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[Article]

Capture-Related Stressors Impair Immune System Function in Sablefish

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Abstract.—The sablefish *Anoplopoma fimbria* is a valuable North Pacific Ocean species that, when not targeted in various commercial fisheries, is often a part of discarded bycatch. Predictions of the survival of discarded fish are dependent on understanding how a fish responds to stressful conditions. Our objective was to describe the immunological health of sablefish exposed to capture stressors. In laboratory experiments designed to simulate the capture process, we subjected sablefish to various stressors that might influence survival: towing in a net, hooking, elevated seawater and air temperatures, and air exposure time. After stress was imposed, the in vitro mitogen-stimulated proliferation of sablefish leukocytes was used to evaluate the function of the immune system in an assay we validated for this species. The results demonstrated that regardless of fishing gear type, exposure to elevated seawater temperature, or time in air, the leukocytes from stressed sablefish exhibited significantly diminished proliferative responses to the T-cell mitogen, concanavalin A, or the B-cell mitogen, lipopolysaccharide. There was no difference in the immunological responses associated with seawater or air temperature. The duration and severity of the capture stressors applied in our study were harsh enough to induce significantly elevated levels of plasma cortisol and glucose, but there was no difference in the magnitude of levels among stressor treatments. These data suggest that immunological suppression occurs in sablefish subjected to capture-related stressors. The functional impairment of the immune system after capture presents a potential reason why delayed mortality is possible in discarded sablefish. Further studies are needed to determine whether delayed mortality in discarded sablefish can be caused by increased susceptibility to infectious agents resulting from stressor-mediated immunosuppression.

The unaccounted-for mortality associated with the capture and release of nontargeted marine fishes as discarded bycatch is one of the primary issues currently affecting commercial fisheries management. Managers need to account for the mortality of discarded fish to accurately assess fish populations and harvest impacts, yet discard mortality rates of specific fisheries are generally unknown (Chopin et al. 1995; Pascoe 1997; NMFS 2003). The sablefish *Anoplopoma fimbria* is a valuable north Pacific Ocean species caught in several commercial fisheries and is often discarded due to size or catch limits. Sablefish are susceptible to overexploitation due to their long lives—ages over 50 years are regularly recorded (Kimura et al. 1993; McFarlane and Beamish 1983)—and their highly migratory nature (Maloney and Heifetz 1997; Rutecki and Varosi 1997). Juvenile sablefish are very susceptible to trawl fisheries during nearshore residency, and their incidental discard can adversely affect sablefish recruitment (Sampson et al. 1997; Sigler et al. 2001). The size-selective price structure of the commercial industry and the minimum size limits also result in the discard of smaller fish, potentially leading to discard mortality (Schirripa and Methot 2002; Davis and Parker 2004).

Delayed mortality is one source of undetected mortality in discarded fishes. Studies of fish held in the laboratory or field after capture have indicated that delayed mortality can vary with the species of fish and the presence of physical injury (Davis 2002, 2005). For

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example, in laboratory studies where fish were held for 60 d after capture-related stressors, delayed mortality was documented for up to 30 d in Pacific halibut *Hippoglossus stenolepis*, 14 d in walleye pollock *Theragra chalcogramma*, and 35 d in sablefish (Davis 2002, 2005). The observance of delayed mortality in discards indicates that capture stressors may induce physiological modifications that result indirectly in future mortality from predation, stress, or disease (Davis 2002).

Fish, like other vertebrates, respond to stressful stimuli through a neuroendocrine cascade that results in physiological and behavioral responses. After the stressor is perceived, a physiological resistance phase follows, leading to either adaptation or compensation (Selye 1950). Situations that push physiological systems beyond homeostasis can result in what Sterling and Eyer (1988) have defined as allostasis, which is the increase or decrease in vital functions by an organism to achieve a new form of stasis (Sterling and Eyer 1988; McEwen 1999). However, the cumulative strain on the organism from maintaining this allostatic response to stressors can result in an allostatic load (McEwen and Stellar 1993). Over time, the maintenance of allostasis reduces the organism's ability to maintain normal physiological functions (Pruett 2003).

Predictions of the survival of discarded fish are dependent on an understanding of how a fish responds to stressful conditions. The capacity of fish to respond to a stressor can be measured by biological, physiological, and behavioral changes that demonstrate the degree of stress expressed (Wedemeyer et al. 1990). In sablefish, commonly measured stress indicators (e.g., plasma cortisol, lactate, and glucose), though useful in quantifying the stress response, have not been correlated directly with capture-related mortality (Davis et al. 2001; Davis 2002; Parker et al. 2003)

Although several techniques have been developed and used for monitoring the immune competence of teleosts (Weyts et al. 999), the Anoplopomatidae have been largely unstudied physiologically, and there are no established techniques to characterize their immunological health. Evaluating the in vitro proliferative responses of pronephric lymphocytes to classic mammalian B- and T-cell mitogens is one of the most commonly used methods to assess the function of the cellular immune system (Luft et al. 1991; Faisal and Hargis 1992). In this study, proliferation was assessed by lymphocyte ^{3}H-thymidine incorporation in an assay we developed for sablefish. We report a number of experiments that evaluated the in vitro conditions necessary to maximize a ^{3}H-thymidine lymphocyte incorporation assay sensitive to B- and T-cell mitogens.

The objective of this study was to examine, in the laboratory, the effects of nonlethal, capture-related stressors on the immune function of the sablefish. A greater understanding of the interactions of capture and environmental stressors and their effects on sablefish is critical for clarifying potential controls on delayed discard mortality. Knowledge of the immune response after the imposition of capture-related stressors may lead to a better understanding of why fish die after capture.

Methods

Fish collection and maintenance.—Juvenile sablefish (20–40 mm total length [TL]) were collected with neuston nets in the spring of 2001 about 50 km offshore of Newport, Oregon, and were reared at the Hatfield Marine Science Center (HMSC) in circular tanks (2.0-m diameter, 0.8-m depth, 31,401 L) supplied with sand-filtered and ultraviolet-sterilized flow-through seawater (29–32‰ salinity, 8–11°C, $O_2 >$ 90% saturation) at a replacement rate of 101 L/min. Fish were fed to satiation on pelletized salmon food three times per week. During the second year, fish were maintained at 30–40 fish per tank in larger circular tanks (4.5-m diameter, 1.0-m depth, 159,041 L) supplied with flow-through seawater (201 L/min, 29–32‰ salinity, $O_2 > 90\%$ saturation, 8°C) and were fed to satiation with a biweekly diet of squid *Loligo opalescens*. The protocols used in this research conformed to the guidelines for ethical treatment of experimental animals prescribed by the American Fisheries Society.

Reagents and mitogens for immune assay.—The medium for isolation and maintenance of leukocytes was prepared as described in Milston et al. (2003) and Misumi (2004) but was adjusted according to sablefish blood osmolality of approximately 380 milliosmols. The tissue culture media (TCM) consisted of a 7% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, and 9% tumor cocktail in Minimum Essential Media (MEM; Invitrogen Co.). The tumor cocktail was prepared by adding 7.5 g of dextrose, 75 mL of essential amino acids, 100 mL of nonessential amino acids, and 100 mL of sodium pyruvate to 630 mL of MEM. The mixture was adjusted to a pH of 7.0 with 10 N NaOH before adding 8.5 g of sodium bicarbonate, 340 mg of penicillin, 0.2 mg of streptomycin sulfate per milliliter of MEM, 0.1 mg/mL gentamycin in MEM, and 34 μL of β-2-mercaptoethanol. The cocktail was dispensed through a 0.45-μm filter (Corning), aliquoted into 45 mL per conical tube, and stored at −20°C.

The nutritional supplement was prepared by a modification of the method of Kaattari and Holland (1990). Fifteen milliliters of TCM were added to 7.5 mL of

heat-inactivated FBS, 0.347 mL of Hepes buffer, 1.0 mL of guanosine in TCM (1.0 mg/mL), and a 1.0-mg/mL mixture containing equal parts adenosine, uridine, and cytidine in TCM. The solution was adjusted to a pH of 7.4 with 10 N NaOH, filtered through a 45-μm filter, and stored at −20°C.

Concanavalin A (Con A; Sigma Chemical Co.), a polyvalent carbohydrate-binding protein from the wonderbean *Canavalia ensiformis*, and lipopolysaccharide (LPS; Sigma) from the bacterium *Escherichia coli* serotype O 55:B5 were obtained as aseptic powders and reconstituted with TCM to stock solutions (10 mg/mL). The stock solutions were diluted further to obtain mitogen doses of 25 μg/well and were filtered (0.45-μm filter) before use.

Isolation and cultivation of pronephric leukocytes.—Individual head kidneys were harvested into TCM under aseptic techniques, and lymphocytes were isolated according to the methods of Ellsaesser and Clem (1986), Arkoosh et al. (1994), Milston et al. (2003), and Misumi (2004). Briefly, 10 mL of a whole head kidney cell suspension (2×10^7 cells/mL) were slowly layered over an equivalent volume of Histopaque-1077 (Sigma) in 50-mL conical tubes and were centrifuged at 500 × gravity (*g*) for 45 min at 17°C. The buffy layer of leukocytes at the interface was removed with a Pasteur pipette, TCM was added for a total volume of 10 mL, and the cells were centrifuged at 500 × *g* for 15 min at 17°C. The leukocyte pellet was resuspended in 10 mL of TCM and was centrifuged again. After the second wash, the supernatant was aspirated and the pellet was resuspended with 0.5 mL of TCM. By use of the trypan blue exclusion method to determine viability, the isolated populations of leukocytes were counted with a hemocytometer and were diluted with TCM to 5×10^7 cells/mL. For the proliferation assay, leukocytes from each sablefish were added in triplicate with or without each mitogen to flat-bottom, 96-well tissue culture plates (Becton Dickinson) at 5×10^6 cells/well. The cultures were maintained at 17°C in a humidified incubation chamber (CBS Scientific Co.) containing a blood gas mixture (10% O_2, 10% CO_2, and 80% N_2). On day 2 of culture, 1 microcurie (μCi) of ^{3}H-thymidine in 10 μL of nutrient supplement was added to each well. Twenty-four hours later, the leukocytes were harvested from their wells with a Wallac Tomtec cell harvester onto a glass-fiber filtermat (Wallac). The filtermat was dried and sealed in a plastic filter bag with 4.5 mL of optiphase scintillation fluid (Wallac). Proliferation was assessed by ^{3}H-thymidine incorporation measured as counts per minute by a Wallac liquid scintillation counter. The proliferative responses were assessed for each individual fish as stimulation indices (SI), which were calculated as the mean ^{3}H-thymidine uptake of the mitogen-stimulated cultures divided by the mean ^{3}H-thymidine uptake of the nonstimulated cultures.

Validation of in vitro culture system.—Experiments were initially conducted to validate the in vitro culture conditions that would support the mitogenic response of sablefish pronephric leukocytes. To determine the optimal dose of mitogen and to establish the peak day of mitogen-induced proliferation, leukocytes from three sablefish were incubated in triplicate at 17°C with concentrations of 5, 25, 150, and 250 μg/well of LPS or 0.5, 5.0, 25.0, and 50.0 μg/well of Con A. On day 0, 2, 4, or 6 of culture, 1 μCi of ^{3}H-thymidine in 10 μL of nutrient supplement was added before each well was subsequently harvested and counted as described above.

For determination of the leukocyte density that produced the greatest SI, pronephric leukocytes ranging from concentrations of 5×10^4 to 5×10^7 cells/mL were stimulated in triplicate with the optimal concentration of either LPS or Con A and were incubated at 17°C. Cells were pulsed with 1 μCi of ^{3}H-thymidine on day 2 of incubation and were harvested and counted on day 3.

The leukocyte density, mitogen concentration, and duration of culture yielding the maximum mitogenic proliferation were used to determine whether a different in vitro temperature would enhance the mitogen-induced proliferative response. The leukocytes were cultured at three temperatures: 8, 12, and 17°C. Cells were pulsed with 1 μCi of ^{3}H-thymidine on day 2 of culture, were harvested 24 h later, and were counted.

To determine whether a greater SI could be produced with hypotonic lysis instead of density centrifugation, we separated leukocytes from erythrocytes as described by Crippen et al. (2001). Briefly, 2 mL of whole-cell suspension were diluted with 9 mL of sterile distilled water, the erythrocytes were lysed for 20 s, and the cells were returned to isotonicity with 1 mL of 10× phosphate-buffered saline. The suspension was centrifuged at 500 × *g* for 7 min at 17°C, the supernatant was aspirated, and the pellet was resuspended in 2 mL of TCM. Isolated leukocytes at a density of 5×10^6 were stimulated with 25 μg/mL of LPS or Con A, and kinetic assays were performed in triplicate at 17°C. On day 0, 3, 5, or 7 of incubation, the cultures were harvested and counted.

Using the culture conditions previously determined to be optimal, we assessed the degree of variability in the proliferative response to a mitogen in 25 nonreproductive, 2–3-year-old sablefish. Pronephric leukocytes of sablefish were stimulated in triplicate with either 25 μg/mL of LPS or Con A, were pulsed with

^{3}H-thymidine on day 2 of culture, and were harvested 24 h later.

Laboratory stressor treatments.—In laboratory experiments designed to simulate the capture process, we investigated the impairment of immune system function in 2-year-old, nonreproductive sablefish. Each experiment involved six unstressed fish remaining at the maintenance temperature of 8°C as a negative control and six fish subjected to a stressor. All experiments were conducted over a 4-month period at the HMSC.

To simulate the stress imposed by trawling in a net and exposure to air that would arise on a commercial vessel, we transferred sablefish (mean TL = 36.8 cm) from the maintenance tank by rapid netting to a tank with towing nets, as described in Olla et al. (1997). Briefly, to simulate the cod ends of trawls, two 2.5-cm, diamond-mesh nylon nets (1.2-m length, 0.7-m diameter) were attached to rotating arms in a large tank (4.5-m diameter, 1-m depth). For each trial, fish were sequestered in one net and towed for 2 h at 1.1 m/s, a speed at which sablefish could not swim (Olla et al. 1997), and at 8°C, the temperature at which the fish were reared. Directly after the tow, the fish were transferred in a net to a large basin (90 × 60 × 30 cm) in a temperature-controlled room and were exposed to 10°C or 16°C air for 15 min.

The effects of hooking for 2 h followed by air exposure for 15 min at 10°C or 16°C were determined in nonreproductive, 2-year-old sablefish (mean TL = 36.6 cm). The fish were transferred from the maintenance tank by rapid netting to a circular tank (2.0-m diameter, 0.8-m depth, 8°C) and were hooked through the upper jaw onto commercial long-line gear, as described in Davis et al. (2001). Fish were maintained on the lines for 2 h, unhooked by hand, and exposed to air as described above.

We simulated the capture of sablefish by trawling and exposure to warmer temperatures during gear retrieval by towing nonreproductive, 2-year-old sablefish (mean TL = 45.1 cm) for 2 h, followed by exposure to elevated seawater temperatures. After towing as described above, the fish were transferred by rapid netting to a tank (3.0 × 1.0 m), containing 10°C or 16°C seawater for 15 min.

To simulate the stress imposed by air exposure on the deck of a fishing vessel, we used dipnets to transfer sablefish (mean TL = 36.8 cm) from the maintenance tank into a large basin (90 × 60 × 30 cm) in a temperature-controlled room. The fish were exposed to air at 10°C or 16°C for either 15 or 30 min and then were placed back into a recovery tank with 8°C seawater (3.0 × 1.0 m) for 2.0 or 1.75 h , respectively. The fish were placed into the recovery tank after air exposure to ensure that the interval between the onset of stress and the immune function measurement were equivalent for all laboratory experiments.

After treatments were performed as described above, the fish were immediately placed in a lethal dose (400 mg/L) of tricaine methanesulfonate. The TL of each fish was recorded before blood and head kidney samples were obtained. Control fish from the maintenance tank (8°C) were sampled concurrently with the treatment groups. Blood was collected from the caudal vein with 3-mL, heparinized Vacutainers; the plasma was separated by centrifugation and stored at −80°C until analyzed for cortisol and glucose levels. Plasma cortisol was assayed according to radioimmunoassay techniques by Redding et al. (1984) and was validated for sablefish by Olla et al. (1997). Plasma glucose was measured with a blood analyzer (NOVA Biomedical Co.).

Data analysis.—Because each fish was sampled individually, each individual was an independent unit and therefore was considered the unit of measure for statistical purposes. Immune assays were performed in triplicate. Therefore, data were derived from triplicate determinations for each fish, and individual proliferative responses were derived after the calculated mean of triplicate determinations of the mitogen-stimulated cultures and the nonstimulated cultures.

For each capture-related laboratory experiment, the sample size was six fish and the mean (±SE) was calculated. Because the data did not fit a normal distribution and variances were not equivalent, data were analyzed with nonparametric tests in SigmaStat software (SPSS 1997). A Kruskal–Wallis one-way analysis of variance by ranks test was used to detect any differences due to treatments, followed by a Dunn's multiple-range test to examine differences between the median values at different levels of capture. Differences were considered statistically significant when *P*-values were less than 0.05.

Results

Proliferation Assay Culture Conditions

Maximum mitogenic responses for both LPS and Con A were observed on day 3 of culture at a mitogen concentration of 25 μg/mL and a density of 5×10^{6} leukocytes/mL. Sablefish kidney leukocytes assessed on day 3 of culture maintained a maximum SI for both LPS and Con A at 17°C. At 8°C and 12°C, the mitogenic responses to LPS were approximately equal at a SI of 1.0, while at 12°C a SI of greater than 2.0 was maintained for Con A. The greatest SI for LPS was induced on day 3 of culture with cells separated by density gradient centrifugation. While the LPS pro-

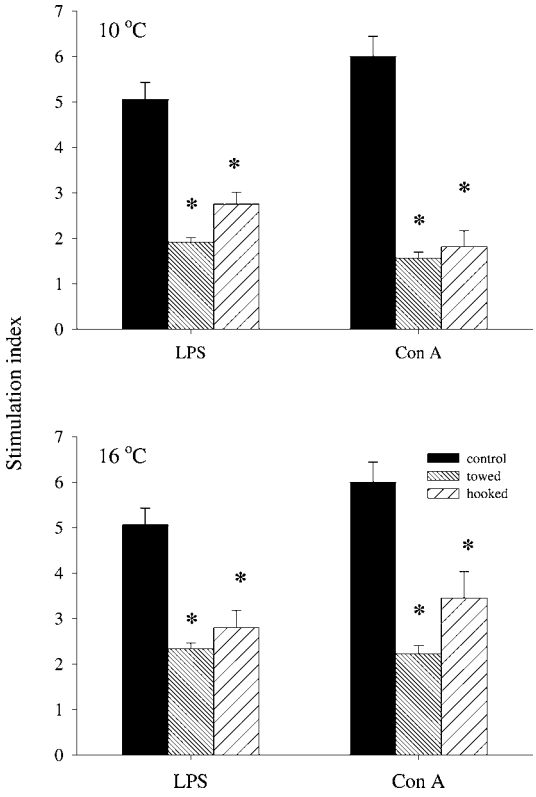

FIGURE 1.—Mean (±SE) stimulation indices (SIs) of control sablefish (unstressed at 8°C) and sablefish subjected to 2 h of simulated hooking or towing at 8°C followed by 15 min of air exposure at 10°C or 16°C. Stress was measured in terms of immunological responses to concanavalin A (Con A) or lipopolysaccharide (LPS). Asterisks denote cases in which the SI of stressed fish was significantly lower than that of control fish ($P < 0.001$).

liferative response was suppressed after hypotonic lysis, the magnitude and kinetics of the response to Con A were relatively unaffected by hypotonic lysis. By use of the culture conditions that were determined to create a functioning assay, assaying 25 nonreproductive, 2–3-year-old sablefish resulted in SIs ranging from 1.7 to 7.8 for LPS and 1.9 to 10.9 for Con A; the mean SI was 5.1 for LPS and 5.8 for Con A.

Laboratory Treatments

Cells from control fish (unstressed at 8°C) were capable of responding to LPS and Con A (SI = 5.0 and 6.0, respectively), while the proliferative responses were significantly depressed ($P < 0.001$) in fish that were towed for 2 h at 8°C followed by 15 min of exposure to 10°C or 16°C air (Figure 1). There was no difference in the immunological response of sablefish

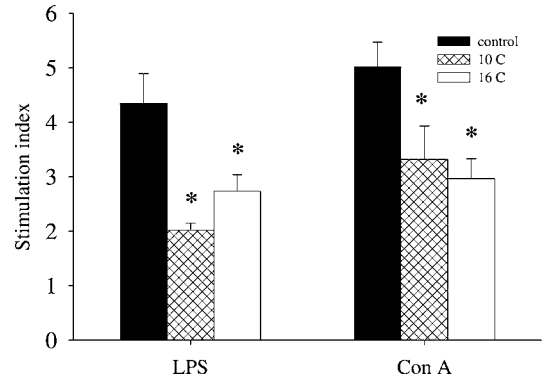

FIGURE 2.—Mean (±SE) stimulation indices (SIs) of control sablefish (unstressed at 8°C) and sablefish subjected to 2 h of simulated towing at 8°C followed by 15 min of exposure to an elevated seawater temperature of 10°C or 16°C. Stress was measured in terms of immunological responses to concanavalin A (Con A) or lipopolysaccharide (LPS). Asterisks denote cases in which the SI of stressed fish was significantly lower than that of control fish ($P < 0.001$).

exposed to air at 10°C or 16°C ($P > 0.5$). Relative to control fish, sablefish that were hooked for 2 h and then transferred to 10°C or 16°C air for 15 min also presented significantly diminished ($P < 0.001$) levels of leukocyte proliferation upon in vitro stimulation with LPS and Con A (Figure 1). No significant difference was discerned between the posthooking 10°C and 16°C air temperature treatments for either of the mitogens ($P > 0.05$). In addition, there was no significant difference in proliferative response between the two capture methods ($P > 0.05$).

Stimulation indices of sablefish towed for 2 h at 8°C followed by a 15-min exposure to an elevated seawater temperature of 10°C or 16°C were diminished significantly ($P < 0.001$) relative to controls for both LPS and Con A (Figure 2). A significant difference in the immunological response associated with seawater temperature was not detected in sablefish. Sablefish that were directly exposed to air temperatures of 10°C or 16°C for 15 or 30 min and then placed in a recovery tank with 8°C seawater also presented a loss of mitogen responsiveness. The SIs for LPS and Con A were significantly lower ($P < 0.05$) than those of control fish (unstressed at 8°C; Figure 3). However, the proliferative responses did not vary significantly ($P > 0.05$) due to air temperature or minutes of air exposure.

Physiological variables measured directly after each treatment indicated that the sablefish were undergoing significant stress responses. Plasma cortisol levels increased significantly ($P < 0.001$) from a mean of 18.0 ng/mL in control fish (unstressed at 8°C) up to

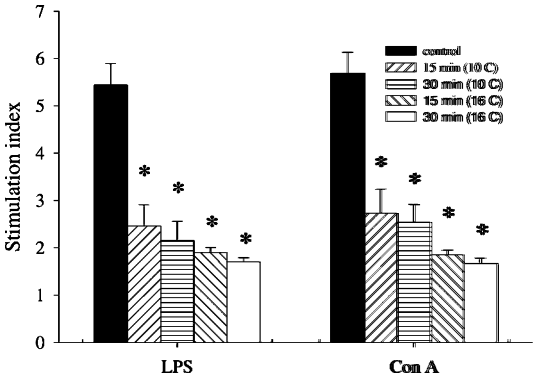

FIGURE 3.—Mean (±SE) stimulation indices (SIs) of sablefish subjected to different air exposure times (15 or 30 min) and air temperatures (10°C or 16°C). Stress was measured in terms of immunological responses to concanavalin A (Con A) or lipopolysaccharide (LPS). Asterisks denote cases in which the SI of stressed fish was significantly lower than that of control fish ($P < 0.001$).

a mean of 170.8 ng/mL in fish towed and held in air at 10°C and 92.4 ng/mL in fish towed and held in air at 16°C. Fish hooked and exposed to elevated air temperatures of 10°C or 16°C had mean cortisol levels of 220.9 and 176.4 ng/mL, respectively. In comparison to the controls, plasma cortisol levels were also significantly elevated ($P < 0.001$) in sablefish exposed to various air temperatures and times and in fish towed and exposed to elevated seawater temperatures (Figure 4). However, no significant differences in cortisol levels among the different treatments were detected (Figure 4). Regardless of capture stressor, the cortisol levels did not vary significantly ($P > 0.05$) due to air temperature or minutes of air exposure. Fish towed and exposed to elevated air temperatures of 10°C or 16°C had mean blood glucose levels of 165.0 and 158.5 mg/dL, respectively (Figure 5). Fish captured by hooking and exposure to elevated air temperatures of 10°C or 16°C had mean blood glucose levels of 114.0 and 121.5 mg/dL, respectively (Figure 5). In these fish, blood glucose levels increased significantly from a mean of 50.0 mg/dL (controls) to elevated glucose levels immediately after the induced stress and did not significantly differ between the two capture methods ($P > 0.05$) (Figure 5). In comparison to the controls, glucose levels were also elevated significantly ($P < 0.001$) in sablefish exposed to various air temperatures and times and in fish towed and exposed to elevated seawater temperatures (Figure 5). However, there were also no significant differences in the magnitude of glucose levels among the different treatments. Regardless of capture stressor, the glucose levels did not vary

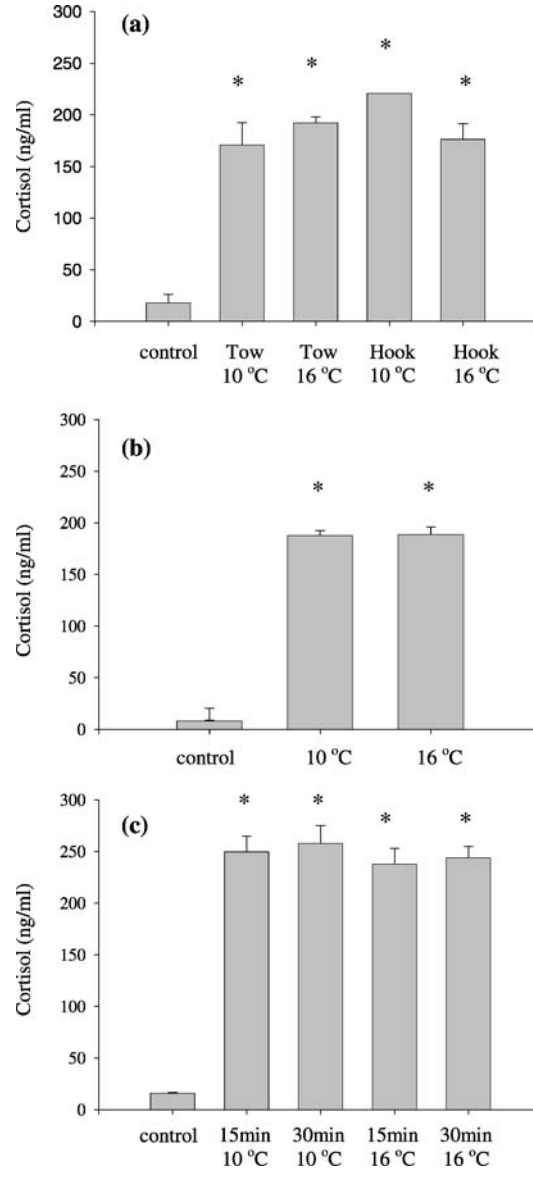

FIGURE 4.—Mean (±SE) cortisol concentrations (ng/mL) of control sablefish (unstressed at 8°C) and sablefish that were **(a)** subjected to 2 h of simulated hooking or towing at 8°C followed by 15 min of air exposure at 10°C or 16°C, **(b)** subjected to 2 h of simulated towing at 8°C followed by 15 min of exposure to an elevated seawater temperature of 10°C or 16°C, and **(c)** subjected to different air exposure times (15 or 30 min) and air temperatures (10°C or 16°C). Asterisks denote cases in which the cortisol concentration of stressed fish was significantly higher than that of control fish ($P < 0.001$).

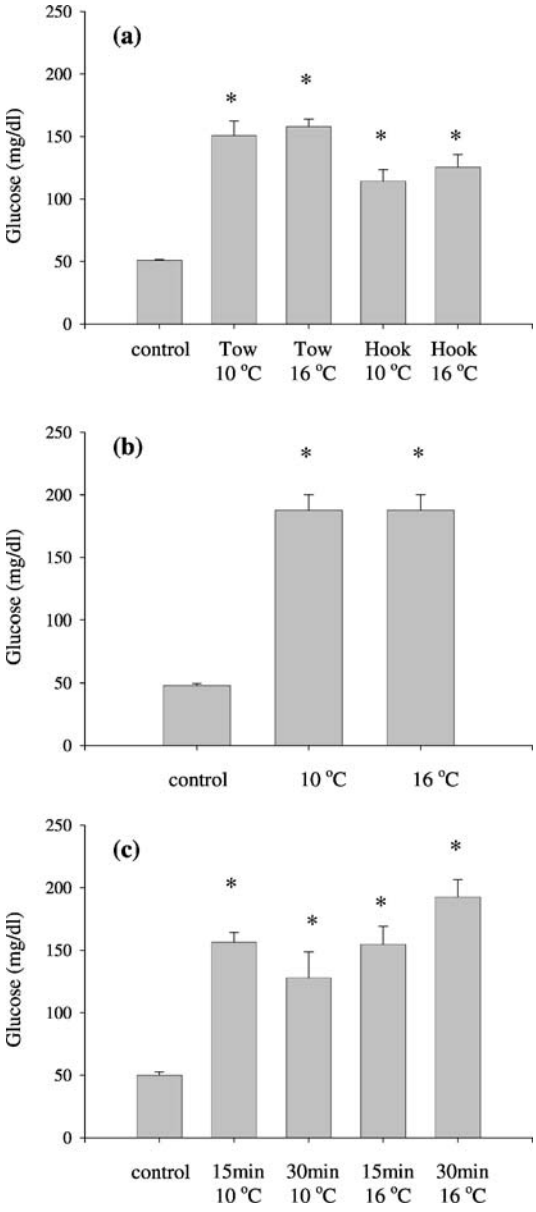

FIGURE 5.—Mean (±SE) glucose concentrations (mg/dL) of control sablefish (unstressed at 8°C) and sablefish that were **(a)** subjected to 2 h of simulated hooking or towing at 8°C followed by 15 min of air exposure at 10°C or 16°C, **(b)** subjected to 2 h of simulated towing at 8°C followed by 15 min of exposure to an elevated seawater temperature of 10°C or 16°C, and **(c)** subjected to different air exposure times (15 or 30 min) and air temperatures (10°C or 16°C). Asterisks denote cases in which the glucose concentration of stressed fish was significantly higher than that of control fish ($P < 0.001$).

significantly ($P > 0.05$) in response to air temperature or minutes of air exposure.

Discussion

Regardless of treatment, the duration and combination of capture and environmental stressors in our study were severe enough to result in a significant reduction in the ability of pronephric leukocytes from stressed sablefish to respond in culture to B- and T-cell mitogens. Immunosuppression during stress is frequently associated with decreased disease resistance to opportunistic pathogens (Wendelaar Bonga 1997), and our study demonstrates the cellular basis at which captured and released sablefish could be more susceptible to infectious diseases. The fact that the immune system is functionally impaired after exposure to capture-related stressors may shed some light on a potential mechanism of delayed mortality in discarded sablefish. If a fish is discarded back into the ocean, its ability to perform at the whole-organism level may be diminished for an extended period of time. Others have documented changes in feeding, social interactions, schooling, swimming ability, orientation, predator evasion, and mortality that corresponded with increased capture intensity in previous laboratory studies in sablefish, Pacific halibut, walleye pollock, and lingcod *Ophiodon elongatus* (for review, see Davis 2002). Davis (2005) suggested that in his laboratory study with sablefish towed in a net and exposed to air, delayed mortality resulted from external skin and fin infections at the site of injury.

Based on the available evidence, there is little doubt that the imposition of a stressor will result in altered immune responsiveness (Kusnecov and Rabin 1994; Dhabhar and McEwen 2001; Schreck 2000; Pruett 2003). The decreased lymphocyte responsiveness to mitogen stimulation and antigen stimulation and a reduction in lymphocyte cytotoxicity have been demonstrated in fish after the imposition of a stressor (Ellis 1981; Wendelaar Bonga 1997). The in vitro stimulation of fish lymphocytes from the peripheral blood, thymus, pronephros, and spleen is one of the most commonly used methods to assess the function of the cellular immune system (Luft et al. 1991; Faisal and Hargis 1992). The mitogen-induced proliferation of pronephric lymphocytes was used in this study, as it can be considered a reasonable first approximation to show that the immune system of a fish is capable of functioning (Ellsaesser and Clem 1986).

In our study, immunosuppression was observed in sablefish that were towed or hooked and then exposed to air or increased temperatures. In these experiments, sablefish were exposed to two sequential stressors for a cumulative duration of 2.25 or 2.5 h. The

combinations of sequential stressors used were nonlethal, resulting in no immediate mortality or delayed mortality in sablefish during previous capture-related laboratory experiments (M. W. Davis, unpublished data). Nonlethal conditions were used to avoid dealing with samples from morbid individuals and to ensure consistency in sample sizes.

There is little information on the immunological response of fish to exposure to two or more sequential stressors (Schreck 2000). Schreck (2000) presented a conceptual model of physiological stress response to sequential stressors, suggesting that if the stressors occur close together temporally then there can be cumulative effects. The cumulative effect of stressors is apparent in other laboratory studies in sablefish, Pacific halibut, walleye pollock, and lingcod, where stress increased with stressor intensity as measured by changes in feeding, orientation, predator evasion, and mortality (Olla et al. 1997, 1998; Davis and Olla 2001, 2002; Davis et al. 2001; Davis 2005). In these studies, the initial capture (towing or hooking) caused stress, which was then magnified by the addition of environmental stressors such as air or increased seawater temperature, resulting in impaired behavior and increased mortality. It was therefore surprising in our study that at treatment severities that were just below lethal levels, increased environmental air or seawater temperatures did not cause further immunosuppression. Increased levels of physiological stress and mortality have been observed to increase under field conditions of increased temperature (Barton and Iwama 1991). However, in sablefish towed for 4 h and then exposed to air, Davis (2005) observed that immediate mortality increased with increased time in air while delayed mortality did not. Because we did not quantify immune function after each successive stressor, we do not know whether the initial capture depressed the immune system so severely that it prevented further immunosuppression that might have been induced with each sequential stressor. However, we cannot disregard the possibility that the sequence of stressors, even at nonlethal severities, were debilitating to the immune system due to the cumulative effect of stressors. Further studies are needed to determine the time course of sablefish immunosuppression after capture.

Sablefish exposed directly to air for 15 or 30 min had decreased mitogen-induced lymphocyte proliferation. These results are consistent with other studies in which salmonids were subjected to nonlethal aerial emersions as brief as 30 s (Maule et al. 1989), which caused a significant depression in immune function. Many nonlethal acute stressors, including confinement, crowding, acute handling, transport, and increased water temperature, can also induce immunosuppression (Wendelaar Bonga 1997). Sublethal levels of stress produced by hypoxia have also caused impairment in immune function (Mellergaard and Nielsen 1995; Ortuno et al. 2002). In previous experiments, sablefish mortality was observed only after 30 min in air and increased dramatically between 30 and 50 min (Davis and Parker 2004).

The duration and severity of the capture stressors applied in our study were harsh enough to induce significantly elevated levels of cortisol and glucose. Davis et al. (2001) observed greater plasma cortisol, lactate, and potassium levels in sablefish that were towed and then subjected to elevated temperature and air than in those that were hooked and subsequently exposed to elevated temperature and air. However, in that study, the stress-induced capture of sablefish encompassed a total of 4 h—two times the duration used in our study. The greater duration of capture in their study possibly contributed to the further increases in cortisol and glucose levels observed after the towing stressor.

Our study demonstrates that the sablefish immune system is functionally impaired after exposure to capture-related stressors. The ability of discarded fish to perform at the whole-organism level may be diminished for an extended period of time. During stress, metabolic energy from performance and investment activities (e.g., growth, reproduction, and immune responses) is reallocated to activities required to restore homeostasis (e.g., cardiac output, oxygen uptake, and hydromineral balance) (Barton and Iwama 1991). Over time, the cumulative strain on a discarded fish from maintaining physiological adaptation reduces the fish's ability to sustain normal immunological functions. Therefore, the ability of the immune system to protect the fish from damage is impaired and may possibly predispose the fish to disease and eventually to death. However, our study is only an initial step towards future research on capture-related stressors and the sablefish immune function response. Discarded sablefish mortality will depend on the extent of the immunosuppression after capture and the presence of infectious agents. Further studies on the time course for the immune response and a disease challenge to ecologically available pathogens are needed to determine whether delayed mortality in sablefish discards can be caused by increased susceptibility to infectious agents resulting from stressor-related immunosuppression.

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