- 12 movement by other crabs, including approach behavior (Tolstoganova 2002). King crab also appeared to 13 produce 'discomfort' sounds when environmental conditions were manipulated. These discomfort sounds
- *14* differ from the feeding sounds in terms of frequency range and pulse duration.
- 15 Snapping shrimp (*Synalpheus parneomeris*) are among the major sources of biological sound in temperate 16 and tropical shallow-water areas (Au and Banks 1998). By rapidly closing one of its frontal chelae 17 (claws), a snapping shrimp generates a forward jet of water and the cavitation of fast moving water 18 produces a sound. Both the sound and the jet of water may function in feeding and territorial behaviors of
- alpheidae shrimp. Measured source SPLs for snapping ship were 183-189 dB re 1 μ Pa·m_{p-p} and
- *20* extended over a frequency range of 2–200 kHz.

21 D.1.2 Sound Detection

- 22 There is considerable debate about the hearing capabilities of aquatic invertebrates. Whether they are able 23 to hear or not depends on how underwater sound and underwater hearing are defined. In contrast to the 24 situation in fish and marine mammals, no physical structures have been discovered in aquatic 25 invertebrates that are stimulated by the pressure component of sound. However, vibrations (i.e., mechan-26 ical disturbances of the water) are also characteristic of sound waves. Rather than being pressure-27 sensitive, aquatic invertebrates appear to be most sensitive to the vibrational component of sound 28 (Breithaupt 2002). Statocyst organs may provide one means of vibration detection for aquatic invert-29 ebrates.
- 30 More is known about the acoustic detection capabilities in decapod crustaceans than in any other marine31 invertebrate group, although cephalopod acoustic capabilities are now becoming a focus of study.
- Crustaceans appear to be most sensitive to sounds of low frequencies (i.e., <1000 Hz) (Budelmann 1992;
 Popper et al. 2001). A study by Lovell et al. (2005) suggests greater sensitivity of the prawn *Palaemon*
- *serratus* to low-frequency sound than previously thought. Lovell et al. (2006) showed that *P. serratus* is
- capable of detecting a 500 Hz tone regardless of the prawn's body size and the related number and size of
- 36 statocyst hair cells. Studies of American lobsters suggest that these crustaceans are more sensitive to
- 37 higher frequency sounds than previously realized (Pye and Watson 2004).
- 38 It is possible that statocyst hair cells of cephalopods are directionally sensitive in a way that is similar to 39 the responses of hair cells of the vertebrate vestibular and lateral line systems (Budelmann and
- *Heresponses of hair cells of the vertebrate vestibular and lateral line systems (Budelmann and Williamson 1994; Budelmann 1996). Kaifu et al. (2008) provided evidence that the cephalopod Octopus*
- *coellatus* detects particle motion with its statocyst. Studies by Packard et al. (1990), Rawizza (1995) and
- 42 Komak et al. (2005) have tested the sensitivities of various cephalopods to water-borne vibrations, some
- 43 of which were generated by low-frequency sound. Using the auditory brainstem response (ABR)

l approach, Hu et al. (2009) showed that auditory evoked potentials can be obtained in the frequency ranges

2 400 to 1500 Hz for the squid Sepiotheutis lessoniana and 400 to 1000 Hz for the octopus Octopus

- *vulgaris*, higher than frequencies previously observed to be detectable by cephalopods.
- 4 In summary, only a few studies have been conducted on the sensitivity of certain invertebrate species to 5 underwater sound. Available data suggest that they are capable of detecting vibrations but they do not
- 6 appear to be capable of detecting pressure fluctuations.

7 D.1.3 Potential Seismic Effects

8 In marine invertebrates, potential effects of exposure to sound can be categorized as pathological, 9 physiological, and behavioral. Pathological effects include lethal and sub-lethal injury to the animals, 10 physiological effects include temporary primary and secondary stress responses, and behavioral effects 11 refer to changes in exhibited behaviors (i.e., disturbance). The three categories should not be considered 12 as independent of one another and are likely interrelated in complex ways.

13 Pathological Effects

14 In water, acute injury or death of organisms as a result of exposure to sound appears to depend on two 15 features of the sound source: (1) the received peak pressure, and (2) the time required for the pressure to 16 rise and decay. Generally, the higher the received pressure and the less time it takes for the pressure to 17 rise and decay, the greater the chance of acute pathological effects. Considering the peak pressure and 18 rise/decay time characteristics of seismic airgun arrays used today, the associated pathological zone for 19 invertebrates would be expected to be small (i.e., within a few meters of the seismic source, at most). Few 20 studies have assessed the potential for pathological effects on invertebrates from exposure to seismic 21 sound.

22 The pathological impacts of seismic survey sound on marine invertebrates were investigated in a pilot 23 study on snow crabs (Chionoecetes opilio) (Christian et al. 2003, 2004). Under controlled field 24 experimental conditions, captive adult male snow crabs, egg-carrying female snow crabs, and fertilized 25 snow crab eggs were exposed to variable SPLs (191-221 dB re 1 µPa_{0-p}) and SELs (<130-187 dB re $1 \mu Pa^2 \cdot s$). Neither acute nor chronic (12 weeks post-exposure) mortality was observed for the adult crabs. 26 27 However, a significant difference in development rate was noted between the exposed and unexposed 28 fertilized eggs/embryos. The egg mass exposed to seismic energy had a higher proportion of less-29 developed eggs than did the unexposed mass. It should be noted that both egg masses came from a single 30 female and any measure of natural variability was unattainable (Christian et al. 2003, 2004).

31 In 2003, a collaborative study was conducted in the southern Gulf of St. Lawrence, Canada, to investigate 32 the effects of exposure to sound from a commercial seismic survey on egg-bearing female snow crabs 33 (DFOC 2004). This study had design problems that impacted interpretation of some of the results 34 (Chadwick 2004). Caged animals were placed on the ocean bottom at a location within the survey area 35 and at a location outside of the survey area. The maximum received SPL was ~195 dB re 1 µPa_{0-p}. The 36 crabs were exposed for 132 hr of the survey, equivalent to thousands of seismic shots of varying received 37 SPLs. The animals were retrieved and transferred to laboratories for analyses. Neither acute nor chronic 38 lethal or sub-lethal injury to the female crabs or crab embryos was indicated. DFOC (2004) reported that 39 some exposed individuals had short-term soiling of gills, antennules and statocysts, bruising of the 40 hepatopancreas and ovary, and detached outer membranes of oocytes. However, these differences could 41 not be linked conclusively to exposure to seismic survey sound. Boudreau et al. (2009) presented the 42 proceedings of a workshop held to evaluate the results of additional studies conducted to answer some

- questions arising from the original study discussed in DFOC (2004). Proceedings of the workshop did not
 include any more definitive conclusions regarding the original results.
- 3 Payne et al. (2007) recently conducted a pilot study of the effects of exposure to airgun sound on various
- 4 health endpoints of the American lobster. Adult lobsters were exposed either 20 to 200 times to 202 dB re
- 5 $1\mu Pa_{p-p}$ or 50 times to 227 dB re $1\mu Pa_{p-p}$, and then monitored for changes in survival, food consumption,
- 6 turnover rate, serum protein level, serum enzyme levels, and serum calcium level. Observations extended
- 7 over a period of a few days to several months. Results showed no delayed mortality or damage to the
- 8 mechanosensory systems associated with animal equilibrium and posture (as assessed by turnover rate).
- 9 In a field study, Pearson et al. (1994) exposed Stage II larvae of the Dungeness crab (*Cancer magister*) to 10 single discharges from a seven-airgun array and compared their mortality and development rates with
- 11 those of unexposed larvae. No statistically significant differences were found in immediate survival, long-
- 12 term survival, or time to molt between the exposed and unexposed larvae, even those exposed within 1 m
- 13 of the seismic source.
- 14 In 2001 and 2003, there were two incidents of multiple strandings of the giant squid (Architeuthis dux) on
- 15 the north coast of Spain, and there was speculation that the strandings were caused by exposure to
- 16 geophysical seismic survey sounds occurring at about the same time in the Bay of Biscay (Guerra et al. 2004). A total of nine giant squid, either stranded or moribund and floating at the surface, were collected
- 17 2004). A total of nine giant squid, either stranded or moribund and floating at the surface, were collected 18 at these times. However, Guerra et al. (2004) did not present any evidence that conclusively links the
- 19 giant squid strandings and floaters to seismic activity in the area. Based on necropsies of seven (six
- 20 females and one male) specimens, there was evidence of acute tissue damage. The authors speculated that
- 21 one female with extensive tissue damage was affected by the impact of acoustic waves. However, little is
- 22 known about the impact of strong airgun signals on cephalopods and the authors did not describe the
- 23 seismic sources, locations, and durations of the Bay of Biscay surveys. In addition, there were no
- 24 controls, the observations were circumstantial, and the examined animals had been dead long enough for
 - 25 commencement of tissue degradation.
 - McCauley et al. (2000a, b) exposed caged cephalopods to noise from a single 20-in³ airgun with maximum SPLs of >200 dB re 1 μ Pa_{0-p}. Statocysts were removed and preserved, but at the time of publication, results of the statocyst analyses were not available. No squid or cuttlefish mortalities were reported as a result of these exposures.
 - 30 Physiological Effects
 - Biochemical responses by marine invertebrates to acoustic exposure have also been studied to a limited degree. Such studies of stress responses could possibly provide some indication of the physiological consequences of acoustic exposure and perhaps any subsequent chronic detrimental effects. Stress responses could potentially affect animal populations by reducing reproductive capacity and adult abundance.
 - 36 Stress indicators in the haemolymph of adult male snow crabs were monitored immediately after exposure 37 of the animals to seismic survey sound (Christian et al. 2003, 2004) and at various intervals after 38 exposure. No significant acute or chronic differences were found between exposed and unexposed 39 animals in which various stress indicators (e.g., proteins, enzymes, cell type count) were measured.
 - 40 Payne et al. (2007), in their study of the effects of exposure of adult American lobsters to airgun sound,
 - 41 noted decreases in the levels of serum protein, particular serum enzymes and serum calcium, in the 42 haemolymph of animals exposed to the sound pulses. Statistically significant differences (P=0.05) were
 - noted in serum protein at 12 days post-exposure, serum enzymes at 5 days post-exposure, and serum

- *l* calcium at 12 days post-exposure. During the histological analysis conducted 4 months post-exposure,
- 2 Payne et al. (2007) noted more deposits of PAS-stained material, likely glycogen, in the hepatopancreas
- 3 of some of the exposed lobsters. Accumulation of glycogen could be due to stress or disturbance of
- *4* cellular processes.
- 5 Price (2007) found that blue mussels (*Mytilus edulis*) responded to a 10 kHz pure tone continuous signal
- 6 by decreasing respiration. Smaller mussels did not appear to react until exposed for 30 min whereas larger
- 7 mussels responded after 10 min of exposure. The oxygen uptake rate tended to be reduced to a greater
- δ degree in the larger mussels than in the smaller animals.
- 9 In general, the limited studies done to date on the effects of acoustic exposure on marine invertebrates 10 have not demonstrated any serious pathological and physiological effects.
- 11 Behavioral Effects
- 12 Christian et al. (2003) investigated the behavioral effects of exposure to airgun sound on snow crabs.
- 13 Eight animals were equipped with ultrasonic tags, released, and monitored for multiple days prior to
- 14 exposure and after exposure. Received SPL and SEL were ~191 dB re 1 μ Pa_{0-p} and <130 dB re 1 μ Pa²·s,
- *15* respectively. The crabs were exposed to 200 discharges over a 33-min period. None of the tagged animals
- 16 left the immediate area after exposure to the seismic survey sound. Five animals were captured in the
- 17 snow crab commercial fishery the following year, one at the release location, one 35 km from the release
- 18 location, and three at intermediate distances from the release location.
- 19 Another study approach used by Christian et al. (2003) involved monitoring snow crabs with a remote
- 20 video camera during their exposure to airgun sound. The caged animals were placed on the ocean bottom
- 21 at a depth of 50 m. Received SPL and SEL were ~202 dB re 1 μ Pa_{0-p} and 150 dB re 1 μ Pa²·s,
- 22 respectively. The crabs were exposed to 200 discharges over a 33-min period. They did not exhibit any
- *23* overt startle response during the exposure period.
- Christian et al. (2003) also investigated the pre- and post-exposure catchability of snow crabs during a commercial fishery. Received SPLs and SELs were not measured directly and likely ranged widely considering the area fished. Maximum SPL and SEL were likely similar to those measured during the telemetry study. There were seven pre-exposure and six post-exposure trap sets. Unfortunately, there was considerable variability in set duration because of poor weather. Results indicated that the catch-per-uniteffort did not decrease after the crabs were exposed to seismic survey sound.
- 30 Parry and Gason (2006) statistically analyzed data related to rock lobster (*Jasus edwardsii*) commercial
- *catches and seismic surveying in Australian waters from 1978 to 2004. They did not find any evidence*
- *32* that lobster catch rates were affected by seismic surveys.
- 33 Caged female snow crabs exposed to airgun sound associated with a recent commercial seismic survey 34 conducted in the southern Gulf of St. Lawrence, Canada, exhibited a higher rate of 'righting' than those 35 crabs not exposed to seismic survey sound (J. Payne, Research Scientist, DFOC, St. John's, 36 Newfoundland, pers. comm.). 'Righting' refers to a crab's ability to return itself to an upright position 37 after being placed on its back. Christian et al. (2003) made the same observation in their study.
- Payne et al. (2007), in their study of the effects of exposure to airgun sound on adult American lobsters,noted a trend for increased food consumption by the animals exposed to seismic sound.
- 40 Andriguetto-Filho et al. (2005) attempted to evaluate the impact of seismic survey sound on artisanal
- 41 shrimp fisheries off Brazil. Bottom trawl yields were measured before and after multiple-day shooting of
- 42 an airgun array. Water depth in the experimental area ranged between 2 and 15 m. Results of the study

did not indicate any significant deleterious impact on shrimp catches. Anecdotal information from
Newfoundland, Canada, indicated that catch rates of snow crabs showed a significant reduction
immediately following a pass by a seismic survey vessel (G. Chidley, Newfoundland fisherman, pers.
comm.). Additional anecdotal information from Newfoundland indicated that a school of shrimp observed
via a fishing vessel sounder shifted downwards and away from a nearby seismic airgun sound source (H.

6 Thorne, Newfoundland fisherman, pers. comm.). This observed effect was temporary.

7 Caged brown shrimp (*Crangon crangon*) reared under different acoustical conditions exhibited differ-8 ences in aggressive behavior and feeding rate (Lagardère 1982). Those exposed to a continuous sound 9 source showed more aggression and less feeding behavior. It should be noted that behavioral responses by 10 caged animals may differ from behavioral responses of animals in the wild.

- 11 McCauley et al. (2000a, b) provided the first evidence of the behavioral response of southern calamari 12 squid (*Sepioteuthis australis*) exposed to seismic survey sound. They reported on the exposure of caged 13 cephalopods (50 squid and 2 cuttlefish) to noise from a single 20-in³ airgun. The cephalopods were 14 exposed to both stationary and mobile sound sources. The two-run total exposure times during the three 15 trials ranged from 69 to 119 min. at a firing rate of once every 10–15 s. The maximum SPL was >200 dB 16 re 1 μ Pa_{0-p}. Some of the squid fired their ink sacs apparently in response to the first shot of one of the 17 trials and then moved quickly away from the airgun. In addition to the above-described startle responses,
- 18 some squid also moved towards the water surface as the airgun approached. McCauley et al. (2000a, b)
- 19 reported that the startle and avoidance responses occurred at a received SPL of 174 dB re 1 μ Pa_{rms}. They
- 20 also exposed squid to a ramped approach-depart airgun signal whereby the received SPL was gradually
- increased over time. No strong startle response (i.e., ink discharge) was observed, but alarm responses,including increased swimming speed and movement to the surface, were observed once the received SPL
- reached a level in the 156–161 dB re 1 μ Pa_{rms} range.
- 24 Komak et al. (2005) also reported the results of a study of cephalopod behavioral responses to local water 25 movements. In this case, juvenile cuttlefish (Sepia officinalis) exhibited various behavioral responses to 26 local sinusoidal water movements of different frequencies between 0.01 and 1000 Hz. These responses 27 included body pattern changing, movement, burrowing, reorientation, and swimming. Similarly, the 28 behavioral responses of the octopus (Octopus ocellatus) to non-impulse sound have been investigated by 29 Kaifu et al. (2007). The sound stimuli, reported as having levels 120 dB re 1 μ Pa rms, were at various frequencies: 50, 100, 150, 200 and 1000 Hz. The respiratory activity of the octopus changed when 30 31 exposed to sound in the 50–150 Hz range but not for sound at 200–1,000 Hz. Respiratory suppression by 32 the octopus might have represented a means of escaping detection by a predator.
- Low-frequency sound (<200 Hz) has also been used as a means of preventing settling/fouling by aquatic invertebrates such as zebra mussels (*Dreissena polymorpha*) (Donskoy and Ludyanskiy 1995) and balanoid barnacles (*Balanus* sp.) (Branscomb and Rittschof 1984). Price (2007) observed that blue mussels closed their valves upon exposure to 10 kHz pure tone continuous sound.

Although not demonstrated in the invertebrate literature, masking can be considered a potential effect of anthropogenic underwater sound on marine invertebrates. Some invertebrates are known to produce sounds (Au and Banks 1998; Tolstoganova 2002; Latha et al. 2005). The functionality and biological relevance of these sounds are not understood (Jeffs et al. 2003, 2005; Lovell et al. 2005; Radford et al. 2007). If some of the sounds are of biological significance to some invertebrates, then masking of those sounds or of sounds produced by predators, at least the particle displacement component, could potentially have adverse effects on marine invertebrates. However, even if masking does occur in some 1 invertebrates, the intermittent nature of airgun sound is expected to result in less masking effect than would occur with continuous sound. 2

3 **D.2** FISH

4 **D.2.1 Acoustic Capabilities**

5 Sensory systems - like those that allow for hearing - provide information about an animal's physical, 6 biological, and social environments, in both air and water. Extensive work has been done to understand 7 the structures, mechanisms, and functions of animal sensory systems in aquatic environments (Atema et 8 al. 1988; Kapoor and Hara 2001; Collin and Marshall 2003). All fish species have hearing and skin-based 9 mechanosensory systems (inner ear and lateral line systems, respectively) that provide information about 10 their surroundings (Fav and Popper 2000). Fav (2009) and some others refer to the ambient sounds to 11 which fishes are exposed as 'underwater soundscapes'. Anthropogenic sounds can have important 12 negative consequences for fish survival and reproduction if they disrupt an individual's ability to sense its 13 soundscape, which often tells of predation risk, prey items, or mating opportunities. Potential negative effects include masking of key environmental sounds or social signals, displacement of fish from their 14

15 habitat, or interference with sensory orientation and navigation.

16 Fish hearing via the inner ear is typically restricted to low frequencies. As with other vertebrates, fish hearing involves a mechanism whereby the beds of hair cells (Howard et al. 1988; Hudspeth and Markin 17

1994) located in the inner ear are mechanically affected and cause a neural discharge (Popper and Fay 18

19 1999). At least two major pathways for sound transmittance between sound source and the inner ear have

20 been identified for fishes. The most primitive pathway involves direct transmission to the inner ear's

21 otolith, a calcium carbonate mass enveloped by sensory hairs. The inertial difference between the dense

22 otolith and the less-dense inner ear causes the otolith to stimulate the surrounding sensory hair cells. This

23 motion differential is interpreted by the central nervous system as sound.

24 The second transmission pathway between sound source and the inner ear of fishes is via the swim 25 bladder, a gas-filled structure that is much less dense than the rest of the fish's body. The swim bladder, 26 being more compressible and expandable than either water or fish tissue, will differentially contract and 27 expand relative to the rest of the fish in a sound field. The pulsating swim bladder transmits this 28 mechanical disturbance directly to the inner ear (discussed below). Such a secondary source of sound 29 detection may be more or less effective at stimulating the inner ear depending on the amplitude and 30 frequency of the pulsation, and the distance and mechanical coupling between the swim bladder and the 31 inner ear (Popper and Fay 1993).

32 A recent paper by Popper and Fay (2010) discusses the designation of fishes based on sound detection 33 capabilities. They suggest that the designations 'hearing specialist' and 'hearing generalist' no longer be 34 used for fishes because of their vague and sometimes contradictory definitions, and that there is instead a 35 range of hearing capabilities across species that is more like a continuum, presumably based on the 36 relative contributions of pressure to the overall hearing capabilities of a species.

37 According to Popper and Fay (2010), one end of this continuum is represented by fishes that only detect 38 particle motion because they lack pressure-sensitive gas bubbles (e.g., swim bladder). These species 39 include elasmobranchs (e.g., sharks) and jawless fishes, and some teleosts including flatfishes. Fishes at 40 this end of the continuum are typically capable of detecting sound frequencies below 1,500 Hz.

41 The other end of the fish hearing continuum is represented by fishes with highly specialized otophysic connections between pressure receptive organs, such as the swim bladder, and the inner ear. These fishes 42 43 include some squirrelfish, mormyrids, herrings, and otophysan fishes (freshwater fishes with Weberian 1 apparatus, an articulated series of small bones that extend from the swim bladder to the inner ear). Rather 2 than being limited to 1.5 kHz or less in hearing, these fishes can typically hear up to several kHz. One 3 group of fish in the anadromous herring sub-family Alosinae (shads and menhaden) can detect sounds to 4 well over 180 kHz (Mann et al. 1997, 1998, 2001). This may be the widest hearing range of any 5 vertebrate that has been studied to date. While the specific reason for this very high frequency hearing is 6 not totally clear, there is strong evidence that this capability evolved for the detection of the ultrasonic 7 sounds produced by echolocating dolphins to enable the fish to detect, and avoid, predation (Mann et al. 8 1997; Plachta and Popper 2003).

9 All other fishes have hearing capabilities that fall somewhere between these two extremes of the 10 continuum. Some have unconnected swim bladders located relatively far from the inner ear (e.g., salmonids, tuna) while others have unconnected swim bladders located relatively close to the inner ear 11 12 (e.g., Atlantic cod, Gadus morhua). There has also been the suggestion that Atlantic cod can detect 38 13 kHz (Astrup and Møhl 1993). However, the general consensus was that this was not hearing with the ear; 14 probably the fish were responding to exceedingly high pressure signals from the 38-kHz source through

15 some other receptor in the skin, such as touch receptors (Astrup and Møhl 1998).

16 It is important to recognize that the swim bladder itself is not a sensory end organ, but rather an 17 intermediate part of the sound pathway between sound source and the inner ear of some fishes. The inner 18 ear of fishes is ultimately the organ that translates the particle displacement component into neural signals 19 for the brain to interpret as sound.

- 20 A third mechanosensory pathway found in most bony fishes and elasmobranchs (i.e., cartilaginous fishes)
- 21 involves the lateral line system. It too relies on sensitivity to water particle motion. The basic sensory unit
- 22 of the lateral line system is the neuromast, a bundle of sensory and supporting cells whose projecting
- 23 cilia, similar to those in the ears, are encased in a gelatinous cap. Neuromasts detect distorted sound
- waves in the immediate vicinity of fishes. Generally, fishes use the lateral line system to detect the 24
- 25 particle displacement component of low frequency acoustic signals (up to 160 to 200 Hz) over a distance 26 of one to two body lengths. The lateral line is used in conjunction with other sensory systems, including
- 27 hearing (Sand 1981; Coombs and Montgomery 1999).

28 **D.2.2** Potential Effects on Fishes

29 Review papers on the effects of anthropogenic sources of underwater sound on fishes have been 30 published recently (Popper 2009; Popper and Hastings 2009a, b). These papers consider various sources of anthropogenic sound, including seismic airguns. For the purposes of this review, only the effects of 31

32 seismic airgun sound are considered.

33 Marine Fishes

34 Evidence for airgun-induced damage to fish ears has come from studies using pink snapper (Pagrus 35 auratus) (McCauley et al. 2000a, b, 2003). In these experiments, fish were caged and exposed to the 36 sound of a single moving seismic airgun every 10 s over a period of 1 h and 41 min. The source SPL at 1 37 m was about 223 dB re 1 μ Pa·m_{p-p}, and the received SPLs ranged from 165 to 209 dB re 1 μ Pa_{p-p}. The 38 sound energy was highest over the 20-70 Hz frequency range. The pink snapper were exposed to more 39 than 600 airgun discharges during the study. In some individual fish, the sensory epithelium of the inner 40 ear sustained extensive damage as indicated by ablated hair cells. Damage was more extensive in fish 41 examined 58 days post-exposure compared to those examined 18 h post-exposure. There was no evidence 42 of repair or replacement of damaged sensory cells up to 58 days post-exposure. McCauley et al. (2000a, 43 b, 2003) included the following caveats in the study reports: (1) fish were caged and unable to swim

- *l* away from the seismic source, (2) only one species of fish was examined, (3) the impact on the ultimate
- survival of the fish is unclear, and (4) airgun exposure specifics required to cause the observed damage
 were not obtained (i.e., a few high SPL signals or the cumulative effect of many low to moderate SPL
- 4 signals).

The fish exposed to sound from a single airgun in this study also exhibited startle responses to short range start up and high-level airgun signals (i.e., with received SPLs of 182 to 195 dB re 1 μ Pa_{rms} (McCauley et al. 2000a, b). Smaller fish were more likely to display a startle response. Responses were observed above received SPLs of 156 to 161 dB re 1 μ Pa_{rms}. The occurrence of both startle response (classic C-turn response) and alarm responses (e.g., darting movements, flash school expansion, fast swimming) decreased over time. Other observations included downward distributional shift that was restricted by the 10 m x 6 m x 3 m cages, increase in swimming speed, and the formation of denser aggregations. Fish behavior expansion to pre-exposure state 15, 20 min after constant of saisming firing

12 behavior appeared to return to pre-exposure state 15–30 min after cessation of seismic firing.

13 Pearson et al. (1992) investigated the effects of seismic airgun sound on the behavior of captive 14 rockfishes (Sebastes sp.) exposed to the sound of a single stationary airgun at a variety of distances. The 15 airgun used in the study had a source SPL at 1 m of 223 dB re 1 µPa · m_{0-p}, and measured received SPLs ranged from 137 to 206 dB re 1 µPa_{0-p}. The authors reported that rockfishes reacted to the airgun sounds 16 17 by exhibiting varying degrees of startle and alarm responses, depending on the species of rockfish and the 18 received SPL. Startle responses were observed at a minimum received SPL of 200 dB re 1 µPa_{0-p}, and 19 alarm responses occurred at a minimum received SPL of 177 dB re 1 µPa_{0-p}. Other observed behavioral 20 changes included the tightening of schools, downward distributional shift, and random movement and 21 orientation. Some fishes ascended in the water column and commenced to mill (i.e., "eddy") at increased 22 speed, while others descended to the bottom of the enclosure and remained motionless. Pre-exposure 23 behavior was reestablished from 20 to 60 min after cessation of seismic airgun discharge. Pearson et al. 24 (1992) concluded that received SPL thresholds for overt and more subtle rockfish behavioral response are 25 180 dB re 1 μ Pa_{0-p} and 161 dB re 1 μ Pa_{0-p}, respectively.

26 Using an experimental hook and line fishery approach, Skalski et al. (1992) studied the potential effects 27 of seismic airgun sound on the distribution and catchability of rockfishes. The source SPL of the single 28 airgun used in the study was 223 dB re 1 μ Pa·m $_{0-p}$, and the received SPLs at the bases of the rockfish 29 aggregations ranged from 186 to 191 dB re 1 µPa_{0-p}. Characteristics of the fish aggregations were 30 assessed using echosounders. During long-term stationary seismic airgun discharge, there was an overall 31 downward shift in fish distribution. The authors also observed a significant decline in total catch of 32 rockfishes during seismic discharge. It should be noted that this experimental approach was quite 33 different from an actual seismic survey, in that duration of exposure was much longer.

34 In another study, caged European sea bass (*Dicentrarchus labrax*) were exposed to multiple discharges 35 from a moving seismic airgun array with a source SPL of about 256 dB re 1 μ Pa·m _{0-p} (unspecified 36 measure type) (Santulli et al. 1999). The airguns were discharged every 25 s during a 2-h period. The 37 minimum distance between fish and seismic source was 180 m. The authors did not indicate any observed 38 pathological injury to the sea bass. Blood was collected from both exposed fish (6 h post-exposure) and 39 control fish (6 h pre-exposure) and subsequently analyzed for cortisol, glucose, and lactate levels. Levels 40 of cortisol, glucose, and lactate were significantly higher in the sera of exposed fish compared to sera of 41 control fish. The elevated levels of all three chemicals returned to pre-exposure levels within 72 h of 42 exposure (Santulli et al. 1999).

43 Santulli et al. (1999) also used underwater video cameras to monitor fish response to seismic airgun44 discharge. Resultant video indicated slight startle responses by some of the sea bass when the seismic

1 airgun array discharged as far as 2.5 km from the cage. The proportion of sea bass that exhibited startle 2 response increased as the airgun sound source approached the cage. Once the seismic array was within

3 180 m of the cage, the sea bass were densely packed at the middle of the enclosure, exhibiting random

4 orientation, and appearing more active than they had been under pre-exposure conditions. Normal

5 behavior resumed about 2 h after airgun discharge nearest the fish (Santulli et al. 1999).

6 Boeger et al. (2006) reported observations of coral reef fishes in field enclosures before, during and after 7 exposure to seismic airgun sound. This Brazilian study used an array of eight airguns that was presented 8 to the fishes as both a mobile sound source and a static sound source. Minimum distances between the 9 sound source and the fish cage ranged from 0 to 7 m. Received sound levels were not reported by Boeger et al. (2006). Neither mortality nor external damage to the fishes was observed in any of the experimental 11 scenarios. Most of the airgun array discharges resulted in startle responses although these behavioral 12 scenarios.

12 changes lessened with repeated exposures, suggesting habituation.

13 Chapman and Hawkins (1969) investigated the reactions of free ranging whiting (silver hake) (Merluccius

bilinearis), to an intermittently discharging stationary airgun with a source SPL of 220 dB re 1 μ Pa·m_{0-p}. Received SPLs were estimated to be 178 dB re 1 μ Pa_{0-p}. The whiting were monitored with an echosounder. Prior to any airgun discharge, the fish were located at a depth range of 25 to 55 m. In

apparent response to the airgun sound, the fish descended, forming a compact layer at depths greater than

18 55 m. After an hour of exposure to the airgun sound, the fish appeared to have habituated as indicated by

19 their return to the pre-exposure depth range, despite the continuing airgun discharge. Airgun discharge

20 ceased for a time and upon its resumption, the fish again descended to greater depths, indicating only

21 temporary habituation.

22 Hassel et al. (2003, 2004) studied the potential effects of exposure to airgun sound on the behavior of 23 captive lesser sandeel (Ammodvtes marinus). Depth of the study enclosure used to hold the sandeel was 24 about 55 m. The moving airgun array had an estimated source SPL of 256 dB re 1 µPa·m (unspecified 25 measure type). Received SPLs were not measured. Exposures were conducted over a 3-day period in a 10 26 $km \times 10$ km area with the cage at its center. The distance between airgun array and fish cage ranged from 27 55 m when the array was overhead to 7.5 km. No mortality attributable to exposure to the airgun sound 28 was noted. Behavior of the fish was monitored using underwater video cameras, echosounders, and 29 commercial fishery data collected close to the study area. The approach of the seismic vessel appeared to 30 cause an increase in tail-beat frequency although the sandeels still appeared to swim calmly. During 31 seismic airgun discharge, many fish exhibited startle responses, followed by flight from the immediate 32 area. The frequency of occurrence of startle response seemed to increase as the operating seismic array 33 moved closer to the fish. The sandeels stopped exhibiting the startle response once the airgun discharge 34 ceased. The sandeel tended to remain higher in the water column during the airgun discharge, and none of 35 them were observed burying themselves in the soft substrate. The commercial fishery catch data were

36 inconclusive with respect to behavioral effects.

Various species of demersal fishes, blue whiting, and some small pelagic fishes were exposed to a moving seismic airgun array with a source SPL of about 250 dB re 1 μ Pa·m (unspecified measure type) (Dalen and Knutsen 1986). Received SPLs estimated using the assumption of spherical spreading ranged from 200 to 210 dB re 1 μ Pa (unspecified measure type). Seismic sound exposures were conducted every 10 s during a one week period. The authors used echosounders and sonars to assess the pre- and post-exposure fish distributions. The acoustic mapping results indicated a significant decrease in abundance of demersal

43 fish (36%) after airgun discharge but comparative trawl catches did not support this. Non-significant

- *i* reductions in the abundances of blue whiting and small pelagic fish were also indicated by post-exposure*i* acoustic mapping.
- 3 La Bella et al. (1996) studied the effects of exposure to seismic airgun sound on fish distribution using
- 4 echosounder monitoring and changes in catch rate of hake by trawl, and clupeoids by gill netting. The
- 5 seismic array used was composed of 16 airguns and had a source SPL of 256 dB re 1 μ Pa · m _{0-p}. The shot
- 6 interval was 25 s, and exposure durations ranged from 4.6 to 12 h. Horizontal distributions did not appear
- 7 to change as a result of exposure to seismic discharge, but there was some indication of a downward shift
- 8 in the vertical distribution. The catch rates during experimental fishing did not differ significantly
- *9* between pre- and post-seismic fishing periods.
- 10 Wardle et al. (2001) used video and telemetry to make behavioral observations of marine fishes (primarily 11 juvenile saithe, adult pollock, juvenile cod, and adult mackerel) inhabiting an inshore reef off Scotland 12 before, during, and after exposure to discharges of a stationary airgun. The received SPLs ranged from 13 about 195 to 218 dB re 1 µPa_{0-p}. Pollock did not move away from the reef in response to the seismic 14 airgun sound, and their diurnal rhythm did not appear to be affected. However, there was an indication of 15 a slight effect on the long-term day-to-night movements of the pollock. Video camera observations indicated that fish exhibited startle responses ("C-starts") to all received levels. There were also 16 17 indications of behavioral responses to visual stimuli. If the seismic source was visible to the fish, they fled
- *18* from it. However, if the source was not visible to the fish, they often continued to move toward it.
- *19* The potential effects of exposure to seismic sound on fish abundance and distribution were also investigated by Slotte et al. (2004). Twelve days of seismic survey operations spread over a period of 1
- mivestigated by Stote et al. (2004). Twelve days of seismic survey operations spread over a period of T month used a seismic airgun array with a source SPL of 222.6 dB re 1 μ Pa·m_{p-p}. The SPLs received by
- the fish were not measured. Acoustic surveys of the local distributions of various kinds of pelagic fish,
- *including herring, blue whiting, and mesopelagic species, were conducted during the seismic surveys.*
- 24 There was no strong evidence of short-term horizontal distributional effects. With respect to vertical
- 25 distribution, blue whiting and mesopelagics were distributed deeper (20 to 50 m) during the seismic
- 26 survey compared to pre-exposure. The average densities of fish aggregations were lower within the
- 27 seismic survey area, and fish abundances appeared to increase in accordance with increasing distance
- 28 from the seismic survey area.
- 29 Fertilized capelin (Mallotus villosus) eggs and monkfish (Lophius americanus) larvae were exposed to
- 30 seismic airgun sound and subsequently examined and monitored for possible effects of the exposure
- 31 (Payne et al. 2009). The laboratory exposure studies involved a single airgun. Approximate received SPLs
- 32 measured in the capelin egg and monkfish larvae exposures were 199 to 205 dB re 1 μ Pa_{p-p} and 205 dB re
- $1 \mu Pa_{p-p}$, respectively. The capelin eggs were exposed to either 10 or 20 airgun discharges, and the monkfish larvae were exposed to either 10 or 30 discharges. No statistical differences in
- 35 mortality/morbidity between control and exposed subjects were found at 1 to 4 days post-exposure in any 36 of the exposure trials for either the capelin eggs or the monkfish larvae
- *36* of the exposure trials for either the capelin eggs or the monkfish larvae.
- 37 In uncontrolled experiments, Kostyvchenko (1973) exposed the eggs of numerous fish species (anchovy,
- 38 red mullet, crucian carp, blue runner) to various sound sources, including seismic airguns. With the
- *39* seismic airgun discharge as close as 0.5 m from the eggs, over 75% of them survived the exposure. Egg
- 40 survival rate increased to over 90% when placed 10 m from the airgun sound source. The range of
- 41 received SPLs was about 215 to 233 dB re 1 μ Pa_{0-p}.
- 42 Eggs, yolk sac larvae, post-yolk sac larvae, post-larvae, and fry of various commercially important fish
 43 species (cod, saithe, herring, turbot, and plaice) were exposed to received SPLs ranging from 220 to 242
- 44 dB re 1 μPa (unspecified measure type) (Booman et al. 1996). These received levels corresponded to

- *l* exposure distances ranging from 0.75 to 6 m. The authors reported some cases of injury and mortality but
- 2 most of these occurred as a result of exposures at very close range (i.e., <15 m). The rigor of anatomical
- *3* and pathological assessments was questionable.
- 4 Saetre and Ona (1996) applied a "worst-case scenario" mathematical model to investigate the effects of
- 5 seismic sound on fish eggs and larvae. They concluded that mortality rates caused by exposure to seismic
- 6 airgun sound are so low compared to the natural mortality that the impact of seismic surveying on
- 7 recruitment to a fish stock must be regarded as insignificant.
- 8 Freshwater Fishes
- 9 Popper et al. (2005) tested the hearing sensitivity of three Mackenzie River fish species after exposure to 10 five discharges from a seismic airgun. The mean received peak SPL was 205 to 209 dB re 1 μ Pa per 11 discharge, and the approximate mean received SEL was 176 to 180 dB re 1 μ Pa² · s per discharge. While 12 the broad whitefish showed no TTS as a result of the exposure, adult northern pike and lake chub 13 exhibited TTSs of 10 to 15 dB, followed by complete recovery within 24 h of exposure. The same 14 animals were also examined to determine whether there were observable effects on the sensory cells of 15 the inner ear as a result of exposure to seismic sound (Song et al. 2008). No damage to the ears of the
- *16* fishes was found, including those that exhibited TTS.
- 17 In another part of the same Mackenzie River project, Jorgenson and Gyselman (2009) investigated the
- 18 behavioral responses of arctic riverine fishes to seismic airgun sound. They used hydroacoustic survey
- 19 techniques to determine whether fish behavior upon exposure to airgun sound can either mitigate or
- 20 enhance the potential impact of the sound. The study indicated that fish behavioral characteristics were
- 21 generally unchanged by the exposure to airgun sound. The tracked fish did not exhibit herding behavior in
- 22 front of the mobile airgun array and, therefore, were not exposed to sustained high sound levels.
- 23 Anadromous Fishes
- 24 In uncontrolled experiments using a very small sample of different groups of young salmonids, including
- 25 Arctic cisco, fish were caged and exposed to various types of sound. One sound type was either a single
- *26* firing or a series of four firings 10 to 15 s apart of a 300-in³ seismic airgun at 2000 to 2200 psi (Falk and
- 27 Lawrence 1973). Swim bladder damage was reported but no mortality was observed when fish were
- 28 exposed within 1 to 2 m of an airgun source with source level, as estimated by Turnpenny and Nedwell
- 29 (1994), of ~230 dB re 1 μ Pa·m (unspecified measure).
- 30 Thomsen (2002) exposed rainbow trout and Atlantic salmon held in aquaculture enclosures to the sounds 31 from a small airgun array. Received SPLs were 142 to 186 dB re 1 μ Pa_{p-p}. The fish were exposed to 124 32 pulses over a 3-day period. In addition to monitoring fish behavior with underwater video cameras, the 33 authors also analyzed cod and haddock catch data from a longline fishing vessel operating in the
- *immediate area.* Only 8 of the 124 shots appeared to evoke behavioral reactions by the salmonids, but
- *35* overall impacts were minimal. No fish mortality was observed during or immediately after exposure. The
- 36 author reported no significant effects on cod and haddock catch rates, and the behavioral effects were
- 37 hard to differentiate from normal behavior.
- 38 Weinhold and Weaver (1972, cited in Turnpenny et al. 1994) exposed caged coho salmon smolts to
- 39 impulses from 330 and 660-in³ airguns at distances ranging from 1 to 10 m, resulting in received levels
- 40 estimated at \sim 214 to 216 dB (units not given). No lethal effects were observed.
- 41 It should be noted that, in a recent and comprehensive review, Hastings and Popper (2005) take issue with 42 many of the authors cited above for problems with experimental design and execution, measurements, and

1 interpretation. Hastings and Popper (2005) deal primarily with possible effects of pile-driving sounds (which, like airgun sounds, are impulsive and repetitive). However, that review provides an excellent and

critical review of the impacts to fish from other underwater anthropogenic sounds.

4 D.2.3 Indirect Effects on Fisheries

5 The most comprehensive experimentation on the effects of seismic airgun sound on catchability of fishes 6 was conducted in the Barents Sea by Engås et al. (1993, 1996). They investigated the effects of seismic 7 airgun sound on distributions, abundances, and catch rates of cod and haddock using acoustic mapping 8 and experimental fishing with trawls and longlines. The maximum source SPL was about 248 dB re 9 $1 \mu Pa \cdot m_{0-p}$ based on back-calculations from measurements collected via a hydrophone at depth 80 m. 10 Nomeasurements of the received SPLs were made. Davis et al. (1998) estimated the received SPL at the sea bottom immediately below the array and at 18 km from the array to be 205 dB re 1 μ Pa_{0-p} and 178 dB 11 12 re 1 µPa_{0-p}, respectively. Engås et al. (1993, 1996) concluded that there were indications of distributional 13 change during and immediately following the seismic airgun discharge (45 to 64% decrease in acoustic 14 density according to sonar data). The lowest densities were observed within 9.3 km of the seismic 15 discharge area. The authors indicated that trawl catches of both cod and haddock declined after the seismic operations. While longline catches of haddock also showed decline after seismic airgun 16 17 discharge, those for cod increased.

18 Løkkeborg (1991), Løkkeborg and Soldal (1993), and Dalen and Knutsen (1986) also examined the 19 effects of seismic airgun sound on demersal fish catches. Løkkeborg (1991) examined the effects on cod 20 catches. The source SPL of the airgun array used in his study was 239 dB re 1 μ Pa·m (unspecified 21 measure type), but received SPLs were not measured. Approximately 43 h of seismic airgun discharge 22 occurred during an 11-day period, with a 5-s interval between pulses. Catch rate decreases ranging from 23 55 to 80% within the seismic survey area were observed. This apparent effect persisted for at least 24 h

24 within about 10 km of the survey area.

Turnpenny et al. (1994) examined results of these studies as well as the results of other studies on rockfish. They used rough estimations of received SPLs at catch locations and concluded that catchability is reduced when received SPLs exceed 160 to 180 dB re 1 μ Pa_{0-p}. They also concluded that reaction thresholds of fishes lacking a swim bladder (e.g., flatfish) would likely be about 20 dB higher. Given the considerable variability in sound transmission loss between different geographic locations, the SPLs that were assumed in these studies were likely quite inaccurate.

- 30 were assumed in these studies were likely quite inaccurate.
- 31 Turnpenny and Nedwell (1994) also reported on the effects of seismic airgun discharge on inshore bass 32 fisheries in shallow U.K. waters (5 to 30 m deep). The airgun array used had a source level of 250 dB re 1
- μ Pa·m_{0-p}. Received levels in the fishing areas were estimated to be 163–191 dB re 1 μ Pa_{0-p}. Using fish
- 34 tagging and catch record methodologies, they concluded that there was not any distinguishable migration
- 35 from the ensonified area, nor was there any reduction in bass catches on days when seismic airguns were
- 36 discharged. The authors concluded that effects on fisheries would be smaller in shallow nearshore waters
- 37 than in deep water because attenuation of sound is more rapid in shallow water.
- 38 Skalski et al. (1992) used a 100-in³ airgun with a source level of 223 dB re 1 μ Pa·m_{0-p} to examine the
- 39 potential effects of airgun sound on the catchability of rockfishes. The moving airgun was discharged
- along transects in the study fishing area, after which a fishing vessel deployed a set line, ran three echo-
- 41 sounder transects, and then deployed two more set lines. Each fishing experiment lasted 1 h 25 min.
- 42 Received SPLs at the base of the rockfish aggregations ranged from 186 to 191 dB re 1 μ Pa_{0-p}. The catch-
- 43 per-unit-effort (CPUE) for rockfish declined on average by 52.4% when the airguns were operating.
- 44 Skalski et al. (1992) believed that the reduction in catch resulted from a change in behavior of the fishes.

1 The fish schools descended towards the bottom and their swimming behavior changed during airgun

- 2 discharge. Although fish dispersal was not observed, the authors hypothesized that it could have occurred
- 3 at a different location with a different bottom type. Skalski et al. (1992) did not continue fishing after
- 4 cessation of airgun discharge. They speculated that CPUE would quickly return to normal in the experi-
- mental area, because fish behavior appeared to normalize within minutes of cessation of airgun discharge.
 However, in an area where exposure to airgun sound might have caused the fish to disperse, the authors
- 7 suggested that a lower CPUE might persist for a longer period.

8 European sea bass were exposed to sound from seismic airgun arrays with a source SPL of 262 dB re 1 9 μ Pa·m_{0-p} (Pickett et al. 1994). The seismic survey was conducted over a period of 4 to 5 months. The 10 study was intended to investigate the effects of seismic airgun discharge on inshore bass fisheries. 11 Information was collected through a tag and release program, and from the logbooks of commercial 12 fishermen. Most of the 152 recovered fish from the tagging program were caught within 10 km of the 13 release site, and it was suggested that most of these bass did not leave the area for a prolonged period. 14 With respect to the commercial fishery, no significant changes in catch rate were observed (Pickett et al.

15 1994).

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