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## Linking “Sink or Swim” Indicators to Delayed Mortality in Red Snapper by Using a Condition Index

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**Abstract.**—The ability of fish to submerge after discarding is often used as a proxy for survival, but this practice underestimates total discard mortality because delayed mortality is overlooked. Fishery managers need a way to link “sink or swim” indicators, or variables observed during capture and release, with delayed mortality rates. We conducted a cage study of red snapper *Lutjanus campechanus* off the coast of Texas to estimate delayed mortality rates and to find factors that could link immediate and delayed mortality. Immediate mortality (17%) was predicted by the interaction of depth and the difference in temperature between surface and bottom waters. Lactate levels were also significant predictors of immediate mortality in fish whose blood was tested. Delayed mortality (64%) was predicted primarily by a condition index consisting of the presence or absence of injuries, symptoms of barotrauma, and fish behaviors immediately after capture. Specific categories included bleeding, protruding intestines, everted stomach, exophthalmia, the presence or absence of flapping and gilling behaviors, and problems with cage submergence. The majority of fatalities occurred within 24 h after fish were placed in the cages. Our mortality estimates indicate that red snapper discard mortality was significantly underestimated in the 2005 stock assessment for red snapper. The use of indices that relate the condition of an individual fish at capture to its probability of delayed mortality is an excellent method for linking immediate and delayed mortality and will likely be applicable to many species that are subject to catch-and-release fishing.

Fish are discarded in almost every commercial and recreational fishery. Fish may be discarded because the species or size of fish is not desired for economic or personal reasons. Fishermen may discard less-desirable fish when more desirable fish are obtained. Fish may also be discarded due to regulations such as minimum size limits, bag limits, or closed seasons that are intended to limit catches or to protect some segment of the population. The assumptions behind these regulations are that very few fish are discarded and that most fish survive the catch-and-discard process without detrimental effects.

Accurate estimates of discard mortality are critical not only to gauge the effectiveness of regulations but also for setting unbiased catch quotas. In fact, the underestimating of discard mortality has been cited as one cause of the collapse of northern Atlantic cod *Gadus morhua* stocks off the coast of eastern Canada

in the early 1990s (Myers et al. 1997). Because discarded fish are often juveniles or young adults, discard mortality mimics recruitment failure in terms of its population effects, since young fish are killed before they can recruit into the adult population (Diamond et al. 1999). Simulations show that stock assessments that exclude discard mortality overestimate spawning stock biomass and yield compared to assessments that include discard mortality (Breen and Cook 2002). Underestimating discard mortality can therefore lead to overestimates of the total allowable catch, which can result in depletion and, in some cases, collapse.

Survival rates of discarded fish are generally estimated over three time scales: (1) over minutes to hours after capture (immediate survival) by surface observation studies that assess the proportion of fish that can submerge immediately after release; (2) over hours to weeks (short-term or delayed survival) by holding fish in cages after capture or by means of telemetry to monitor fish after release; or (3) over days to years (long-term survival) by marking and recapturing released fish (Pollock and Pine 2007). However, using submergence data in place of survival data can be misleading, as the ability to swim immediately after release is not a direct measure of survival. Fish that submerge immediately after release may still die afterwards, while fish that are unable to submerge may survive (Patterson et al. 2002). Fishery managers therefore need some way to link “sink or swim” indicators observed during capture and release with

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longer-term survival rates that can be used in stock assessments.

An example of the need for such links is the directed fisheries for red snapper *Lutjanus campechanus* in the Gulf of Mexico (hereafter, the Gulf). The red snapper is the most economically important reef fish species in the Gulf. Red snapper have been classified as overfished since 1984 (Goodyear and Phares 1990), and fishery managers have implemented a complex series of measures to regulate the catch and rebuild the stock of red snapper, including size limits, bag limits, trip limits, fleet quotas, and seasonal closures (see Hood et al. 2007 for historical review). Unfortunately, all of these measures result in discarding of red snapper because of red snapper bycatch outside of the legal limits or seasons. In the 2005 stock assessment, release mortality in the recreational fisheries was estimated to be 40% for the western Gulf (Louisiana and Texas) and 15% for the eastern Gulf (Mississippi, Alabama, and Florida) based on literature estimates and the depth distribution of the recreational fisheries (GMFMC 2005). One major criticism of these estimates was that surface observation studies were treated as direct estimates of discard mortality and given equal weight to cage studies where short-term mortality was actually measured. There are concerns that the discard mortality rates used in the assessment are underestimates, introducing a large source of uncertainty into the assessment and biasing the resulting management actions.

Our objectives in this study were to estimate immediate and delayed mortality of red snapper caught in the recreational fisheries and to investigate sink or swim indicators observed during capture and release that could be used to predict delayed mortality. To accomplish our objectives, we caught fish off the coast of Texas at three depths and seasons and held them in cages for 1–7 d. We collected data on fishing parameters and fish condition, including physical injuries, fish behavior, and physiological status (symptoms of barotrauma and blood cortisol, lactate, and osmolality levels). Metrics on fish condition were combined into a condition index, which was tested as a predictor of immediate and delayed mortality. Our hypotheses were that delayed mortality would be higher than immediate mortality and that we could find predictors of delayed mortality based on capture and release conditions.

## Methods

*Field methods.*—Red snapper were caught on or near the bottom via hook and line in July, September, and October 2002 at three petroleum production platforms owned by British Petroleum, located 48.28

km (30 mi) off Port Aransas, Texas. Platforms were situated in 30, 40, and 50 m of water, respectively: MI-668 (27°56.141 N, 96°38.746 W), MI-703 (27°53.733 N, 96°25.679 W), and MU-762A (27°41.787 N, 96°34.823 W). When fish were landed, we recorded two types of data: (1) data on fishing conditions, including depth, season, air temperature on the deck where fish were handled, temperature of surface and bottom waters, type of bait, and type of hook, and (2) data on fish condition, including fish size (fork length [FL], mm), the elapsed time between hooking and landing the fish (fight time), the elapsed time between landing the fish and when the fish was returned to the water (air time), the elapsed time between landing and when the cage containing the fish was about 3 m below the water surface (surface time), the total time between hook-up and return to depth (handling time), the activity level of the fish on deck and in the cage (flapping, gilling, or motionless), external condition of the fish (extruded stomach, bulging eyes, distended anus, or bleeding), the number of attempts to draw blood, and the amount of blood drawn. In July, fight time was not measured, so we estimated fight time from the average for each platform in September and October. All fish were tagged by means of a Floy tag inserted in the muscle tissue below the dorsal fin, and the air was released from the swim bladder by means of a large-gauge needle or the Floy tag gun coated with antibiotic. About 0.5 cm<sup>3</sup> of blood was drawn from the caudal vein in two-thirds of the fish (“experimental fish”) by means of a 21-gauge needle, and the blood was immediately placed on ice. The other one-third of the fish were considered control fish, so no blood was drawn. When on the boat, fish were kept in the shade whenever possible, and the air temperature in the shade was recorded whenever a fish was caught.

We placed all fish in collapsible individual cages, which were sent down to 20-m depth as quickly as possible by means of weights to push the cages along a line (the “zip” line) tied from the boat to one of the legs of the platform. Pressure at a depth of 20 m is three times higher than surface pressure, so many of the symptoms of barotrauma should be largely relieved, especially with venting. The 20-m depth was therefore a compromise between our ability to observe caged fish by means of scuba and full recompression of the fish. Any problems with submergence of the cages due to currents or other problems were recorded. Cages (1.00 × 1.00 × 0.67 m) were constructed of polyvinyl chloride piping, with chicken wire on the top and bottom and 44.5-mm nylon webbing on the sides. When 10 cages had been sent down or 2 h had elapsed since the first fish reentered the water, scuba divers were deployed to suspend cages to top and bottom

ropes attached to the platform legs at 20 m. After immediate placement on the platform, scuba divers checked the fish for general condition about every 24 h. Cages with dead fish were retrieved as soon as possible, and dead red snapper were replaced with newly caught fish until the end of the sampling period. Individual fish were in the experiment for a minimum of 1 d and up to about 7 d. However, inclement weather and logistical difficulties in July prevented final cage retrieval for 13 d and allowed only 2 d of observations at platform MU-762A (50-m platform) in September. Cages with live fish at the end of the sampling period were taken to the surface via lift bag on the zip line, and fish were euthanized.

We measured water temperature and salinity at depth at each platform during each sampling period by means of a Sea-Bird SBE 19 conductivity–temperature–depth profiler (Sea-Bird Electronics, Bellevue, Washington). The only exception was that measurements were not taken at platform MU-762A during September. For purposes of the analysis, the measurements taken at platform MI-703 in September were used for platform MU-762A in September. True controls for the processes of hooking, handling, barotrauma, and release would be to capture fish by means of a different gear type that avoids these procedures (Pollock and Pine 2007). To capture true control fish, we set out baited traps. Traps were similar to the fish cages used for discard fish but included a bait bag and a funnel so that fish could enter but not exit. Traps were deployed on the bottom at various times during the sampling periods.

*Laboratory analysis.*—Three assays were conducted on fish blood. Blood lactate was measured while on the boat by means of whole blood with an Accutrend Lactate Meter (precision = 0.2 mmol/L). Because the lower threshold for detection of this kit is 0.8 mmol/L, any blood sample that registered as below threshold limits was given a lactate reading of 0.4 mmol/L. Whole blood was transferred to micro-centrifuge tubes and centrifuged for 5 min at 5,500 revolutions/min. After separation in the centrifuge, plasma was pipetted off and separated into aliquots for osmolality and cortisol analyses. Samples were then frozen for storage. Osmolality analysis was performed by means of procedures from Farrell et al. (2001). Prior to running each batch of samples, the osmometer (Wescor Vapro 5100 C) was calibrated by means of appropriate 290- and 1,000-mmol/kg standards until readings stabilized. Plasma samples were vortexed, and 10  $\mu$ L of the sample were tested in duplicate or triplicate depending on the amount of blood available. Any samples with more than a 10% difference in readings were tested until readings stabilized. Cortisol analysis was per-

formed by means of two different procedures. The first procedure involved a radioimmunoassay (RIA) method from Irwin et al. (1999), and we used a competitive binding Coat-A-Count cortisol kit (Diagnostic Products Corporation, Los Angeles, California). The second procedure used for cortisol was an enzyme immunoassay procedure (Cayman Chemical, Ann Arbor, Michigan). Red snapper specific standards for both assays were made following the method of Mitsuma et al. (1972). Blood samples were tested in duplicate when available.

*Statistical analysis.*—Because drawing blood could potentially increase the probability of mortality, we first used chi-square ( $\chi^2$ ) tests to test for differences in immediate or delayed mortality between blood control and experimental fish during each sampling period. There was no significant difference ( $\chi^2$ ,  $P > 0.05$ ) in either immediate or delayed mortality between control and experimental fish in September or October, but we did find a significant difference in delayed mortality between experimental and control fish in July ( $P = 0.04$ ). We therefore wanted to limit the mortality estimates to blood control fish only. Unfortunately, due to logistical problems, we had only four control fish in July. To increase our sample size but to ensure that our blood-drawing procedure did not affect the results, we included as control fish any experimental fish sampled in July that had one or fewer attempts at drawing blood and air times of less than 3 min. These experimental fish in July plus the control fish in each month were called “quasicontrol” fish. No significant difference was found in the percent mortality or the length frequency distribution ( $\chi^2$ ,  $P > 0.05$ ) between control and quasicontrol fish, so quasicontrol fish were used to estimate mortality and in all other analyses of factors affecting mortality.

Mortality was calculated as  $1 - S$ , where  $S$  (survival) is calculated as  $N_t/N_0$  ( $N_t$  = the number of fish at the end of the experiment;  $N_0$  = the number of fish at the beginning of the experiment). Standard errors (SEs) were calculated by means of equation (17) in Pollock and Pine (2007). For the purposes of this article, we use the term “mortality” to refer to the probability of dying rather than the instantaneous mortality rate ( $\log_e S$ ). Any fish that was dead when landed, gut hooked, or bleeding profusely from the gills was excluded from the analysis. All fish whose cages were put on the ropes attached to the platform were considered to have survived initially unless a subsequent check on the same day found that the fish was dead. Fish that were missing out of the cage were excluded from the analyses. Because the size limit refers to the total length (TL) rather than the FL, we converted the size limit to FL by means of a conversion equation

TABLE 1.—Number of red snapper captured for a survival study at three Gulf of Mexico petroleum platforms (30-, 40-, and 50-m depths). Control fish had no blood drawn, while experimental fish had about 0.5 cm<sup>3</sup> of blood drawn from the caudal vein. Because of the low number of control fish in July, quasicontrol fish are those with only one attempt at drawing blood and an “air time” (time between landing and return to the water) of less than 3 min.

Season	Depth (m)	Control			Quasicontrol		
		Control	Experimental	Total	Control	Experimental	Total
Jul	30	2	35	37	10	27	37
	40	0	9	9	4	5	9
	50	2	34	36	7	29	36
	Subtotal	4	78	82	21	61	82
Sep	30	22	25	47	22	25	47
	40	23	32	55	23	32	55
	50	16	19	35	16	19	35
	Subtotal	61	76	137	61	76	137
Oct	30	9	21	30	9	21	30
	40	14	18	32	14	18	32
	50	14	24	38	14	24	38
	Subtotal	37	63	100	37	63	100
Total		102	217	319	119	200	319

estimated from measurements of 2,665 red snapper in Texas waters in 2005:  $TL = 1.0653 \cdot FL + 0.472$  ( $R^2 = 0.994$ ). A two-way analysis of variance (ANOVA) with unequal sample size was run on fish length to determine differences by season and depth.

We used logistic regression (forward stepwise regression by means of the likelihood ratio statistic for entering and removing variables) in the Statistical Package for the Social Sciences (SPSS) version 15 (SPSS, Chicago, Illinois) to determine which factors significantly contributed to immediate and delayed mortality. We used the following variables in the logistic regression: depth, temperature differential between surface and bottom waters (temperature differential), the interaction of depth and the temperature differential, air time, fish length, air temperature, and condition index, as well as all of the individual variables in the index. To create the fish condition index, we first coded the presence or absence of flapping and gilling behaviors, extruded intestines, everted stomach, exophthalmia, bleeding, or problems with cage resubmergence as binary variables, giving one point for each healthy response (maximum of seven points). Everted stomach was considered the healthy condition in the index, due to the higher initial survival of fish with everted stomachs (S. L. Diamond, unpublished data). The sum of these variables was used as the condition index. For delayed mortality, we used only those fish that survived initially. We also looked at correlations between environmental variables to reduce the number of parameters in the logistic models by means of ANOVAs and Tukey’s honestly significant difference (HSD) tests (SPSS version 15). For all logistic models, hook type and hook location were not

used because almost all of the fish were caught with circle hooks in the mouth.

Since most of the quasicontrol fish had no blood drawn, the logistic regressions for blood parameters were performed only on experimental fish. However, due to the small amount of blood drawn from each fish, problems with blood clotting in the syringe before blood could be frozen and processed, and problems with the cortisol RIA, which resulted in cortisol values well below the standard graphs, very few fish had concurrent data for lactate, osmolality, and cortisol, resulting in small sample sizes. We ran these logistic equations with lactate, osmolality, cortisol, and all of the same parameters as in the logistic regressions for quasicontrol fish, including depth, air time, air temperature, temperature differential, the interaction of depth and the temperature differential, the fish condition index, and individual variables in the index. We also included the number of attempts to draw blood to see if the blood-drawing procedure contributed significantly to mortality. To understand more of the mechanisms behind fish stress and the discard experience, we also looked at the correlation between blood factors and other parameters (e.g., depth, air temperature, fight time, and air time) by means of the correlation function in SPSS. Finally, we analyzed the timing of mortality once fish were caged to see if delayed mortality was due to caging or to the fishing process. Due to variable visibility conditions underwater, inclement weather, or logistical problems, we sometimes did not check the fish condition each day. If fish were found dead upon our return, we considered the fish to have been dead on the day after it was last seen alive.

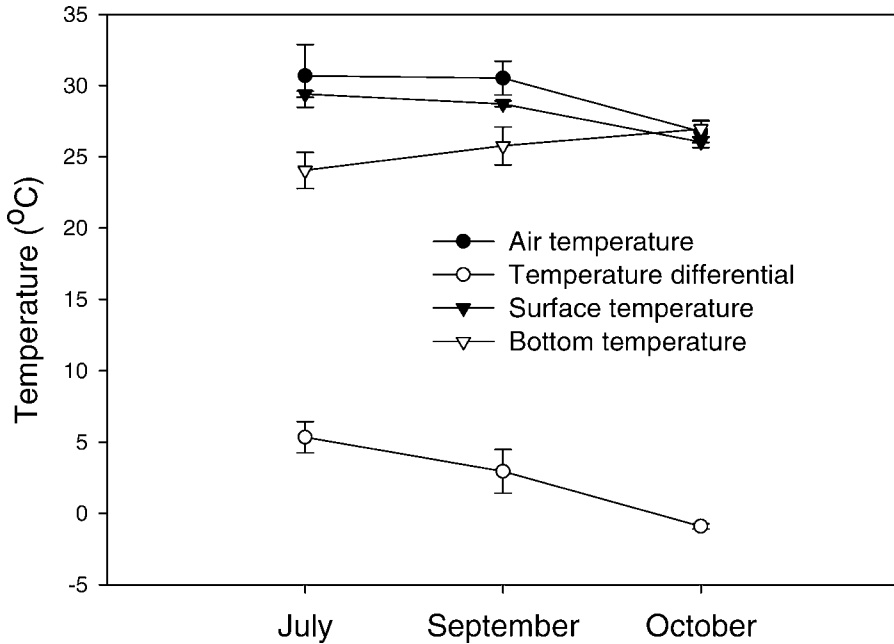


FIGURE 1.—Average ( $\pm$ SD) monthly air temperatures measured in the shade where red snapper were measured on deck. Surface and bottom water temperatures were measured with a Sea-Bird SBE 19 conductivity–temperature–depth profiler. July and September air temperatures were significantly different from October air temperatures, while surface and bottom water temperatures and the temperature differential between surface and bottom waters differed significantly each month.

**Results**

A total of 320 red snapper were caught during 17 d of sampling. Fish caught were fairly evenly divided by month and platform (Table 1). A total of 119 quasicontrol fish were used in the mortality analyses. Despite repeated attempts, no fish were captured in the traps, so we had no procedural control fish.

*Fishing Conditions*

Mean air temperatures for the July and September sampling periods were similar to each other but significantly warmer than air temperature for the October sampling period ( $P < 0.05$ ) based on Tukey’s HSD tests (Figure 1). Surface water temperatures and the temperature differential between surface and bottom waters differed significantly in each of the three sampling periods, decreasing over time ( $P < 0.001$ ). Bottom water temperature increased significantly each season ( $P < 0.01$ ) and was the only environmental parameter that differed significantly by depth, with higher bottom temperatures at the 30-m platform than at the 40- or 50-m platform. The ocean in July and September had a pronounced thermocline, while the ocean in October was well mixed (Figure 1).

Mean fight time of quasicontrol fish was generally 40–60 s and increased slightly with depth. Air time

averaged around 2.5 min at all platforms. Submergence of the cages took less time at the 30-m platform than at the other platforms and averaged 30–45 s. Some problems with cage submergence were encountered on sampling days when surface currents were very strong. Overall handling time (from initial hook-up to resubmergence to 3 m below the surface) was around 4 min at each platform. Fight time, air time, surface time, and handling time were not significantly different by month or depth ( $P > 0.05$ ) but did vary significantly between control and experimental fish, particularly in July.

*Fish Condition*

Fish ranged in length from 19.7 to 51.6 cm FL. Mean FL of quasicontrol fish captured during the experiment was 34.4 cm (37.1 cm TL). Fish length was significantly different by depth and season, with a significant interaction term (depth  $P = 0.0488$ , season  $P < 0.001$ , depth  $\times$  season interaction  $P = 0.0091$ ). Mean FLs of fish captured in October (32.1 cm) were significantly smaller based on Tukey’s HSD than fish captured in either July (35.3 cm) or September (35.7 cm). Mean FLs of captured fish were 33.7 cm (36.2 cm TL) at the 30-m platform, 35.1 cm (37.9 cm TL) at the 40-m platform, and 34.7 cm (37.4 cm TL) at the 50-m

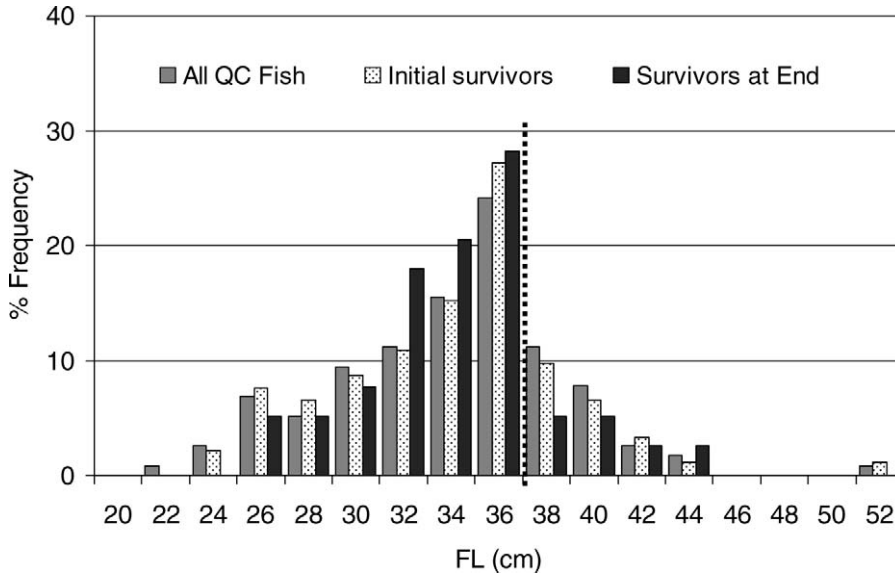


FIGURE 2.—Length frequencies (fork length [FL]) of red snapper: all quasicontrol (QC) fish captured (gray bars), survivors of immediate mortality (pattered bars), and survivors of delayed mortality (black bars). The dashed line represents the legal size limit of 37.7 cm FL (40.6 cm total length).

platform. The length frequency distributions of fish that survived initially and after several days were not significantly different from the distribution of all quasicontrol fish caught ( $P = 0.95$ ,  $P = 0.48$ , respectively; Figure 2).

Out of the 119 quasicontrol red snapper, 53% exhibited external signs of trauma, with 42% showing everted stomachs, 12% experiencing bulging eyes, 2.5% suffering intestinal protrusion from the anus, and 2.5% with bleeding (Figure 3). Only 35% of fish were flapping on deck, and 51% showed either gulping or decreased respiration. In general, fish showed more symptoms of injury, barotrauma, and behavioral inhibition in July and at the 50-m platform than in other seasons and depths. There was no clear relationship between the condition index and immediate mortality, although the relationship between the condition index and delayed mortality was significant ( $P = 0.0183$ , adjusted  $R^2 = 0.884$ ; Figure 4). The equation for the relationship was delayed mortality =  $a / \{1 + \exp^{-(x - x_0)/b}\}$ , where  $a = 1.1517$ ,  $b = 1.8193$ , and  $x_0 = 6.5470$ . Fish condition significantly decreased as depth increased ( $P = 0.027$ ), and condition was significantly negatively correlated with lactate ( $P = 0.003$ ), osmolality ( $P = 0.020$ ), cortisol ( $P = 0.014$ ), and fight time ( $P = 0.047$ ).

Combining sites and seasons, mean lactate level for experimental fish was 1.48 mmol/L, mean osmolality level was 443.77 mmol/kg, and mean cortisol level was 0.31  $\mu\text{g/mL}$ . Based on ANOVAs, lactate levels were

not different by depth but showed a strong but nonsignificant trend by season ( $P = 0.051$ ). Osmolality levels were highest at the 50-m platform by depth and lowest in July by season, but these differences were not significant. Cortisol levels were positively correlated with increasing depth (correlation analysis,  $P = 0.004$ ), but trends were not significant by season. Lactate levels were significantly positively correlated with air temperature ( $P = 0.043$ ), FL ( $P = 0.009$ ), and osmolality ( $P = 0.004$ ). Osmolality levels were positively correlated with lactate levels ( $P = 0.004$ ).

*Mortality*

Overall mortality of control fish (no blood drawn) combined over season and depth was  $17.7\% \pm 3.8\%$  (mean  $\pm$  SE) at the initial check and  $67\% \pm 4.7\%$  at the final check, while mortality of all fish combined (control and experimental) was  $23\% \pm 2.4\%$  and  $74\% \pm 2.5\%$ , respectively (Figure 5). Mortality of the quasicontrol fish was very similar to that of control fish (initial:  $16.2\% \pm 3.4\%$  [mean  $\pm$  SE]; immediate delayed:  $64.4\% \pm 4.4\%$ ). Immediate mortality of quasicontrol fish was significantly different by season ( $P = 0.015$ ), with lowest mortality in October (5%) and highest mortality in July (24%; Figure 6). Delayed mortality was also lowest in October (59%) and highest in July (76%), but the differences were not significant ( $P = 0.37$ ). By depth, immediate mortality was lowest at the 40-m platform (7%) and highest at the 50-m platform (27%) and delayed mortality was lowest at the

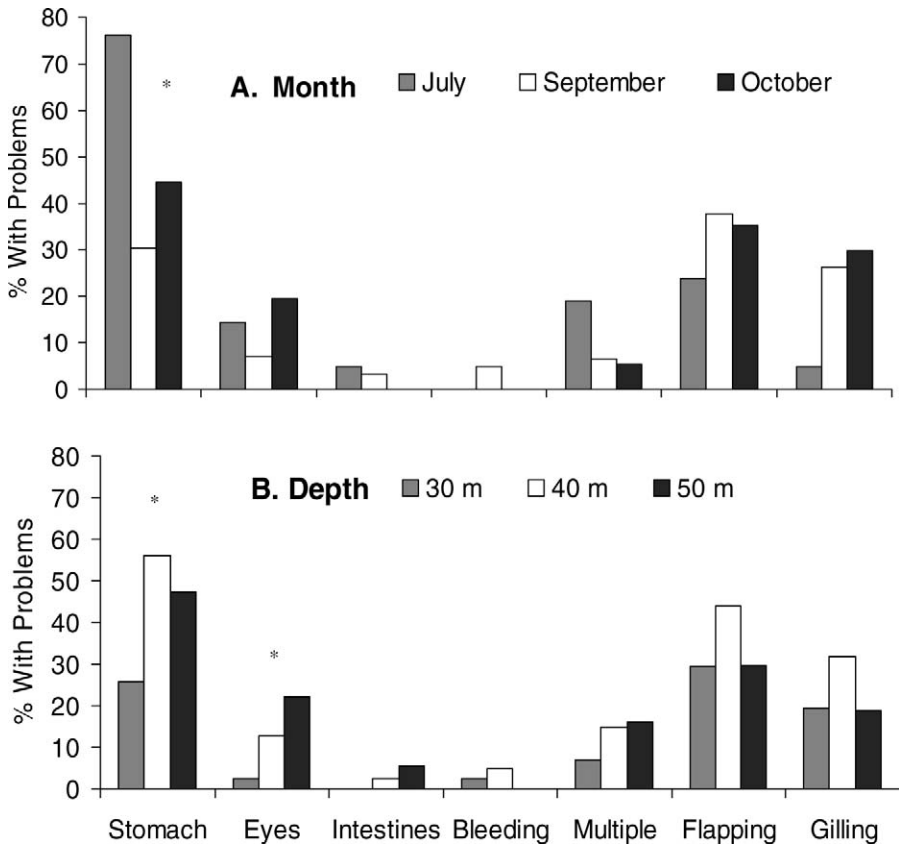


FIGURE 3.—Symptoms of barotraumas, injury, and behavioral impairment in red snapper by month and depth at three Gulf of Mexico petroleum platforms (30-, 40-, and 50-m platforms): stomach = stomach eversion; eyes = exophthalmia; intestines = intestines protruding out of the anus; multiple = more than one symptom; flapping = the ability of the fish to flap while on deck; and gilling = the presence of normal gilling activity.

30-m platform (61%) and highest at the 40-m platform (71%), but these differences were not significant (Figure 6).

Logistic binary regressions of immediate mortality conducted on quasicontrol data showed that significant parameters for immediate mortality were everted stomach and the interaction of depth and the temperature differential (Table 2). The model was an adequate fit to the data (Hosmer–Lemeshow statistic = 0.197). The proportion of variability explained by the full model was 0.180 (Nagelkerke  $R^2$ ). The odds ratios showed that for every 1°C increase in the difference between surface and bottom water temperatures by 1 m of depth, red snapper were 0.3% more likely to die. Fish without everted stomachs were almost five times more likely to die than fish whose stomachs were everted. Very similar results were obtained when control fish only were used in the analysis. In addition, substituting season and depth, season and platform, or

sea surface temperature for temperature differential also produced very similar results due to correlations between these parameters.

Logistic binary regressions run on delayed mortality in fish that survived initially showed that the fish condition index was the only significant predictor of delayed mortality (Table 3). The model was an adequate fit to the data (Hosmer–Lemeshow statistic = 0.581). The proportion of variability explained by the full model was very small (Nagelkerke  $R^2 = 0.05$ ). The odds ratios showed that for every improved level in the condition index, fish were 41% less likely to suffer delayed mortality. Virtually the same results were obtained when control fish only were used in the analysis, and as with immediate mortality, substituting season and depth, platform, surface temperature, or the temperature differential between surface and bottom waters produced very similar results.

When blood parameters were included, logistic



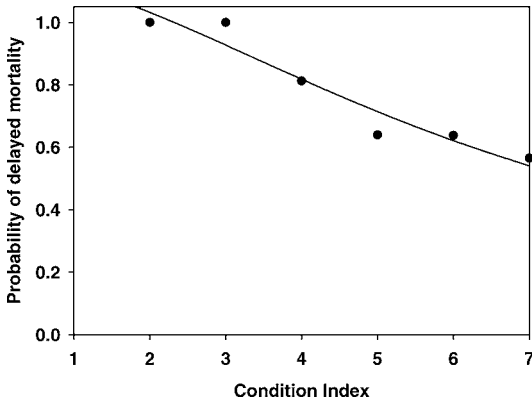


FIGURE 4.—Mean probability of delayed mortality of quasicontrol red snapper by condition level. The condition index consisted of the following factors: stomach eversion, exophthalmia, protruding intestines, bleeding, ability to flap normally, ability to gill normally, and problems with cage submergence. The normal or healthy condition was given one point except in the case of everted stomach, where the presence of eversion was given one point due to higher survival (S. L. Diamond, unpublished data). The index was the sum of points. Delayed mortality was measured after 1–7 d of holding in the cage.

regression results showed that the binary logistic model was an adequate model to describe the immediate mortality of experimental fish ( $P < 0.001$ ), although sample size was only 36 fish (Table 2). With all parameters included, the model explained much of the variability in immediate mortality (Nagelkerke  $R^2 = 0.626$ ). Significant parameters were lactate and the depth  $\times$  temperature differential interaction. Based on the odds ratio, every unit increase in lactate (mmol/L) resulted in almost sixfold increase in mortality, while every increase in temperature by meter resulted in a 0.5% increase in mortality.

The logistics model including blood data of experimental fish was also an adequate fit for delayed mortality ( $P < 0.001$ ), although sample size was only 41 fish (Table 2). Temperature differential and the condition index were found to be significant predictors of delayed mortality in experimental fish. The model explained some of the variability in mortality (Nagelkerke  $R^2 = 0.313$ ). The odds ratios showed that for every 1°C increase in the difference between surface and bottom temperatures, red snapper were 1.6 times more likely to die, while for each one-point improvement in condition level, the fish were about 70% less likely to die.

The number of hours of survival varied because fish were captured on different days and so were kept for a variable amount of time (a rightward truncated distribution); however, mortality was generally calcu-

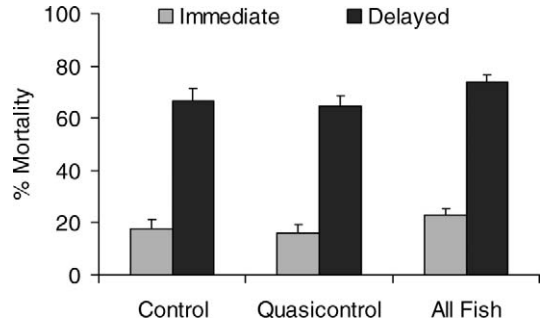


FIGURE 5.—Immediate and delayed mortality of control, quasicontrol, and all red snapper combined by season and depth. Immediate mortality was the percentage of fish that died during the first day of capture, while delayed mortality was the percentage of fish that died after 1–7 d in the cage. All fish included control fish, quasicontrol fish, and fish whose blood was drawn.

lated for between 2 and 5 d. The highest mortality occurred between initial capture and 24 h after capture, regardless of depth or season (Figure 7). Generally, if fish survived for 48 h, then they most likely survived regardless of the amount of time spent in the experiment.

**Discussion**

Our experiment shows that delayed mortality, which often occurs in the first 24–48 h after fish are caught, is much higher than immediate mortality, making it likely that surface observations of a fish’s ability to submerge are underestimates of delayed discard mortality. For instance, immediate mortality of red snapper in this study was 17%, while delayed mortality was 64%. Furthermore, out of the 100 red snapper that initially survived the discard process, 60% later died. In addition, the factors that were significant in predicting immediate mortality were different than the factors that predicted delayed mortality. Based on factors that were significant at predicting mortality, immediate mortality was related to the environmental conditions of capture, such as depth and thermal stress, while delayed mortality was related to the condition of the fish, including injury, barotrauma, and behavioral impairment. Therefore, the use of indices that relate the condition of an individual fish at capture to its probability of delayed mortality is an excellent method for linking immediate and delayed mortality, a result that also has been found with fish that have been towed in a net (Davis and Ottmar 2006; Davis 2007).

*Factors Affecting Mortality*

One of the main parameters found to be a significant indicator of increased immediate mortality was the

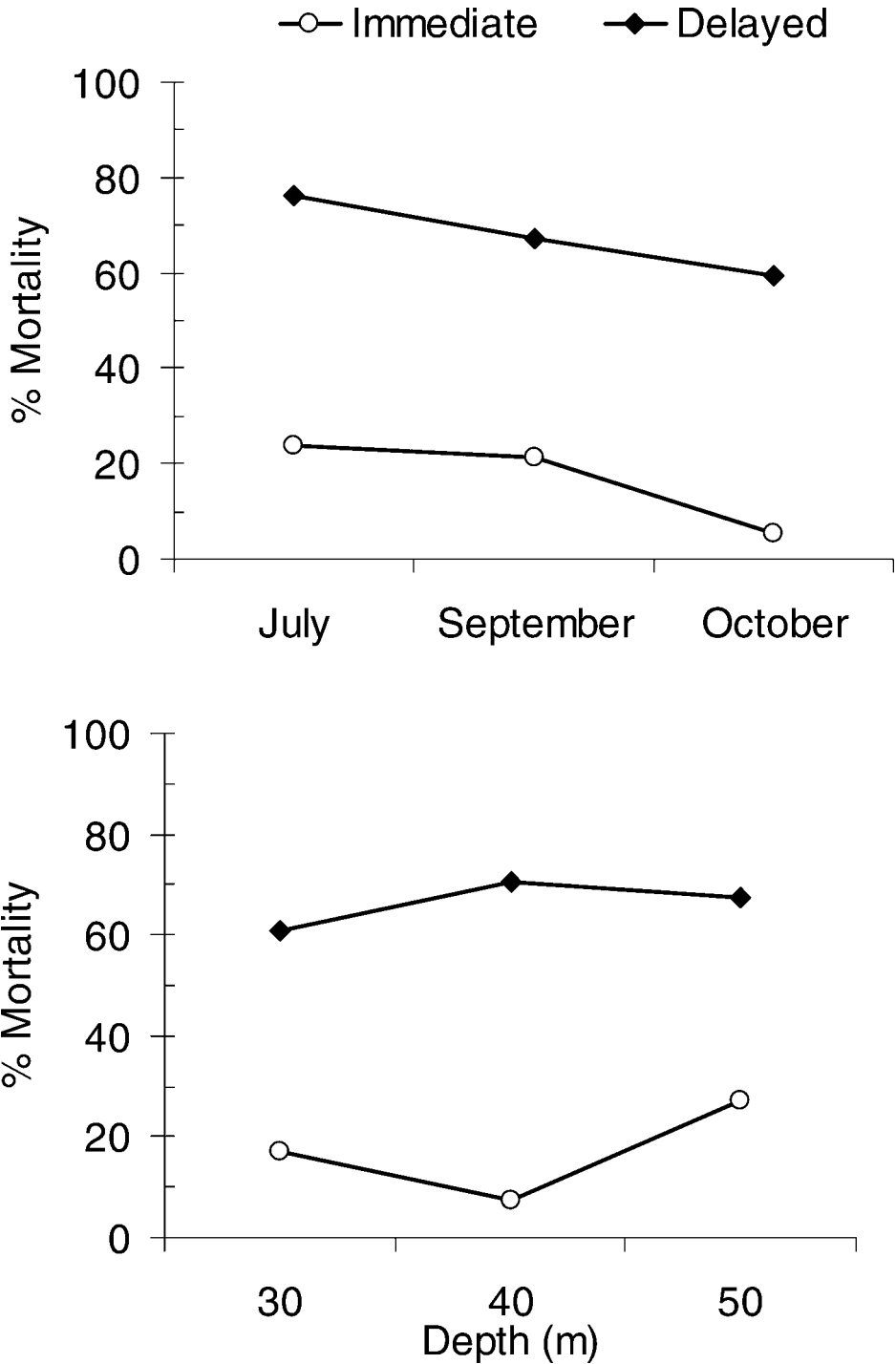


FIGURE 6.—Immediate and delayed mortality by petroleum platform depth and season for quasicontrol red snapper. Immediate mortality was the percentage of fish that died during the first day of capture, while delayed mortality was the percentage of fish that died after 1–7 d in the cage.

TABLE 2.—Results of binary logistic regression analyses ( $b$  = regression coefficient) of immediate and delayed mortality of quasicontrol red snapper (no blood parameters included). Variables included in the model were depth; air time; air temperature; ability to flap and gill normally; absence of everted stomach, protruding intestines, or bulging eyes; temperature differential between sea surface and bottom waters (temperature differential); and interaction of depth and temperature differential.

Mortality	Variable	$b$	df	$P$	Odds ratio (exp[ $b$ ])
Immediate	Everted stomach	-1.559	1	0.016	0.210
	Depth (m) × temperature differential (°C)	0.003	1	0.016	1.003
	Constant	-3.420	1	<0.001	0.033
Delayed	Condition index	-0.372	1	0.056	0.689
	Constant	2.764	1	0.016	15.575

interaction of depth and the temperature differential. Thermal stress has been implicated as a major cause of mortality for many species of marine and freshwater fish captured in the field (Plumb et al. 1988; Barton and Iwama 1991; Muoneke and Childress 1994; Schisler and Bergersen 1996; Ross and Hokenson 1997). Thermal stress can operate in two ways: (1) water temperatures encountered during capture and discard may be outside the range of temperature tolerances for the species and (2) temperatures encountered may be different than the temperature to which the fish is acclimated. Red snapper discarded in the Gulf can encounter both sources of thermal stress. For example, red snapper mortality in this experiment was highest in July and lowest in October. Fish caught in July were subjected to surface water temperatures of about 30°C, which is at the upper limit of their optimal temperature range based on red snapper distribution patterns in the Gulf (S. L. Diamond, unpublished data). Due to the thermocline that develops during the summer months off Texas, these surface water temperatures were 4–6°C higher than the bottom water temperatures to which fish were acclimated. Other studies have found that displacement from cooler temperatures into water that is 3–5°C warmer can cause mortality in fish (Piper et al. 1982). Although September air and surface water temperatures were similar to those in July, the temperature difference between the surface and bottom waters was less by about 1.5°C, which reduced the temperature differential by 32%. Both immediate and delayed mortality rates in September decreased by about 12% compared to July, from 24% to 21% for immediate mortality and from 76% to 67% for delayed mortality (Figure 6). In October, the waters were well-mixed and cooler, with cooler water at the surface, warmer water at depth, and a temperature differential of

TABLE 3.—Results of binary logistic regression analyses ( $b$  = regression coefficient) of immediate and delayed mortality of experimental red snapper, including lactate, osmolality, and cortisol. Variables included in the model were depth; air time; air temperature; ability to flap and gill normally; absence of everted stomach, protruding intestines, or bulging eyes; temperature differential between sea surface and bottom waters (temperature differential); number of attempts to take blood; and interaction of depth and temperature differential.

Mortality	Variable	$B$	df	$P$	Odds ratio (exp[ $b$ ])
Immediate	Depth (m) × temperature differential (°C)	0.005	1	0.075	1.005
	Lactate (mmol/L)	1.771	1	0.009	5.876
	Constant	-6.390	1	0.004	0.002
Delayed	Temperature differential (°C)	0.502	1	0.039	1.652
	Condition index	-1.296	1	0.028	0.274
	Constant	7.480	1	0.026	1,772.153

less than 1°C. Immediate mortality was 79% lower (from 24% to 5%) and delayed mortality was 22% lower (from 76% to 59%) in October than July.

As in our study, immediate mortality is often found to significantly increase as depth increases (Gitschlag and Renaud 1994; St. John and Syers 2005; Rummer 2007) due to catastrophic decompression syndrome (Rummer and Bennett 2005). Catastrophic decompression syndrome is the collection of injuries such as internal bleeding and damage to organs caused by the expansion of the swim bladder as the fish is pulled from several atmospheres of pressure to the surface. Increased depth of capture increases bladder size, decreases the body cavity space available to organs, and increases compaction injuries to vital organs in red snapper (Rummer and Bennett 2005). The impact of depth is strongly influenced by water temperature, as fish are found to be more impaired and to recover more slowly when subjected to both deeper depths and a large temperature differential than when subjected to changes in depth alone (Campbell et al., in review).

Other significant parameters in immediate mortality were the presence of stomach eversion in quasicontrol fish and lactate levels in the fish with blood drawn. Although the finding that stomach eversion was correlated with lower immediate mortality was unexpected, stomach eversion may allow more room for the expanded air bladder and may lessen compaction injuries to the other organs. However, other consequences of everted stomach, such as rupture of the stomach, infection, or problems with blood supply, may lessen the beneficial impacts over the longer term. With the exception of stomach eversion, our results were similar to those found in other studies (see Davis

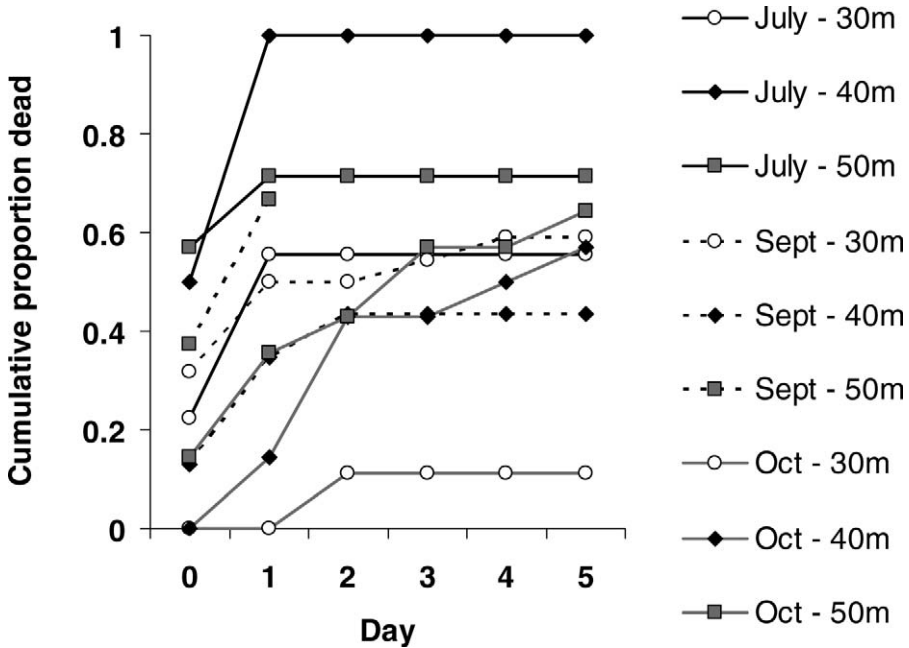


FIGURE 7.—Timing of red snapper mortality (cumulative proportion dead in relation to number of days of holding in cages) by season and Gulf of Mexico petroleum platform. If a cage was not checked every day and the fish was dead at the next condition check, the fish was considered to have died the day after it was last seen alive. Fish were sampled in July (solid black lines), September (dotted lines), and October (dotted–dashed lines) at platforms MI-668 (30 m; open circles), MI-703 (40 m; black triangles), and MU-762A (50 m; gray squares).

2005 and Rummer 2007 for reviews). The correlation of lactate levels with mortality in the presence of elevated temperatures has been seen in other fish, such as Pacific halibut *Hippoglossus stenolepis* (Davis and Schreck 2005) and sablefish *Anoplopoma fimbria* (Davis et al. 2001), although only under specific conditions. Lactate is a metabolic byproduct of exhaustive exercise (Kieffer 2000), which in itself can cause mortality through intracellular acidosis (Wood et al. 1983) or reduced heart rate and arrhythmia (Cooke et al. 2002). Similar to our findings, other blood factors such as cortisol are not well correlated with mortality in most studies of discarded fish (Davis 2002).

In all of our tests, the interaction of depth and season, sea surface temperature, and the difference in temperature between surface and bottom waters were surrogates for each other in the logistic models because these parameters were significantly correlated. We also found that there was a fixed effect of platform in this experiment beyond the interaction of depth and season. Fish at the 40-m platform (MI-703) showed lower immediate mortality, higher delayed mortality, a higher incidence of barotrauma and physical injuries, and less behavioral inhibition than would be expected based on

depth and temperatures alone. This platform was different in other ways as well; we caught only eight fish there in July compared to 35–40 fish at the other two platforms, despite expending equal or higher fishing effort. It is uncertain whether these differences in the 40-m platform were caused by different water conditions, different activities around the platform, or other reasons.

*Condition Indices*

A condition index is one of the best ways to link sink or swim indicators with delayed mortality because such an index makes it possible to gauge the probability of mortality of an individual fish based on its own status. A good index should incorporate the three sources of individual variability in discard mortality: physical condition and injury, physiological condition, and behavioral responses (Davis 2005). Physical condition includes symptoms of barotrauma (e.g., intestinal protrusion or exophthalmia) along with injuries (e.g., bleeding or wounds). Physiological condition includes factors such as cortisol levels or other indicators of stress, gut fullness, or reproductive condition. Behaviors such as flapping, swimming, and ability to orient are indications that fish are capable of at least some

normal functioning. Davis (2005) recommends additional testing of basic reflexes such as the vestibular–ocular and gag responses, as these reflexes indicate the functioning of other organs or systems that might be adversely affected by the capture and discard process. Behavioral response tests can also help predict the likelihood of increased predation due to discarding, as these simple metrics are related to burst swimming speed and other predator avoidance behaviors (Ryer et al. 2004; Campbell et al., in review).

The condition index we used in this experiment was a simple sum of points for the “healthy” condition (i.e., the lack of bleeding, protruding anus, or bulging eyes; stomach eversion; and the ability to flap and gill normally). Although submergence ability was not measured in this study, we added one point to the condition index for quick submergence of the cage to make the condition index more transferable to surface observation studies where submergence ability can be directly measured. Metrics such as the ones listed here are easy to measure under field conditions and can be assessed by observers without much additional handling time or air exposure. In addition, since many of the studies previously conducted included data on fish condition, it may be possible to retroactively estimate delayed discard mortality from past observational studies. As more information is gathered on the mechanisms of delayed discard mortality, the condition indices can be further refined to include weighting of factors that are more relevant or more highly correlated with discard mortality.

#### *Management Implications*

The management implications of underestimating delayed mortality rates are illustrated by the recreational fisheries for red snapper. The red snapper stock assessment (GMFMC 2005) used discard mortality estimates of 15% and 40%, respectively, for the eastern and western Gulf. The stock assessment therefore underestimates discard mortality because these are estimates of immediate mortality instead of delayed mortality. Based on our estimates, the average delayed discard mortality would be over 60%. With mortality rates such as these, the size limit for red snapper in the Gulf may be preventing rebuilding of the stock by leading to the mortality of too many undersized fish, particularly if commercial mortality rates are equally high. In addition, since discard mortality is underestimated, the total allowable catch projected by the stock assessment is probably overestimated, allowing excessive catches and further undermining the stock recovery and rebuilding process. Due to the likelihood of overestimating yield and stock biomass resulting from underestimation of discard mortality in stock

assessments, it is imperative that fishery managers avoid the use of submergence ability as a proxy for discard mortality in red snapper stock assessments.

#### *Caveats*

Because we were unsuccessful with our trapping efforts, we have no true controls for this experiment. As a result, we can neither separate the different components of discard mortality (e.g., hooking, handling, and barotrauma) nor subtract mortality due to natural nonpredation mortality. However, the short duration of the experiment makes it unlikely that natural mortality would be a major factor. In addition, the continued survival of fish during the 13 d in July that we were unable to retrieve the cages after the experiment concluded indicates that fish can survive in the cages without being fed.

All cage experiments have two characteristics that bias the estimates of mortality: (1) cage effects that increase mortality relative to actual fishing conditions and (2) predator exclusion that decreases mortality relative to actual fishing conditions. Cage effects can increase delayed mortality through starvation, stress, and infection. For example, skin infections are a cause of delayed mortality in trawl-caught sablefish (Davis 2005). In the current experiment, cage stress could have resulted from handling, being penned against the cage by strong currents, starvation, or venting. However, although some red snapper did show signs of cage stress, such as fin erosion and skin lesions, almost all of the mortality took place during the first 2 d, indicating that cage effects were not a major factor in the estimates of delayed mortality. In addition, the survival of fish in the cages for 13 d after the end of the July experiment as mentioned above also suggests that cage effects were not a major source of bias in these mortality estimates. However, predation of released fish can be a significant contributor to mortality under actual fishing conditions (Campbell et al., unpublished data). Red snapper in the cages were protected from predators (e.g., dolphins *Tursiops truncatus* and barracudas *Sphyræna* spp.) that target discarded fish; thus, the mortality of these fish was decreased by an unknown amount because of the protection afforded by the cages. In addition, any sublethal effects of discarding, such as slower swimming speeds or other behavioral impairment, would not be detrimental to caged fish, as they would be if discarded fish were swimming freely. Since the effects of these competing biases cannot be measured at this time, our cage experiment represents a compromise between underestimating and overestimating mortality.

Many studies show that the relative amount of stress an individual is undergoing can be measured with

lactate, osmolality, and cortisol levels (Pankhurst and Sharples 1992; Olla et al. 1997, 1998; Pottinger 1998; Davis et al. 2001; Barton 2002; Davis 2005). However, the feasibility of these stress factors as predictors of mortality varies depending on the study or the species of interest. For example, Davis et al. (2001) showed a lack of concordance between mortality and levels of cortisol, glucose, sodium, and potassium in sablefish. While these variables did generally correspond to the types and magnitudes of stressors, the values peaked at lower stressor levels than those producing mortality. Lactate levels have been correlated with mortality in Pacific halibut but only under specific conditions of elevated temperature (Davis and Schreck 2005). While we found that lactate level was a significant factor in predicting initial mortality, we are cautious about these results. Concerns about the small total sample sizes ( $n = 41$  fish for immediate mortality and 36 fish for delayed mortality), the timing of our procedure, and correlations among blood factors and other significant parameters make us suspect that these results may be biased and that the blood-drawing procedure itself contributed to delayed mortality in experimental fish. Due to the uncontrollable logistics of field experiments, such as increased air and handling times when drawing blood and extremely fast blood clotting times in the summer, we do not recommend using blood stress factors as predictors of delayed mortality in the field unless there is some way to quantify the additional stress and possible mortality attributable to the blood-drawing procedure.

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