Exhibit B.2.a
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Stress and delayed mortality induced in Pacific halibut by exposure to hooking, net towing, elevated sea water temperature and air: implications for management of bycatch

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Abstract.- The levels of stress and eventual mortality in Pacific halibut *Hippoglossus stenolepis* that resulted from simulated capture by hooking or towing in a net, followed by abrupt exposure to elevated sea water temperature and air were determined under laboratory conditions. Abrupt exposure to 16°C sea water and air after either method of capture increased capture-induced stress with mortality at 33% for hooked fish and 78 % for fish towed in a net, with these deaths occurring up to 30 d after experimental treatment, suggesting that delayed mortality should be considered in any study of Pacific halibut bycatch mortality. Stress induced by hooking or towing in a net followed by air exposure was reflected in cessation of negative phototaxis and feeding, both of which resumed after 5 d with no mortality occurring. The results of this study clearly show that seasonal increases in temperature associated with thermoclines and deck conditions have the potential for markedly increasing mortality of Pacific halibut that might otherwise survive capture and release in colder seasons. Strategies for effective management of Pacific halibut bycatch need to include consideration of seasonal temperature elevation and how this factor might increase mortality.

Pacific halibut *Hippoglossus stenolepis* form the basis for an economically important fishery along the west coast of the United States and Canada and throughout Alaskan waters. Fishing regulations require that all Pacific halibut bycatch be released back into the ocean with the estimation of the survival of released fish being a major concern for the management of this species. While a system of visual classification for Pacific halibut condition and potential mortality has been implemented, it is not clear how accurately this system may predict mortality of fish that are released after capture (Hoag 1975; Kaimmer and Trumble 1998). Modification of this condition index would require better estimates of Pacific halibut delayed mortality as it is related to fishing practices. Previous in situ studies of delayed mortality in Atlantic halibut *Hippoglossus hippoglossus* (Neilson et al. 1989) and Pacific halibut (Oddsson et al. 1994; Trumble et al. 1995; Kaimmer and Trumble 1998) have held fish in nets or tanks for 2 - 10 d after experimental treatment. However, studies on other marine fish species have shown that delayed mortality can occur up to 14 d after capture (Lockwood et al. 1983;Van Beek et al. 1990; Soldal et al. 1993; Olla et al. 1998). These results suggest that in situ measurement of delayed mortality should be made over an extended period of time, even though this may generally be difficult under ocean conditions. An early preliminary study of delayed mortality in Pacific halibut that had been captured by longline, tagged and held in cages in a shallow bay for up to 77 d showed delayed mortality up to 73 d (Peltonen 1969). However, fish often died for reasons apparently not related to capture and tagging, as holding conditions in the field included increasing temperature and decreasing salinity.

Studies on other commercially important marine fish species have shown that stress and mortality in bycatch may result from a variety of causes including net entrainment, mesh passage, crushing and wounding, sustained swimming until exhaustion, changes in pressure, hooking and exposure to air (Chopin and Arimoto 1995; Murphy et al. 1995; Olla et al. 1997). Trawling has been considered to be a greater source of Pacific halibut bycatch mortality than hooking (Williams and Wilderbuer 1995). The mortality associated with trawling was linked with time on deck, length of fish, total weight of catch, tow depth and tow duration, with typical tow durations during fishing operations that impact Pacific halibut ranging up to 4 h and time on deck ranging up to 1 h, although complete mortality could be noted after 20 min on deck (Hoag 1975; Richards et al. 1995; Trumble et al. 1995). The principle causes for hooking mortality have been suggested to be injuries from hooking and from release methods (Williams and Wilderbuer 1995; Kaimmer and Trumble 1998). Longlines are typically soaked for up to 24 h before hauling fish on deck.

Temperature is one of the key environmental factors that could interact with capture-induced stress to affect survival (Barton and Iwama 1991; Ross and Hokenson 1997; Olla et al. 1998). The magnifying effect of temperature on stress induced by capture has been described in detail for sablefish *Anoplopoma fimbria* (Olla et al. 1998; Davis et al. 2001). This effect is most likely to occur during warmer seasons when captured fish that are hauled on deck may be exposed to thermoclines ranging from 5 - 16°C and with even warmer deck temperatures (Olla et al. 1998). Previous in situ studies on bycatch in Atlantic halibut (Neilson et al. 1989) and Pacific halibut (Oddsson et al. 1994; Richards et al. 1995; Trumble et al. 1995; Williams and Wilderbuer 1995) have not reported temperatures through the water column or on deck and temperature may have been, at least in part, a causative factor in the observed mortality effects. This temperature information is needed for management decisions where more accurate estimates of halibut bycatch mortality could be made on a seasonal basis. A preliminary attempt to assess seasonal mortality for Pacific halibut bycatch produced by the deepwater flatfish fishery in the Gulf of Alaska during 1993 and 1994 indicated that there was a 7 - 8 % increase in mortality rates during the warmer April - September months when compared to the cooler October - March months (G. H. Williams, International Pacific Halibut Commission, personal communication).

The aim of this study was to determine under laboratory conditions the relative degree to which stress and eventual mortality was induced in non-reproductive Pacific halibut by capture with hooking or net towing followed by exposure to air, and to document the role that exposure to elevated temperature plays in increasing mortality resulting from capture stressors. Pacific halibut were observed for up to 60 d after experimental treatment to assess the potential for delayed mortality that resulted from experimental treatments.

Methods

Pacific halibut (70 - 85 cm fork length [FL]) that were non-reproductive were captured in the spring on commercial longline gear offshore from Newport, Oregon and a total of 72 fish used in the studies were held in six tanks at a density of 12 fish per tank in the laboratory for up to 6 months prior to experimentation. Holding tanks were circular (4.5-m diameter, 1.0-m depth, 15,904 L volume), with a 3-cm layer of smooth, dark river rock (<15 mm diameter) spread over the bottom. Tanks were supplied with flow-through sea water (20 L min^{-1}, 4.0 - 5.5°C, 30 - 32 ppt salinity, O_2 > 90 % saturation). Fish were fed to satiation on whole dead common squid *Loligo opalescens* twice per week. Half of the tank was covered with a black opaque cover and since fish were negatively phototactic, they generally remained under the cover and only came into the light side of the tank to feed. The light side of the tank had light conditions (daylight fluorescent, 5000°K) of 1.0 μmol photons m^{-2} s^{-1}, while the dark side decreased to 0.002 μmol photons m^{-2} s^{-1} at the farthest side of the tank.

The combined effect on mortality of being hooked for a 24-h period, followed by abrupt exposure to either the same temperature at which the fish were hooked or to elevated sea water temperature, and then exposure to air was determined in Pacific halibut (Figure 1). Fish were transferred by dip net from a holding tank to a rectangular tank (1.5-m wide, 6.7-m long, 1.0-m depth, 10,050 L volume) supplied with flow-through sea water (20 L min^{-1}, 5.0 $\pm$ 0.2°C, 30 - 32 ppt salinity, O_2 > 90 % saturation). Fish were restrained in a foam-lined holding box in the water and hooked in less than 30 s through the upper jaw onto commercial longline gear consisting of 10-mm rope, 3-mm nylon ganglions, swivel snaps and Mustad circle hooks (13/0). Fish were held on the lines near the bottom of the tank for 24 h in darkness and then they were placed in the holding box, unhooked and placed into a dip net in the water within 10 s. Fish were then transferred to a circular tank (3.0-m diameter, 1.0-m depth, 7,068 L volume) which contained sea water at either 5.0 or 16.0°C and held for 30 min, followed by 15 min in air (16.2 $\pm$ 0.3°C). After treatment, fish were transferred to circular tanks that were similar to the holding tanks and that had been divided in half with clear acrylic partitions. Two fish were held in each partitioned section for 30 d. Half of the tank was covered with an black opaque cover and negative phototaxis was measured in the fish every 24 h by moving the cover over the opposite half of the tank that fish rested in and observing whether the fish moved to the dark side. Fish were offered food daily and uneaten food was taken out of the tank after 4 h. Mortality was noted when it occurred and dead fish were removed from the tank. All fish were transferred back into original holding tanks after 30 d and further mortality and feeding were monitored in groups of fish.

The combined effect on mortality of being towed in a net for a 4-h period, followed by abrupt exposure to either the same temperature at which the fish were towed or to elevated sea water temperature, and then exposure to air was determined in Pacific halibut (Figure 1). Fish were transferred by dip net from a holding tank into towing nets, located in a tank, previously described (Olla et al. 1997; 1998). In brief, the apparatus had two nets suspended at the ends of two rotating arms in a tank (4.5-m diameter, 1-m depth) to simulate cod-ends of fishing trawls. The nets were cylindrical (1.2-m length, 0.7-m diameter) and constructed with 2.5-cm nylon diamond mesh. Nets were towed for 4 h at 5.0 ± 0.2°C in lighted conditions (1.0 μmol photons m^{-2} s^{-1}) at 1.1 m s^{-1}, a speed at which halibut could not swim. During towing, fish rested in the net with a head forward orientation. If fish slid towards the back of the net during towing, they became oriented perpendicular to the main axis of the net and invariably died, probably from an inability to breath as the water current and the net pressed their opercula closed. These fish were not included in the reported experiments. Following towing, fish were exposed to 5.0 or 16.0°C for 30 min, followed by 15 min in air (16.3 ± 0.4°C). Recovery was assessed as described for fish that had been hooked, followed by exposure to elevated sea water temperature and air.

The time course for elevation of body core temperature was determined by transferring Pacific halibut (78 - 85 cm FL) from a rearing temperature of 5.0°C to a circular tank

(3.0-m diameter, 1.0-m depth, 7,068 L volume) containing heated sea water (16.0°C). An ultrasonic temperature transmitter (30 mm x 16 mm; Sonotronics) was manually inserted into the stomach of a fish 5 min prior to transfer to the heated sea water to insure that the transmitter reached initial body core temperature. Change in body core temperature of a free swimming fish was monitored every 5 min for 30 min using an ultrasonic receiver (Sonotronics). At the end of a trial, the transmitter was removed from the fish and inserted into another fish.

Results

When Pacific halibut were hooked for 24 h at 5.0°C, abruptly transferred to 5.0°C for 30 min and then to air for 15 min (16.2°C), no mortality occurred with fish surviving for at least 60 d ($N = 12$, Figure 2). The overt signs of stress in surviving fish were a cessation of feeding and negative phototaxis which both resumed by 5 d after experimental treatment. In contrast, hooking and abrupt transfer of fish to 16.0°C, followed by transfer to air for 15 min (16.2°C) caused significant mortality, with 6 out of 18 fish dying (33 % mortality; one-tailed Sign test, $P = 0.016$; Figure 2), between 2 and 30 d after hooking (mean $\pm$ 1 SE = 10.0 $\pm$ 4.5 d). All surviving fish resumed feeding and negative phototaxis within 30 d after hooking and elevated temperature treatment and no further mortality was noted after 30 d (Figure 3).

When Pacific halibut were towed in a net for 4 h at 5.0°C, abruptly transferred to 5.0°C for 30 min and then to air for 15 min (16.3°C), no mortality occurred for at least 60 d ($N = 12$, Figure 2). Feeding and negative phototaxis in the surviving fish ceased but resumed by 5 d after net towing. In contrast, net towing and abrupt transfer of fish to 16.0°C, followed by transfer to air for 15 min (16.3°C) caused significant mortality, with 14 out of 18 fish dying (78 % mortality, one-tailed Sign test, P <0.001, Figure 2), between 1 and 27 d after towing (16.1 ± 3.0 d). All surviving fish resumed feeding and negative phototaxis within 30 d after towing and elevated temperature treatment and no further mortality was noted after 27 d (Figure 3). The mean time it took for delayed mortality to occur apparently did not differ between fish that were hooked (16.1 ± 3.0 d) or towed (10.0 ± 4.5 d) and exposed to elevated temperature and air (t_{11} = 1.17, P = 0.267; Figure 3).

The time course of increase in body core temperature was determined to estimate the internal temperatures of Pacific halibut during exposure to elevated sea water temperature. For fish (N = 5) that had been acclimated to 5.5°C, abrupt transfer to 16.0°C resulted in body core temperature rising to 7.7 ± 0.3°C after 15 min; by 30 min body core temperature had reached 10.5 ± 0.1°C (Figure 40). This rise in temperature was statistically significant (Friedman's test, F_5 = 25.0, P = 0.0001). No mortality was observed for these fish up to 60 d after exposure to elevated temperature.

Discussion

For Pacific halibut, hooking or towing in a net, followed by air exposure did not result in mortality, while exposure of fish to elevated temperature and air after either hooking or net towing resulted in mortality, which was higher for the net towed fish than for the hooked fish. Similar results were obtained with sablefish towed in a net and exposed to elevated temperature and air (Olla et al. 1998; Davis et al. 2001). We presume that significant mortality would not result from exposure only to 16°C, since no mortality was observed for any of the five Pacific halibut that were abruptly exposed to 16°C for 30 min during body core temperature measurements. Significant mortality would be expected to result as Pacific halibut approached an upper threshold of temperature tolerance, but determination of such a temperature threshold was not within the scope of this study.

Delayed mortality in Pacific halibut occurred up to 30 d after experimental treatment, with the average time to death apparently not differing between fish that had been hooked or towed and then abruptly exposed to elevated temperature and air. In an earlier study, delayed mortality was observed for walleye pollock *Theragra chalcogramma* for up to 14 d after capture by towing (Olla et al. 1997). Other studies have also reported delayed mortality after capture ranging from 3 - 14 d for a variety of species (Lockwood et al. 1983; Van Beek et al. 1990; Soldal et al. 1993; Olla et al. 1998). Since delayed mortality that resulted from post-capture stress was variable, this should be investigated for each species of interest (Chopin and Arimoto 1995; Olla et al. 1997). For both Atlantic halibut *Hippoglossus hippoglossus* (Neilson et al. 1989) and Pacific halibut (Oddsson et al. 1994; Trumble et al. 1995; Kaimmer and Trumble 1998) exposed to capture stresses, in situ studies on delayed mortality in fish held in cages or tanks were conducted for 2 - 10 d after experimental treatment. Our results clearly show that observations of post-capture mortality in Pacific halibut should be made over a longer period of time (up to 30 d) to insure that realistic results are obtained from laboratory or field studies of bycatch mortality. An early preliminary study of delayed mortality in Pacific halibut that had been captured by longline, tagged and held in cages in a shallow bay for up to 77 d showed delayed mortality up to 73 d, with fish not feeding for 60 d (Peltonen 1969). However, it was difficult to link the results of this study to the effects of particular factors since fish were exposed to increasing temperature and decreasing salinity during holding, did not resume feeding until after 60 d and often died for reasons that appeared to be unrelated to capture or tagging.

Mortality appeared to be lower for fish that had been hooked and then exposed to elevated temperature and air than for fish that were towed in a net and then exposed to elevated temperature and air. In an earlier field study, capture of Pacific halibut by trawling was considered to be a greater source of bycatch mortality than by hooking, although temperature was not measured during the trials (Williams and Wilderbuer 1995). While the difference in mortality observed in the field trials may have been caused in part by the effects of crushing in the trawl, our laboratory results suggest that elevated temperature could explain at least part of the differences among capture methods observed in the field.

Comparisons of experiments conducted in the laboratory with possible results obtained in the field must be made with caution. The experiments in this study were designed to simulate some of the stressors that could be associated with bycatch processes and were generally conservative in their effects relative to field conditions of capture. Also, relatively minor stressors were equally present in all the laboratory experiments which would not be present in the field, including handling in nets and transport to tanks between capture, temperature and air treatments and these were considered part of the overall stressor treatments. We recognize that during capture, fish in the laboratory were not subjected to stressors that could be present in the field, e.g., depth and pressure changes, crushing in a trawl, dragging of longline gear by currents and heavy seas, changes in temperature during hauling that generally are less rapid than those simulated in the laboratory, unhooking processes, handling on deck during discard of bycatch, the presence of predators and exposure to thermocline conditions after release from capture in a fishing operation. Conclusions resulting from this study emphasize principles of stressor action rather than attempts to precisely predict the stress that may result from capture in the field under a wide range of conditions. We have chosen to assess bycatch stress under controlled conditions where there is the possibility of understanding the additive nature of stressors and resulting mortality. Once this information is available, then experiments can be designed to investigate bycatch processes under field conditions where control of individual stressors is difficult and complex mixtures of stressors are present. Future research in our laboratory with Pacific halibut will consider possible correlations between measures of mortality and chemical measures of physiological stress which could be used as surrogate measures to predict mortality under actual fishing conditions, where holding fish for extended periods of time to determine mortality is not generally possible (Morgan and Iwama 1997; Olla et al. 1998; Davis et al. 2001).

Stress induced in fish as a result of bycatch processes may often result from a combination of several stressors, including capture, environmental factors and handling (Chopin and Arimoto 1995; Murphy et al 1995; Olla et al. 1997). The magnification of stress in fish caused by interactions of stressors is probably a common occurrence (Wedemeyer et al. 1990; Barton and Iwama 1991). With regards to the prediction of Pacific halibut bycatch mortality after release back into the ocean, the results of the present study suggest that as part of accounting for the interaction of stressors, it would be necessary to measure water and deck temperatures when conducting in situ studies of stress in halibut bycatch. Similar conclusions were reached in previous studies with sablefish in which temperature magnified the stress induced by capture (Olla et al. 1998; Davis et al. 2001).

Previous studies of mortality in bycatch Pacific halibut caught in the field have attempted to relate a three-level visual inspection index of fish condition and potential mortality to capture and handling stressors, but with limited quantitative success (Hoag, 1975; Kaimmer and Trumble 1998). In situ studies of bycatch mortality in Atlantic halibut (Neilson et al. 1989) or Pacific halibut (Oddsson et al. 1994; Richards et al. 1995; Trumble et al. 1995; Williams and Wilderbuer 1995) caught with trawl or longline have not included direct observation of temperature and its effects, but instead have measured the effects of indicator variables such as time on deck, tow depth, tow duration and release methods which may have been associated with elevated temperature. Since the visual index of halibut condition is an essential component in the estimation of bycatch mortality used in stock management models, a high priority for research would be to improve the accuracy of this index. Future refinement of the visual condition index for bycatch halibut should include consideration of the effects of exposure to elevated temperature during capture and handling, as well the use of longer holding times for fish to assess potential delayed mortality.

The thermal history of Pacific halibut during capture, as measured by body core temperature, would be an important factor in the prediction of bycatch mortality. As would be predicted, data on the warming of Pacific halibut showed a slow increase in body core temperature. It is expected that the slope of this warming curve would be dependant on body size and the history of exposure to temperature differentials between the depth of capture and conditions on deck during handling (Spigarelli et al. 1977). Seasonal increases in temperature associated with thermoclines and deck conditions (see Olla et al. 1998) would probably increase mortality of Pacific halibut that might otherwise survive capture and release in cooler seasons.

Obviously under fishing conditions, there are many possible combinations of thermocline and deck temperatures and the effects of this matrix of conditions on Pacific halibut body core temperature should be investigated in further detail. Also it would be relevant to investigate the possible effects of below freezing temperatures on Pacific halibut bycatch, such as would be found on deck during colder seasons in Alaska.

In this study, Pacific halibut were exposed to 16°C because this was a reasonable surface water temperature in the coastal waters off Oregon and Washington during the summer months (Olla et al. 1998). While bottom and surface water temperatures during the summer months in Alaska would be lower than those of Washington and Oregon, temperatures on deck during daylight hours would probably be similar in Alaska, Washington and Oregon, ranging between 15 - 30°C. These elevated temperatures represent a chronic stress, as acclimation of fish to elevated temperature probably does not occur during time periods of exposure less than 2 h that are relevant to bycatch processes. Acclimation of fish to temperature is a physiological process that requires altered gene expression in the broadest sense, occurring in a minimum of 6 - 8 h (Hazel 1993). Abrupt exposure to 16°C after capture was probably not the most realistic way to expose fish when compared to fishing operations, where fish may be retrieved over variable time periods up to 1 h through various gradients of temperature change and subsequent exposure to deck conditions ranging up to 1 h for trawl-caught fish and less than 5 min for hook-caught fish, followed by fish descending down through the thermocline. However, simulation of a range of temperature gradients was not possible in this study because of the limited availability of sea water facilities to hold large numbers of fish. Clearly, information about changes in body core temperature in field-caught Pacific halibut is needed. Ultimately, the most useful predictor of bycatch mortality during warmer seasons may be a combination of measures of the intensity of capture stressors and the interaction of body core temperature. The results of this study emphasize the significance of these interactions in prediction of stress induction and eventual mortality.

Strategies for fisheries management of Pacific halibut bycatch should consider seasonal environmental factors and how they might increase mortality associated with capture. In Observer Programs required for some fisheries that produce Pacific halibut bycatch, in addition to recording characteristics of the capture and handling processes and fish condition and length, it would be useful to record surface water and deck temperatures and Pacific halibut body core temperatures in an effort to quantify factors controlling bycatch mortality. Significant reduction in Pacific halibut bycatch mortality could possibly be obtained by altering fishing practices to reduce elevated temperature exposure during warmer seasons or restricting fisheries that produce bycatch to seasons of cooler temperatures, although caution must be observed in exposing fish to freezing temperatures until more information is available on the possible effects of such cold temperatures. Data for Pacific halibut bycatch mortality rates in the deepwater flatfish fishery in the Gulf of Alaska during 1993 and 1994 indicated that there was a 7 - 8 % increase in mortality rates during the warmer April - September months when compared to the cooler October - March months (G. H. Williams, International Pacific Halibut Commission, personal communication). For all fisheries during 1999 in the Bering Sea and the Gulf of Alaska, 2,658 metric tons of Pacific halibut bycatch mortality occurred during the warmer April - September period while 3,886 metric tons occurred during the cooler October - March period [1]. If there are future shifts in fishing activity into warmer months because of considerations for political, economic or endangered species factors, then total Pacific halibut bycatch mortality would be predicted to increase.

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Footnotes

1. National Oceanographic and Atmospheric Administration, National Marine Fisheries Service, Alaska Region. 2000. 1999 catch statistics for Bering Sea, Aleutian Islands and Gulf of Alaska. Website: http://www.fakr.noaa.gov/1999/1999.html. Accessed 2/2/01.

Figure Captions

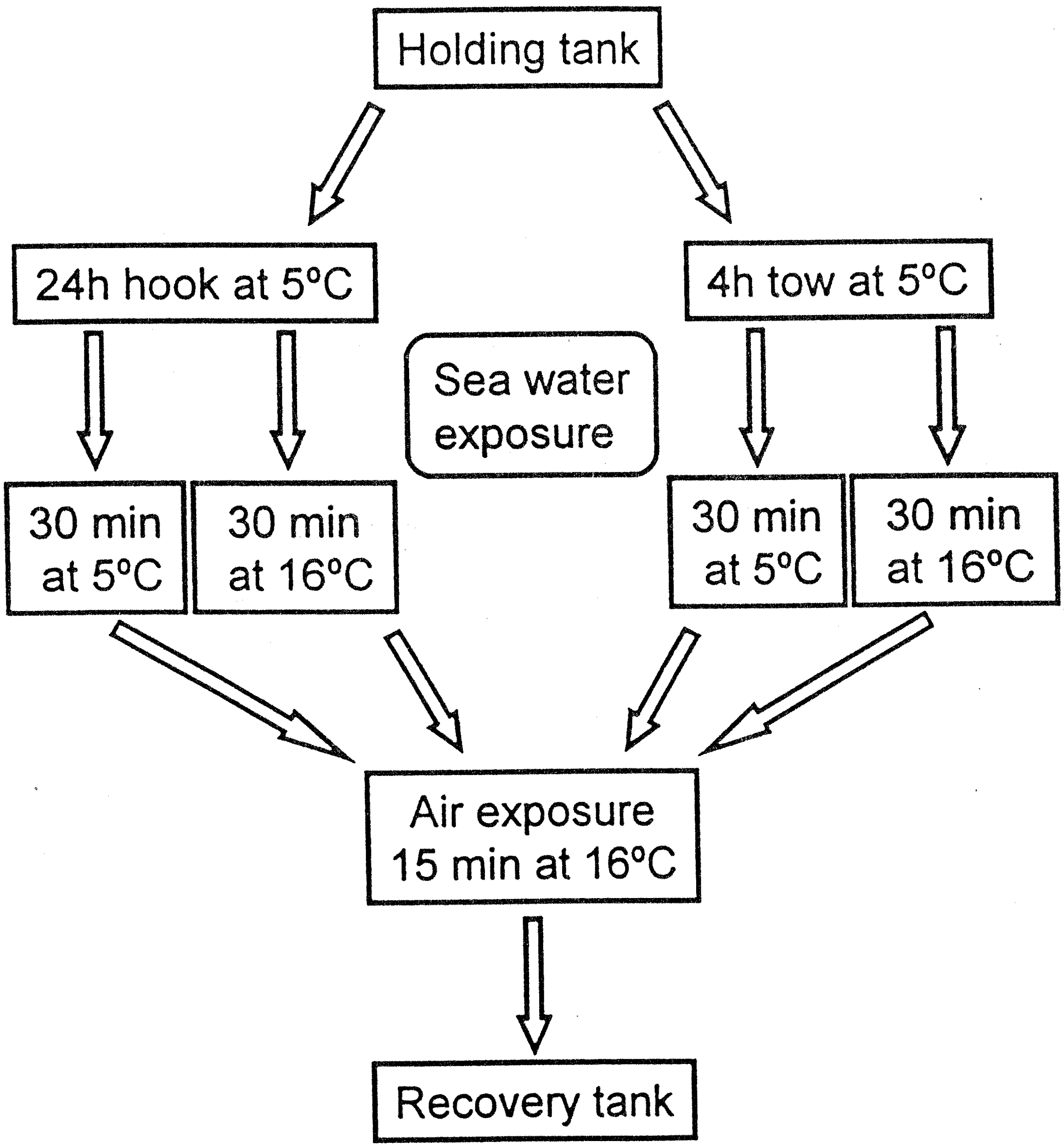
Figure 1. Flow chart of experimental stressor treatments for Pacific halibut *Hippoglossus stenolepis*. Fish were exposed to hooking for 24 h at 5°C followed by 5 or 16°C sea water for 30 min, air exposure for 15 min and transfer into recovery tanks; and exposed to towing for 4 h at 5°C followed by 5 or 16°C sea water for 30 min, air exposure for 15 min and transfer into recovery tanks.

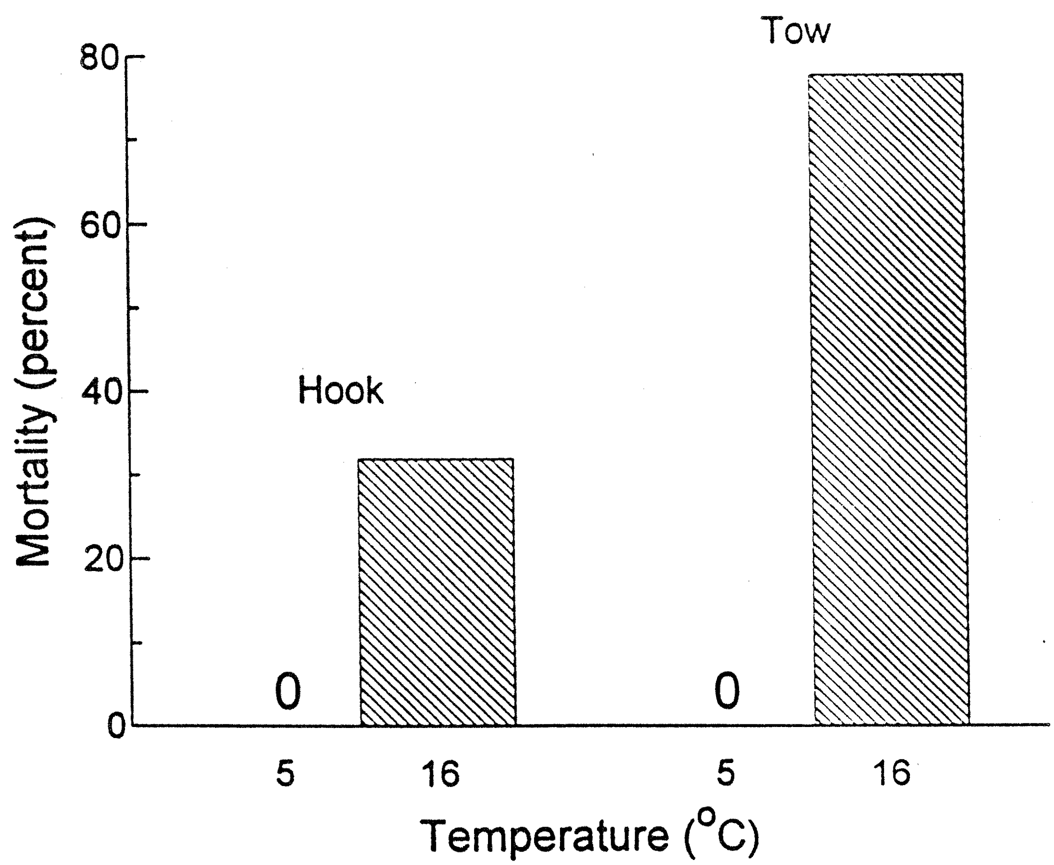
Figure 2. Pacific halibut *Hippoglossus stenolepis*. Effect of sea water temperature (5 and 16°C) on mortality (percent) in fish exposed to hooking for 24 h at 5°C followed by sea water temperature and air exposure (Hook); and exposed to towing for 4 h at 5°C followed by seawater temperature and air exposure (Tow). Mortality at 5°C for Hook and Tow treatments was 0 %.

Figure 3. Pacific halibut *Hippoglossus stenolepis*. Time course (d) post stress induction for cumulative mortality (percent) in fish exposed to hooking for 24 h at 5°C followed by 16°C sea water and air exposure (Hook); and exposed to towing for 4 h at 5°C followed by 16°C seawater and air exposure (Tow). Note that fish were observed for up to 60 d, with no mortality occurring after 27 d for towed fish and 30 d for hooked fish.

Figure 4. Pacific halibut *Hippoglossus stenolepis*. Time course (min) for increase of body core temperature (°C) in fish (78 - 85 cm FL) that were abruptly exposed to 16°C sea water for 30 min. Values are means $\pm$ 1 S.E.; data point at 30 min includes error bar.

Pacific halibut stressor treatments




Tow
Hook
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Mortality (percent)
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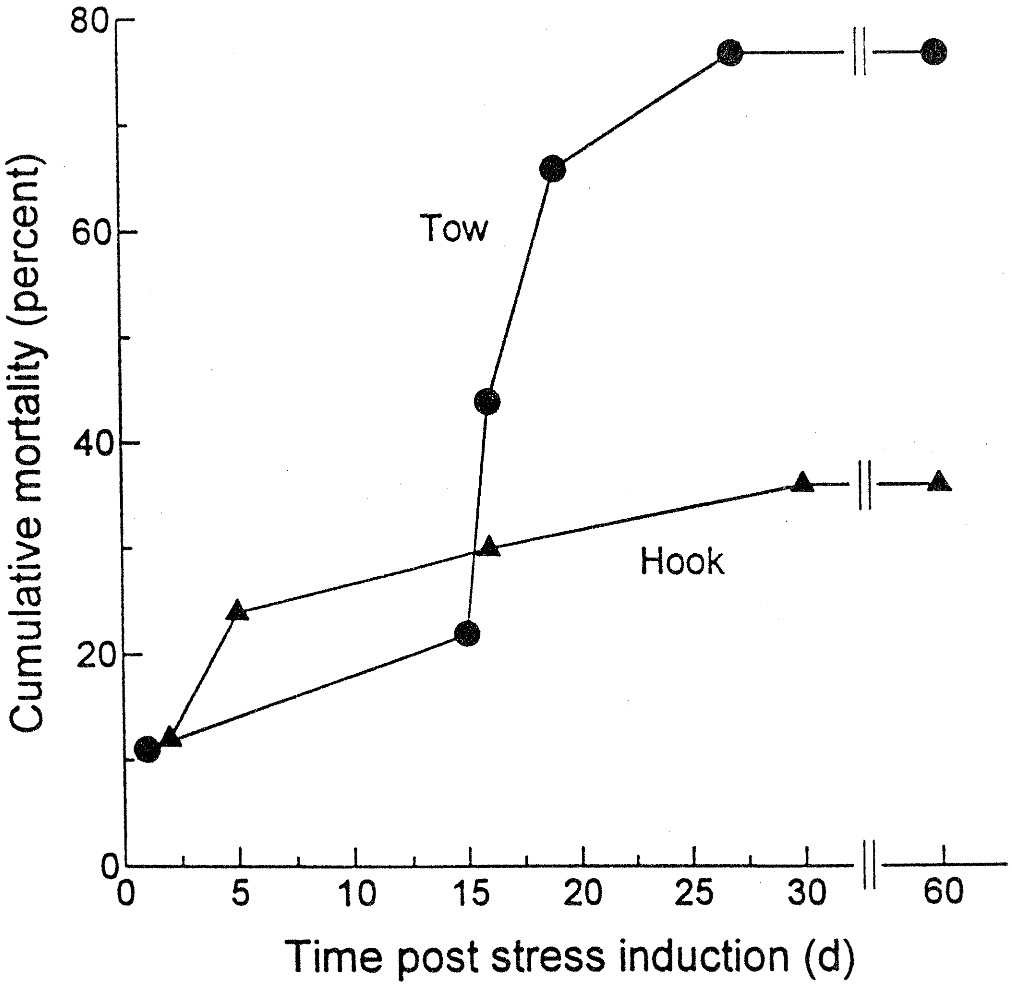
Temperature (°C)


Cumulative mortality (percent)
80
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Tow
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5
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15
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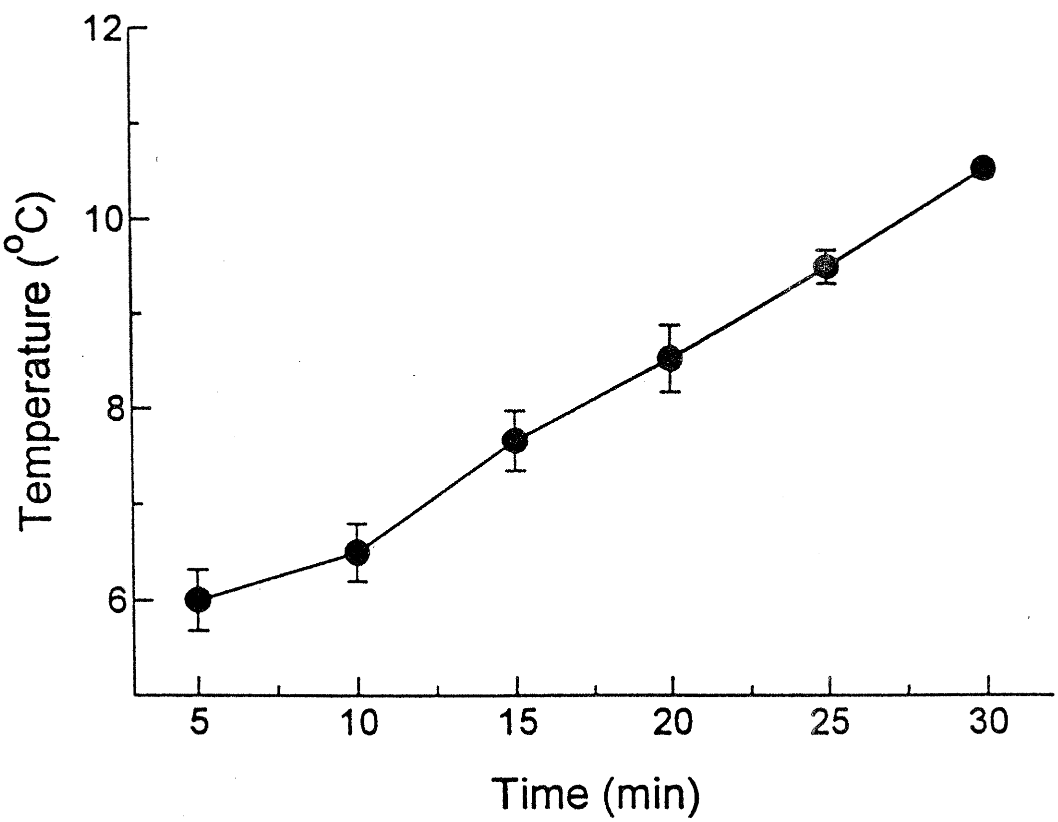
25
30
60
Time post stress induction (d)



12
10
8
6
Temperature (°C)
5
10
15
20
25
30
Time (min)