Recovery potential of black rockfish, *Sebastes melanops* Girard, recompressed following barotrauma

A L Pribyl[1], C B Schreck[2], M L Kent[3], K M Kelley[4] and S J Parker[5]*

1 Oregon Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR, USA

2 U.S. Geological Survey, Oregon Cooperative Fish and Wildlife Research Unit, Oregon State University, Corvallis, OR, USA

3 Department of Microbiology, Oregon State University, Corvallis, OR, USA

4 Environmental Endocrinology Laboratory, Department of Biological Sciences, California State University, Long Beach, CA, USA

5 Oregon Department of Fish and Wildlife, Newport, OR, USA

Abstract

Overfished species of rockfish, *Sebastes* spp., from the Northeast Pacific experience high bycatch mortality because of 'barotrauma', a condition induced from the rapid change in pressure during capture. Field experiments show that it may be possible for rockfish to recover from barotrauma if quickly recompressed; however, no work has followed the physiological recovery of rockfish after recompression or determined whether it is possible for rockfish to survive barotrauma in the long term. Barotrauma was induced in adult black rockfish, *Sebastes melanops* Girard, from a simulated depth of 35 m, followed by recompression. Blood and selected tissues (eye, heart ventricle, head kidney, liver, rete mirabile and gonad) were sampled at days 3, 15 and 31 post-recompression to evaluate the tissue- and physiologic-level response during recovery. No mortality from barotrauma occurred during the experiments, and feeding resumed in 80% of both treatment and control fish. The primary injury in treatment fish was the presence of a ruptured swimbladder and/or a ruptured tunica externa (outer layer of swimbladder), which was slow to heal. Blood plasma was analysed for glucose,

sodium, chloride, potassium, calcium, phosphorus, insulin-like growth factor-1 and cortisol. Plasma analyses indicated no strong effects because of barotrauma, suggesting overall handling stress outweighed any effect from barotrauma. Rockfish with ruptured swimbladders may face compromised competency in the wild; however, it appears the majority of black rockfish decompressed from 35 m have a high potential for recovery if recompressed immediately after capture. This research suggests recompression could be a valuable bycatch mortality reduction tool for rockfish in recreational fisheries.

Keywords: barotrauma, decompression, histology, recompression, rockfish.

Introduction

Pacific rockfish, *Sebastes* spp., are physoclists, indicating that they have a closed swimbladder from which gas cannot escape unless it is torn or ruptured. This functions well for fish species inhabiting deep water because they can inflate their swimbladder at depth and are not limited to gulping air from the surface. However, when physoclist fish are captured and undergo a forced ascent, gas in the closed swimbladder expands as pressure is decreased (Boyle's Law), causing a variety of pressure-related injuries collectively called barotrauma. Depending on the degree of pressure change, barotrauma in physoclist fish can involve

Correspondence *A L Pribyl, NOAA SWFSC, 3333 N. Torrey Pines Ct., La Jolla, CA 92037, USA (e-mails: alena.pribyl@ noaa.gov; alena.pribyl@oregonstate.edu)*
*Present address: National Institute of Water and Atmospheric Research, PO Box 893, Nelson7040, New Zealand.

275

bloating, ruptured swimbladder, crushed organs, eversion of the oesophagus or stomach and exophthalmia (Gotshall 1964; Rummer & Bennett 2005; Parker, McElderry, Rankin & Hannah 2006; Hannah, Parker & Matteson 2008a; Jarvis & Lowe 2008; Pribyl, Kent, Parker & Schreck 2011). Many physoclist fish, such as Pacific rockfish, must be discarded because of fishing regulations to prevent overfishing. Many rockfish populations are in decline, and seven species of Pacific rockfish are currently listed as 'depleted' (PFMC 2008). Rockfish that are discarded often have a high mortality rate because they are too buoyant from barotrauma to submerge on their own (Hannah et al. 2008a); these fish often suffocate or succumb to thermal shock and/or predation on the surface. As rockfish populations decline, it is important to develop and test techniques to reduce this high level of discard mortality.

Recompression is a technique used by some recreational fishermen to reduce discard mortality in physoclist fish species that suffer from barotrauma. Recompression involves any method that will help fish overcome their buoyancy to submerge to a depth where they can swim back down on their own. If a fish can re-submerge close to its original capture depth, the expanded gases will be compressed again, relieving the fish of its excessive buoyancy. Devices that are often used by recreational fishermen to recompress physoclist fish include barbless, weighted hooks and weighted cages (Theberge & Parker 2005). Recent studies have shown that these devices are effective at increasing the short-term survival of Pacific rockfish with barotrauma (Hannah & Matteson 2007; Jarvis & Lowe 2008); however, little is known about the potential for long-term survival, including if tissue injuries incurred as a result of barotrauma (Pribyl et al. 2011) can heal over time, or how rockfish respond and recover at a physiologic level. Thus far, only one study has investigated long-term survival in black rockfish that have been recompressed; however, the focus was on mortality and the healing rate of swimbladders (Parker et al. 2006). No study has undertaken a complete macroscopic and physiologic evaluation of recovery in Pacific rockfish after recompression.

The objective of this study was to determine the potential for long-term recovery in black rockfish decompressed and recompressed from 35 m using both macroscopic and physiologic measures. This information can be used to help determine whether recompression is a good tool to reduce discard mortality in rockfish that suffer from barotrauma. Although black rockfish are not considered depleted, they are long lived and are commonly captured in the recreational fishery off the West coast of North America. Black rockfish also commonly experience a wide array of both macroscopic and tissue-level barotrauma indicators, such as everted oesophagus, exophthalmia, ruptured swimbladder (Parker et al. 2006; Hannah et al. 2008a; Pribyl, Schreck, Kent & Parker 2009; Pribyl et al. 2011), emphysema in the heart ventricle, emboli in the rete mirabile and emboli in the vessels of the head kidney (Pribyl et al. 2011), which have also been observed in several other species of rockfish. Thus, black rockfish could serve as a model for the recovery process in other species of rockfish with similar barotrauma indicators. Metabolic function after barotrauma has not been studied in rockfish and could provide insight into physiologic imbalances that may occur as a result of barotrauma. By observing the recovery process at the macroscopic, tissue and physiologic levels over a month long recovery period, we can better understand a rockfish's potential for long-term recovery from barotrauma and determine whether recompression is a good tool for reducing discard mortality.

Materials and methods

Fish collection

Adult black rockfish were collected off the coast of Newport, Oregon, by hook and line from depths < 15.2 m, which is shallow enough that most rockfish do not suffer from barotrauma. Only rockfish with no or minimal indicators of barotrauma (air in pharyngo-cleithral membrane) were collected to make sure that rockfish in our experiments had no pressure-related injuries from capture that might confound our experiment. Each rockfish was individually tagged using PIT tags as described in Parker & Rankin (2003). Total lengths ranged from 36 to 49 cm, which is generally the length at first maturity (Love, Yoklavich & Thorsteinson 2002); thus, these fish were classified as adults. Upon return to Newport, rockfish were immediately transferred into 2.4-m-diameter flow-through tanks (106 000 L) at the Hatfield Marine Science Center (HMSC) where fish were held until they became neutrally buoyant and actively feeding (minimum 30 days). Cessation of feeding is a

common response to stress (physical or perceived), and resumption of feeding can be an indicator that fish have recovered from the stress (Rice 1990). Neutral buoyancy indicates that the swimbladder is functioning. Fish were held for a minimum of 30 days as a precaution to ensure recovery from any minor stressors as described in Parker *et al.* (2006). Other studies (McElderry 1979; Parker *et al.* 2006) have used this collection and holding technique on hundreds of black rockfish, and no noticeable effect from capture was seen in control fish in these studies. A total of 60 black rockfish were used in this study.

Fish density was 10–15 fish per tank, and flow rate was 12–15 L min⁻¹. Dissolved oxygen (> 80% saturation), salinity (range: 34–37 ppt) and temperature (range: 9.4–14.4 °C) were monitored daily. Rockfish were fed a diet of thawed Atlantic silversides, pink shrimp and California market squid three times a week, and tanks were cleaned by syphoning debris daily.

Recompression experiments

Recompression experiments were designed to model the capture and release of rockfish by recreational fishermen using a recompression device. For each recompression experiment, six black rockfish were placed in each of two large flow-through hyperbaric aquaria (1302 L) and adjusted to 4.5 ATA (approximately 35 m depth) following a standard protocol for rockfish as described by Parker *et al.* (2006). This protocol involved increasing pressure by 1 ATA min⁻¹ until the hyperbaric aquaria reached 4.5 ATA. By increasing pressure at this rate, a strong pressure difference between the environment and the swimbladder was created to maximize the rate of acclimatization. Rockfish became negatively buoyant with this protocol but showed no outward signs of stress. Sea water was provided from an 8700-L header tank fed through two 3-hp pumps. Controlling the inflow and outflow of sea water through the hyperbaric aquaria regulated pressure. For further information, see Parker *et al.* (2006) where the hyperbaric aquarium system is described in great detail.

The 35 m simulated depth is within the depth range that black rockfish are commonly captured in the recreational fishery. Black rockfish were neutrally buoyant (indicating acclimatization to the 35 m depth) within 10 days. One chamber served as a treatment chamber and the other chamber as a control chamber. Once neutrally buoyant, fish in the treatment chamber were exposed to a simulated capture event by decreasing pressure to 1 ATA (0 m) over a 90-s period to induce decompression and simulate the speed at which a fisherman is likely to reel up a fish. After the simulated capture, the fish were held at surface pressure for 3 min to simulate the time it takes to unhook the fish and place it in a recompression device. The fish were then immediately recompressed (30 s) to 4.5 ATA. Control fish remained at 4.5 ATA during this time. After a minimum recovery time of 6 h post-recompression for treatment fish, both treatment and control fish were slowly brought to surface pressure with a 10% pressure reduction every 2–3 h over a period of 72 h (Fig. 1a). This rate of ascent was determined by McElderry (1979) as slow enough for black rockfish to adjust to with no physical damage. Observations of neutral buoyancy at each pressure change confirmed that fish could adjust physiologically to this rate of ascent.

This protocol was reviewed and approved by Oregon State University's Institutional Animal Care and Use Committee (ACUP #: 3238). Standard recreational fishing practices involve the discard of rockfish species listed as 'depleted' back into the ocean. If captured at depths > 30 m (Hannah *et al.* 2008a), the majority of depleted rockfish species will suffer from barotrauma and will be unable to submerge on their own. This means rockfish that are suffering from barotrauma are left on the water's surface for an indefinite length of time until they die from suffocation, thermal shock, barotrauma injuries or predation. In contrast, our study has rockfish at surface pressure for only 3 min before they are immediately recompressed to their acclimatization depth, relieving them of the excessive gas pressure and all external barotrauma injuries. To study the ability of rockfish to recover from barotrauma, it was necessary to simulate what would occur to the rockfish as a matter of standard recreational fishing practices. To reduce suffering of study animals as much as possible, the surface interval was reduced to the minimum length of time we felt was plausible for a recreational fisherman to unhook and recompress their fish. Shortly after being recompressed, rockfish in our study showed no outward signs of stress.

In addition to the 60 black rockfish used in our recompression experiments, blood samples from an additional ten black rockfish captured using hook and line off the coast of Newport, OR, were
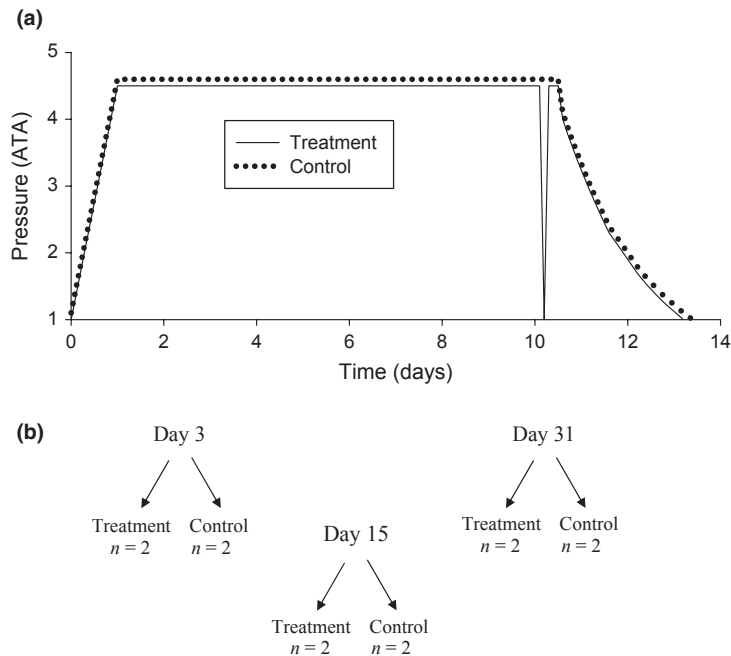
(a)

(b)

Figure 1 Experimental design of pressure chamber experiments. (a) Pressure regime for treatment and control fish in pressure chambers. (b) Fish sampling design; this experiment was replicated five times for a total of *n* = 60.

collected. These rockfish were captured from depths ranging between 12–18 m and immediately sampled for blood with a caudal venipuncture using heparinized BD Vacutainers® (Becton, Dickinson and Company). Blood samples were collected within 5 min of initial hooking, which should have been before many of the metabolites and hormones had a chance to respond to the capture stress. Cortisol, for example, has a lag time of several minutes after perception of a stressor (Wendelaar Bonga 1997). These samples are referred to as our 'field baseline' throughout the paper. These blood samples were processed as described below.

Sample collection

Once at surface pressure (3 days post-recompression), two rockfish from each chamber were removed, examined for external barotrauma indicators and sampled for blood via a caudal venipuncture using heparinized BD Vacutainers®. External barotrauma indicators recorded followed Hannah *et al.* (2008a) and included everted oesophagus, swollen abdomen, inflated pharyngo-cleithral membrane, air bubbles in the pharyngo-cleithral membrane, exophthalmia and ocular emphysema. Blood samples were immediately placed on ice and centrifuged (1000 *g* × 6 min) within 15 min of collection; plasma was then stored at −80 °C. Following

blood sampling, rockfish were immediately euthanized in an overdose of tricaine methane sulphonate (MS-222), and the following organs were collected: eye, liver, head kidney, gonad, rete mirabile and heart ventricle. All fish were dissected within 20 min of euthanasia. Tissue samples were fixed in Davidson's solution (Kent & Poppe 1998) at a ratio of no more than 1:10 (tissue/solution). During dissection, fish were also examined for ruptures in the swimbladder and haemorrhage.

The remaining four treatment rockfish and four control rockfish were transported to the 2.4-m-diameter tanks described above for recovery. Two treatment and two control fish each were netted from the recovery tanks and subsequently sampled as described above at 15 and 31 days post-recompression (Fig. 1b). All samples were endpoint samples. No fish were re-sampled. A 31-day experimental period was used because it allowed us to evaluate longer-term survival and still complete several replicates of the experiment. This experiment was replicated five times, for a total of 30 treatment fish and 30 control fish or a total of 10 fish sampled per treatment and sample day. Through this experimental design, both treatment and control fish experienced the same handling and confinement stress, with the only difference being that treatment fish also experienced forced decompression and subsequent recompression.

Sample processing

Plasma samples were thawed and analysed for general metabolites, insulin-like growth factor-1 (IGF-1) and cortisol. IGF-1 levels were analysed because in several fish species, IGF-1 is strongly correlated with growth rate (Beckman, Shimizu, Gadberry, Parkins & Cooper 2004; Dyer, Barlow, Bransden, Carter, Glencross, Richardson, Thomas, Williams & Carragher 2004; Cameron, Moccia, Azevedo & Leatherland 2007). Thirty days was not an adequate length of time to observe any measurable changes in growth using either otoliths or fish length (rockfish are slow-growing), and IGF-1 could be a good indicator of disruptions in growth rate. Cortisol was analysed because it is the primary stress hormone produced by fish (Barton 2002). Metabolites measured included glucose, sodium, chloride, potassium, calcium and phosphorus. Plasma metabolite measurements were conducted at Oregon State University's Veterinary Diagnostic Laboratory on a Hitachi 917 chemical analyzer. The chemical analyzer used a colorimeter to determine the level of individual analytes. For sodium, potassium and chloride, ion-specific electrodes were used. All assays were conducted at 37 °C. Cortisol was analysed using a radioimmunoassay following procedures in Redding, Schreck, Birks & Ewing (1984). IGF-1 was analysed using a radioimmunoassay according to Shimizu, Swanson, Fukada, Hara & Dickhoff (2000) and Galima (2004).

For metabolites, only plasma from day 3 and day 31 rockfish were analysed because we were primarily interested in the overall change over time. All plasma samples were analysed for IGF-1, and all samples except for the first replicate (insufficient plasma was collected during the first replicate) were also analysed for cortisol.

Tissues were fixed for a minimum of 30 days, embedded in paraffin and then sectioned to a thickness of 5–7 μm. For the eye, slides were sectioned by Oregon State University's Veterinary Diagnostic Laboratory, with a vertical cross-section that showed the entire circumference of the eye including the cornea, iris, lens, retina, choroid and sclera. Only eyes from the first three experiments were sectioned for histology ($n = 36$) because of cost. All slides were stained with haematoxylin and eosin Y. Slides were evaluated with a Leica compound light microscope (Model DM LB; Leica Microsystems).

Statistics

For blood plasma analyses, values from the two pseudoreplicate fish within each experiment were averaged. This pooling of pseudoreplicates yielded a final sample of $n = 5$ per treatment and sample day, although the sample number represents twice as many fish. For plasma cortisol, this yielded $n = 4$ per treatment and sample day. A Welch's test was used to test for differences between treatment, control and field baseline groups because of unequal sample variances between groups. Dunnett's *post hoc* test was used to determine between which groups there were differences.

To determine whether there was a difference in blood plasma measurements as a result of the presence or absence of variables such as ruptured swimbladder, ruptured tunica externa, feeding and inflammation in the rete mirabile, Mann–Whitney tests and logistic regression models were used. Results were similar using both methods; thus, only results from the Mann–Whitney tests are presented here. For these calculations, measurements at the level of the individual fish were used because we were not determining the differences between fixed treatment groups ($n = 60$ for IGF-1, $n = 48$ for cortisol, $n = 40$ for metabolites). Because multiple tests were run utilizing data from the same experimental unit (i.e. individual fish), a correction to control for false discovery rates was applied as described in Waite & Campbell (2009). Non-parametric tests were used because of non-normal distributions in several blood plasma analytes. All analyses were conducted using SPSS v.17.0.

Results

Macroscopic observations

During decompression in the hyperbaric pressure chambers, treatment rockfish exhibited the macroscopic barotrauma indicators of everted oesophagus and exophthalmia. While we estimate about 80% of the fish experienced an everted oesophagus and about 50% experienced exophthalmia, we could not get an accurate count of the number of fish with different barotrauma indicators because the fish were moving rapidly during the surface interval. Once treatment fish were recompressed to their original depth, all external indicators of barotrauma disappeared. The only indication that treatment fish had experienced barotrauma was that treatment fish

were negatively buoyant (because of ruptured swimbladders). There was no indication of barotrauma or negative buoyancy in control rockfish during or after the experiments.

No mortalities occurred in treatment or control fish for the duration of the experiments (n = 60). By day 31, 80% of both treatment and control fish had resumed feeding. There was also no difference in the resumption of feeding between treatment and control fish prior to day 31. No external injuries were visible on treatment or control fish over the entire sampling period. Once dissected, the only macroscopic injury observed was the presence of a ruptured swimbladder and/or a ruptured tunica externa (the outer layer of the swimbladder) in treatment fish. The proportion of treatment fish with a ruptured swimbladder was 80% at day 3 post-recompression, 20% at day 15 post-recompression and 50% at day 31 post-recompression. The proportion of treatment fish with a ruptured tunica externa was 100% at day 3 post-recompression and only declined to 80% in fish sampled at days 15 and 31 post-recompression. No control fish had a ruptured swimbladder or a ruptured tunica externa.

Histology

The rete mirabile was the only organ to show injury at the histological level (Fig. 2). Severe injury (emboli, haemorrhage) of the rete mirabile was present in two of 30 treatment fish; one of the fish was sampled 3 days post-recompression, and the other fish was sampled 15 days post-recompression. Chronic, multifocal inflammation of the rete mirabile was also present in both treatment and control fish throughout the recovery period. Chronic inflammation in the rete mirabile was characterized by large aggregations of macrophages and lymphocytes throughout the tissue, often obliterating capillaries (Fig. 2b). Inflammation was primarily located in the capillary portion of the rete mirabile. Eye, head kidney, liver, heart ventricle and gonad showed no injury because of the forced decompression.

Blood plasma analyses

Blood plasma analyses of glucose, sodium, chloride, potassium, calcium and phosphorus showed no difference between treatment and control groups at each sample day (Dunnett's test, $P > 0.5$ for all 12
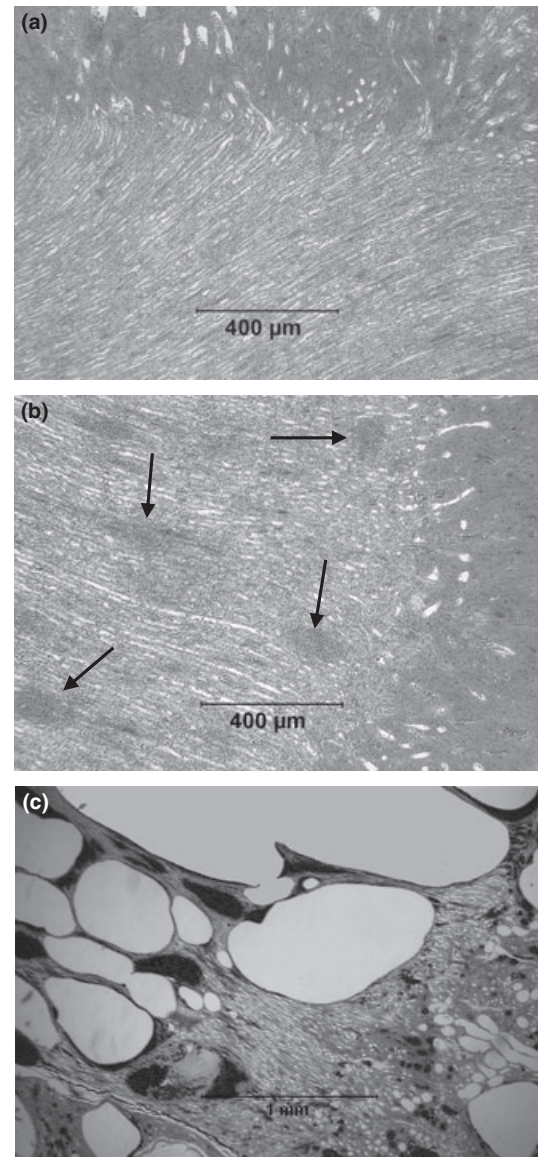

Figure 2 Histology of the rete mirabile in black rockfish. (a) Normal rete mirabile. (b) Chronic, multifocal inflammation; arrows indicate areas of inflammation. (c) Large emboli and haemorrhage associated with barotrauma. Multifocal inflammation of the rete mirabile was present in both treatment and control fish throughout the recovery period, while only two individuals of the 30 treatment fish exhibited more severe injury as shown in panel c.

tests). There were, however, some differences between treatment and/or control groups and the field baseline group (Table 1). Glucose levels for the day 3 control group and day 31 treatment and control groups were elevated compared to the field baseline group (Dunnett's test, $P < 0.05$ for all three tests). Statistical power for determining

Table 1 Mean value $\pm$ SEM for blood plasma metabolites in black rockfish, according to sample day and treatment (n = 5 for each row)

Sample day, treatment	Glucose (mg dL^{-1})	Na (mM)	K (mM)	Cl (mM)	Calcium (mg dL^{-1})	Phosphorus (mg dL^{-1})
Field baseline	28.9 $\pm$ 1.6	187.6 $\pm$ 1.1	3.5 $\pm$ 0.1	155.6 $\pm$ 1.1	12.3 $\pm$ 0.3	8.5 $\pm$ 0.5
03, control	34.0 $\pm$ 1.2	178.9 $\pm$ 4.8	3.4 $\pm$ 0.2	152.1 $\pm$ 3.3	10.5 $\pm$ 0.5[a]	8.0 $\pm$ 0.5
03, treatment	38.4 $\pm$ 3.3[a]	174.7 $\pm$ 4.7	3.4 $\pm$ 0.5	146.7 $\pm$ 3.4	9.9 $\pm$ 0.3[a]	7.9 $\pm$ 1.0
31, control	46.9 $\pm$ 3.5[a]	186.6 $\pm$ 2.7	2.9 $\pm$ 0.1[a]	153.1 $\pm$ 2.2	12.1 $\pm$ 0.3	9.8 $\pm$ 0.5
31, treatment	50.8 $\pm$ 4.2[a]	184.1 $\pm$ 2.1	3.0 $\pm$ 0.1	151.0 $\pm$ 1.4	11.7 $\pm$ 0.3	9.5 $\pm$ 0.6

Field baseline refers to field-captured rockfish (n = 10).

Na, sodium; K, potassium; Cl, chloride.

[a]Difference at α = 0.05 between the specified value and the field baseline value (Dunnett's test).

differences among treatment groups for glucose was > 0.8 for both day 3 and day 31. Potassium levels for the day 31 treatment group was depressed relative to the field baseline group (Dunnett's test, P = 0.001). Statistical power for potassium was 0.071 at day 3 but increased to 0.93 at day 31. Calcium levels at day 3 for both treatment and control groups were depressed relative to the field baseline group (Dunnett's test, P < 0.05 for both tests); however, they showed no differences by day 31 (Dunnett's test, P > 0.4 for both tests). Statistical power for determining differences among treatment groups for calcium at day 3 was 0.99 but declined to 0.17 at day 31. No differences were found for sodium, chloride or phosphorus among treatment groups. Statistical power was < 0.8 for these analytes.

Levels of IGF-1 (Fig. 3) showed no differences between treatment, control and field baseline groups at days 15 and 31 (Dunnett's test,

P > 0.2 for all tests); however, for day 3, the treatment group had lower IGF-1 levels than the field baseline group (Welch's test, df = 2, P = 0.048; Dunnett's test, P = 0.056). Statistical power for determining differences among groups of IGF-1 ranged from 0.43 at day 3 to 0.71 at day 15 to 0.13 at day 31. Levels of cortisol (Fig. 4) also showed no differences between treatment and control groups within each sample day (Dunnett's test: P > 0.1 for all three tests); however, cortisol levels in the control group were elevated relative to the field baseline group on days 15 and 31 (Dunnett's test, P < 0.05 for both tests). Statistical power of cortisol ranged from 0.32 at day 3 to 0.88 at day 15 to 0.98 at day 31. There also appeared to be a trend of higher cortisol levels in all control and treatment groups as compared with the field baseline group.

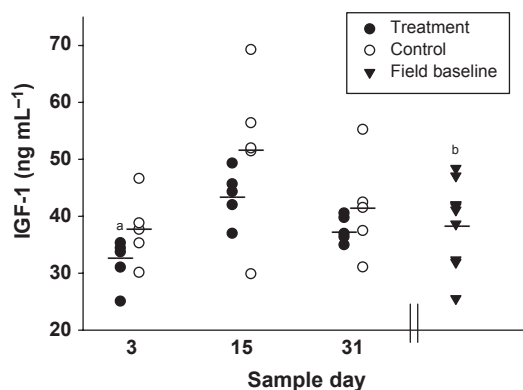Analyses to determine whether the presence or absence of injury (ruptured swimbladder, ruptured

Figure 3 Individual insulin-like growth factor-1 (IGF-1) values in treatment, control and field baseline black rockfish. Lines indicate mean values for each group. There were no significant differences between control and treatment groups; at day 3, the treatment group exhibited a slightly lower mean IGF-1 concentration as compared with the field baseline group ([a,b]values are significantly different, P < 0.05).
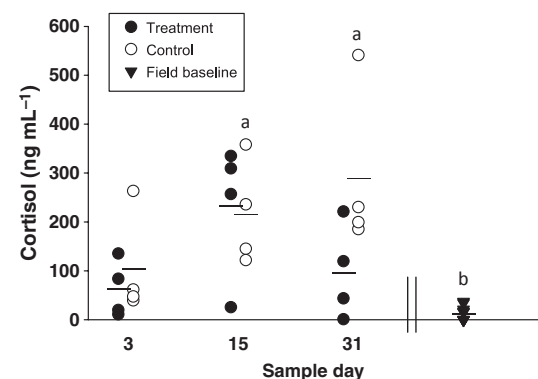

Figure 4 Individual cortisol values for treatment, control and field baseline black rockfish. Lines indicate mean values for each group. There were no significant differences between control and treatment groups; at days 15 and 31, the control group exhibited higher mean cortisol concentrations as compared with the field baseline group ([a,b]values are significantly different, P < 0.05).

tunica externa, inflammation in the rete mirabile or feeding) affected levels of blood plasma analytes yielded significant associations (Table 2). Fish with inflammation in the rete mirabile had lower cortisol levels compared to fish with no rete mirabile inflammation. Fish that were feeding had higher levels of glucose, calcium, phosphorus and cortisol compared to fish that were not feeding (Table 2). There was also an indication that calcium, cortisol and IGF-1 levels may be affected by the presence of a ruptured swimbladder, and calcium levels may be affected by rete mirabile inflammation; however, these associations were not statistically significant once a correction for false discovery rates was applied.

Discussion

Although several species of rockfish are known to survive barotrauma in the short-term if they are recompressed (Hannah & Matteson 2007; Jarvis & Lowe 2008), until now it was unknown what the potential was for long-term physiologic recovery. This study provides new insights into the ability of rockfish to survive and recover from barotrauma in the long term if they are recompressed. The data on feeding suggest the digestive system recovered in rockfish that experienced barotrauma (and possible oesophageal eversion). The histological data show tissue injuries from barotrauma, such as emphysema in the heart ventricle and emboli in the head kidney, are no longer present after recompression, suggesting that little, if any, tissue injury occurs to

Table 2 Summary of *P*-values from Mann–Whitney *U* tests to determine whether there is a difference in blood plasma analytes between the presence and absence of injuries or feeding in black rockfish

Plasma analyte	Ruptured swimbladder	Ruptured tunica externa	Rete mirabile inflammation	Feeding
Glucose[a]	0.960	0.840	0.416	0.001[a]
Na	0.063	0.088	0.351	0.077
Cl	0.072	0.130	0.666	0.940
K	0.950	0.582	0.264	0.111
Ca[a]	0.016[b]	0.057	0.011[b]	0.000[a]
P[a]	0.395	0.119	0.102	0.005[a]
Cortisol[a]	0.036[b]	0.061	0.002[a]	0.006[a]
IGF-1	0.056	0.137	0.667	0.105

IGF-1, insulin-like growth factor-1.
[a]Significant difference in the plasma analyte between the presence and absence of the indicator at α = 0.05.
[b]With a false discovery rate correction, the *P*-value needs to be ≤0.006 to be significant at α = 0.05.

these organs if gases are recompressed quickly. However, injury in the rete mirabile suggests the rete mirabile may be subject to higher gas pressures than other organs. The plasma metabolite and plasma hormone data suggest barotrauma did not contribute any additional stress to the handling stress already experienced by all rockfish in the experiment. The new findings from our study suggest a high proportion of black rockfish have the potential to recover physiologically from barotrauma if recompressed.

The cessation of feeding in fish is a common response to physical or perceived stress (Rice 1990), and resumption of feeding can be an indicator that fish have at least partially recovered from stress. Many of the rockfish in our study that experienced barotrauma also experienced eversion of the oesophagus, a severe physical stress. Research has found that eversion of the oesophagus appears to correspond to internal organ displacement in several species of rockfish, including black rockfish (Hannah, Rankin, Penny & Parker 2008b); organ displacement would likely affect the ability of rockfish to feed and digest food. Our feeding data suggest organs associated with the digestive system recover over time when rockfish are recompressed.

Previous research found tissue emphysema in the heart ventricle and emboli in the head kidney and rete mirabile of black rockfish sampled immediately after forced decompression (Pribyl *et al.* 2011). Recompression appeared to eliminate these internal injuries in the heart ventricle and head kidney. It is likely that emboli in blood vessels disappeared once rockfish were recompressed, because the excess gas would have been compressed. If there was no tissue injury as a result of the emboli, it is unlikely that there would be evidence of the emboli after recompression. Emphysema in the heart ventricle would cause tissue injury, and it was surprising that there was no evidence of this. One possibility is that emphysema in the heart ventricle is a minor injury and was able to heal within the 3-day period post-recompression.

The rete mirabile was affected by forced decompression in a small percentage of rockfish. The rete mirabile is a gas-concentrating organ that resides in the secretory portion of the rockfish swimbladder and consists of a large network of venous and arterial capillaries. Gas from the venous capillaries diffuses into the arterial capillaries and increases gas partial pressures. Blood then flows from the rete capillaries into the gas gland where secretions of

lactic acid further increase the dissolved gas partial pressures by way of the Bohr and Root effects (Evans 1998). The gas molecules then diffuse into the secretory portion of the swimbladder and inflate it. Previous research found emboli in the capillary and gas gland portion of the rete mirabile in 67% of black rockfish captured at depths between 26–46 m (Pribyl *et al.* 2011). We observed a much lower proportion of rockfish with emboli in the rete mirabile; however, the emboli we observed were much more extensive than previously described and accompanied by haemorrhage. This suggests that most emboli in the rete mirabile will be eliminated with recompression, except in the rare cases where the emboli have already caused significant injury to the capillaries by rupturing them, resulting in haemorrhaging. It is unlikely the rete mirabile was functional in these rockfish because the capillaries were completely destroyed by the haemorrhage and emboli. This injury would leave rockfish unable to inflate their swimbladder and thus negatively buoyant.

Chronic, multifocal inflammation was also present in the rete mirabile; however, this condition is likely not related to barotrauma because it was present in both control and treatment rockfish. This condition was also observed histologically in a previous study that sampled black rockfish in the wild (Pribyl *et al.* 2011). Because chronic inflammation takes days to weeks to develop, and the black rockfish in the previous study were sampled immediately upon capture, it was concluded that this is likely an underlying condition in black rockfish that is perhaps associated with frequent diving behaviour (Parker, Olson, Rankin & Malvitch 2008). It is unknown to what extent the inflammation might impair blood flow in the rete.

The primary tissue injury in rockfish that were recovering from barotrauma was a ruptured swimbladder and/or tunica externa. This injury has been observed in several other studies (Parker *et al.* 2006; Hannah *et al.* 2008a; Jarvis & Lowe 2008). The greater number of rockfish with ruptured swimbladders at day 31 compared to day 15 in our study is an artefact of endpoint sampling and illustrates that there is a large amount of variation in swimbladder healing rates. With larger sample sizes, this variation may have been reduced. Our range of percentage healed swimbladders does encompass previous research on swimbladder healing rates in black rockfish decompressed from 4.0 ATA (Parker *et al.* 2006); Parker *et al.* (2006) observed 77% of

swimbladders in black rockfish (*n* = 90) had healed enough to hold gas after a 21-day recovery period, compared to our 50–80% healing rates. Our combined data indicate that there will be a percentage of rockfish that will take an extended period of time to heal their swimbladder or may not be able to heal their swimbladder at all. Under laboratory conditions, this injury does not appear to be fatal, although food is available and predators are absent. It is unknown how this injury will affect rockfish survival in the wild. Life history characteristics may play an important role in how this injury will affect different rockfish species. Rockfish with swimbladders that are unable to hold gas will not be able to maintain neutral buoyancy in the water column and will likely need to expend more energy to find prey and escape predation. However, many rockfish species exhibit benthic, sedentary and solitary lifestyles (Love *et al.* 2002); thus, negative buoyancy may not have a large impact on their survival. Rockfish species that are semi-pelagic and often in the water column would likely be affected to a greater degree. A long-term tagging study would be necessary to determine to what extent a ruptured swimbladder would affect survival of different species in the wild.

The lack of a difference between treatment and control fish in the resumption of feeding, plasma metabolite concentrations, IGF-1 and cortisol indicates that barotrauma and subsequent recompression did not appear to contribute additional stress beyond the handling stress of the experiment. Handling stress was apparent in all experimental rockfish because there were differences in glucose, calcium, IGF-1 and cortisol levels between experimental rockfish and field baseline rockfish. Rockfish were not able to be fed while they were in the pressure chambers; thus, both treatment and control rockfish experienced a 2-week fasting period, along with the handling and confinement stress of being placed in the pressure chambers. Except for glucose and cortisol, all plasma indicators returned to field baseline levels by the end of the recovery period, suggesting rockfish were recovering from the experimental stress. However, it is also important to keep in mind that for many of these tests, statistical power was low; thus, a larger sample size may have found differences.

Plasma glucose is part of the primary and secondary stress response, and levels can increase quickly following a stressor. The handling of fish prior to sampling was likely the cause of elevated

glucose levels observed in both treatment and control fish relative to field baseline levels. Fish sampled on day 31 likely had higher glucose levels than fish sampled earlier because they had resumed feeding; Olsen, Sundell, Ringø, Myklebust, Hemre, Hansen & Karlsen (2008) found that plasma glucose levels were higher in Atlantic cod, *Gadus morhua* L., that had been fed and subjected to an acute stressor compared to cod that had been food deprived and subjected to an acute stressor. Calcium levels were depleted in experimental fish at day 3 but returned to field baseline values by day 31. This is also likely due to the fasting period all experimental fish experienced; about half of plasma calcium is bound to plasma proteins (Andreasen 1985; Björnsson, Young, Lin, Deftos & Bern 1989). Thus, a decline in calcium would be expected after fasting because protein levels would be depressed. Indeed, there were differences in glucose, calcium and phosphorus levels between fish that were feeding and fish that were not feeding.

The hormone IGF-1 has been correlated with growth rate in several fish species including barramundi, *Lates calcarifer* (Bloch), Atlantic salmon, *Salmo salar* L., Coho salmon, *Oncorhynchus kisutch* (Walbaum), Arctic charr, *Salvelinus alpinus* L., and Southern bluefin tuna, *Thunnus maccoyii* (Castelnau) (Beckman *et al.* 2004; Dyer *et al.* 2004; Cameron *et al.* 2007). Thus, IGF-1 has the potential to be a good indicator of normal growth for teleost fish. Although IGF-1 levels in rockfish did not differ between treatment and control rockfish, treatment rockfish sampled at day 3 did have depressed IGF-1 values compared to field baseline rockfish, suggesting experimental stress may have had a short-term effect. Whether alterations in other components of the growth-regulatory endocrine system (Kelley, Schmidt, Berg, Sak, Galima, Gillespie, Balogh, Hawayek, Reyes & Jamison 2002; Wood, Duan & Bern 2005; Kelley, Royal, Galima, Sak, Reyes, Zepeda, Hagstrom, Truong & Lowe 2006) or growth itself may be perturbed by the decompression/recompression treatment is not yet understood.

Cortisol is the primary corticosteroid produced in teleost fish and is part of the primary stress response (Wendelaar Bonga 1997). Because cortisol levels typically increase dramatically from baseline levels during a stress response, cortisol has been widely used as a stress indicator for fish. Elevated cortisol levels in both treatment and control fish relative to field baseline levels indicate that experimental fish were stressed throughout the recovery period, although the variance was high. This elevation in cortisol, as with glucose, is likely due, in part, to capture and handling stress prior to sampling. Interestingly, results also indicated that rockfish with no rete mirabile inflammation had higher levels of cortisol than rockfish with rete mirabile inflammation. This finding could be a result of the anti-inflammatory properties of cortisol (MacKenzie, Iliev, Liarte, Koskinen, Planas, Goetz, Molsa, Krasnov & Tort 2006; Castillo, Teles, Mackenzie & Tort 2009), where rockfish with higher cortisol levels may experience reduced inflammation.

Knowledge of the recovery process from barotrauma at multiple levels gives us insight into the potential for long-term survival in black rockfish that are recompressed. Our findings indicate that the primary problem black rockfish that are recompressed following barotrauma will experience is negative buoyancy from a ruptured swimbladder. However, the majority of black rockfish that are recompressed appear to be able to heal their swimbladder over time. Because many rockfish species are quite sedentary, it is unclear how large an impact a ruptured swimbladder will have on survival in the wild. Long-term tagging studies would be necessary to estimate the degree to which injuries such as a ruptured swimbladder affect survival. Altogether, this study shows a remarkable resiliency in the ability of rockfish to survive and recover from barotrauma both macroscopically and physiologically over a 31-day period if recompressed. It is always best for fishermen to move away from areas where depleted rockfish species are being captured; however, when depleted species have been captured and need to be discarded, the use of recompression devices (such as barbless weighted hooks or weighted cages) to assist excessively buoyant rockfish to return to depth is likely to provide rockfish with a chance of survival they might not otherwise have.

Acknowledgements

References

Andreasen P. (1985) Free and total calcium concentrations in the blood of rainbow trout, *Salmo gairdneri*, during 'stress' conditions. *Journal of Experimental Biology* 118, 111–120.

Barton B.A. (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* 42, 517–525.

Beckman B.R., Shimizu M., Gadberry B.A., Parkins P.J. & Cooper K.A. (2004) The effect of temperature change on the relations among plasma IGF-1, 41-kDa IGFBP, and growth rate in postsmolt coho salmon. *Aquaculture* 241, 601–619.

Björnsson B.T., Young G., Lin R.J., Deftos L.J. & Bern H.A. (1989) Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma calcium regulation, osmoregulation, and calcitonin. *General and Comparative Endocrinology* 74, 346–354.

Cameron C., Moccia R., Azevedo P.A. & Leatherland J.F. (2007) Effect of diet and ration on the relationship between plasma GH and IGF-1 concentrations in Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture Research* 38, 877–886.

Castillo J., Teles M., Mackenzie S. & Tort L. (2009) Stress-related hormones modulate cytokine expression in the head kidney of gilthead seabream (*Sparus aurata*). *Fish & Shellfish Immunology* 27, 493–499.

Dyer A.R., Barlow C.G., Bransden M.P., Carter C.G., Glencross B.D., Richardson N., Thomas P.M., Williams K.C. & Carragher J.F. (2004) Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. *Aquaculture* 236, 583–592.

Evans D.H. (1998) The physiology of fishes. In: *CRC Marine Science Series* (ed. by M.J. Kennish & P.L. Lutz), pp. 30–39. CRC Press LLC, Boca Raton, FL.

Galima M.M. (2004) Catch-and-release stress: impacts on the endocrine physiology of the California sheephead, *Semicossyphus pulcher*. Master's Thesis. California State University, Long Beach.

Gotshall D.W. (1964) Increasing tagged rockfish (genus *Sebastodes*) survival by deflating the swim bladder. *California Fish and Game* 50, 253–260.

Hannah R.W. & Matteson K.M. (2007) Behavior of nine species of Pacific rockfish after hook-and-line capture, recompression, and release. *Transactions of the American Fisheries Society* 136, 24–33.

Hannah R.W., Parker S.J. & Matteson K.M. (2008a) Escaping the surface: the effect of capture depth on submergence success of surface-released Pacific rockfish. *North American Journal of Fisheries Management* 28, 694–700.

Hannah R.W., Rankin P.S., Penny A.N. & Parker S.J. (2008b) Physical model for the development of the external signs of barotrauma in Pacific rockfish. *Aquatic Biology* 3, 291–296.

Jarvis E.T. & Lowe C.G. (2008) The effects of barotrauma on the catch-and-release survival of southern California nearshore and shelf rockfish (Scorpaenidae, *Sebastes* spp.). *Canadian Journal of Fisheries and Aquatic Sciences* 65, 1286–1296.

Kelley K.M., Schmidt K.E., Berg L., Sak K., Galima M.M., Gillespie C., Balogh L., Hawayek A., Reyes J.A. & Jamison M. (2002) Comparative endocrinology of the insulin-like growth factor-binding proteins. *Journal of Endocrinology*, 175, 3–18.

Kelley K.M., Royal T.D., Galima M.M., Sak K., Reyes J.A., Zepeda O., Hagstrom R.K., Truong T. & Lowe C.G. (2006) Insulin-like growth factor-binding proteins (IGFBPs) in fish: beacons for (disrupted) growth endocrine physiology. In: *Fish Endocrinology* (ed. by M. Reinecke, G. Zaccone & B.G. Kapoor), pp. 167–195. Science Publishers, UK.

Kent M.L. & Poppe T.T. (1998) *Diseases of Seawater Netpen-Reared Salmonid Fishes*. Pacific Biological Station, Nanaimo, BC.

Love M.S., Yoklavich M. & Thorsteinson L.K. (2002) *The Rockfishes of the Northeast Pacific*. University of California Press, Berkeley.

MacKenzie S., Iliev D., Liarte C., Koskinen H., Planas J.V., Goetz F.W., Molsa H., Krasnov A. & Tort L. (2006) Transcriptional analysis of LPS-stimulated activation of trout (*Oncorhynchus mykiss*) monocyte/macrophage cells in primary culture treated with cortisol. *Molecular Immunology* 43, 1340–1348.

McElderry H.I. (1979) *A Comparative Study of the Movement Habits and Their Relationship to Buoyancy Compensation in Two Species of Shallow Reef Rockfish (Pisces, Scorpaenidae)*, pp. 168. Department of Biology, University of Victoria, Victoria, BC.

Olsen R.E., Sundell K., Ringø E., Myklebust R., Hemre G.-I., Hansen T. & Karlsen Ø. (2008) The acute stress response in fed and food deprived Atlantic cod, *Gadus morhua* L. *Aquaculture* 280, 232–241.

Parker S.J. & Rankin P.S. (2003) Tag location and retention in black rockfish: feasibility of using PIT tags in a wild marine species. *North American Journal of Fisheries Management* 23, 993–996.

Parker S.J., Mcelderry H.I., Rankin P.S. & Hannah R.W. (2006) Buoyancy regulation and barotrauma in two species of nearshore rockfish. *Transactions of the American Fisheries Society* 135, 1213–1223.

Parker S.J., Olson J.M., Rankin P.S. & Malvitch J.S. (2008) Patterns in vertical movements of black rockfish *Sebastes melanops*. *Aquatic Biology* 2, 57–65.

PFMC (2008) *Pacific Coast Groundfish Fishery Stock Assessment and Fishery Evaluation*, Vol. 1. Pacific Fishery Management Council, Portland, OR.

Pribyl A.L., Schreck C.B., Kent M.L. & Parker S.J. (2009) The differential response to decompression in three species of

nearshore Pacific rockfish. *North American Journal of Fisheries Management* **29,** 1479–1486.

Pribyl A.L., Kent M.L., Parker S.J. & Schreck C.B. (2011) The response to forced decompression in six species of Pacific rockfish. *Transactions of the American Fisheries Society* **140,** 374–383.

Redding J.M., Schreck C.B., Birks E.K. & Ewing R.D. (1984) Cortisol and its effects on plasma thyroid-hormone and electrolyte concentrations in fresh-water and during seawater acclimation in yearling coho salmon, *Oncorhynchus kisutch. General and Comparative Endocrinology* **56,** 146–155.

Rice J.A. (1990) Bioenergetics modeling approaches to evaluation of stress in fishes. *American Fisheries Society Symposium* **8,** 80–92.

Rummer J.L. & Bennett W.A. (2005) Physiological effects of swim bladder overexpansion and catastrophic decompression on red snapper. *Transactions of the American Fisheries Society* **134,** 1457–1470.

Shimizu M., Swanson P., Fukada H., Hara A. & Dickhoff W.W. (2000) Comparison of extraction methods and assay validation for salmon insulin-like growth factor-I using commercially available components. *General and Comparative Endocrinology* **119,** 26–36.

Theberge S. & Parker S. (2005) *Release Methods for Rockfish.* Oregon Sea Grant, Oregon State University, Corvallis, OR.

Waite T.A. & Campbell L.G. (2009) Controlling the false discovery rate and increasing statistical power in ecological studies. *Ecoscience* **13,** 439–442.

Wendelaar Bonga S.E. (1997) The stress response in fish. *Physiology Review* **77,** 591–625.

Wood A.W., Duan C. & Bern H. (2005) Insulin-like growth factor signaling in fish. *International Reviews in Cytology* **243,** 215–285.