THE EFFECTS OF BAROTRAUMA AND DEEPWATER-RELEASE MECHANISMS ON THE REPRODUCTIVE VIABILITY OF YELLOWEYE ROCKFISH IN PRINCE WILLIAM SOUND, ALASKA

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Abstract

Previous research has shown that releasing sport-caught Yelloweye Rockfish *Sebastes ruberrimus* with a deepwater-release mechanism (DRM) can alleviate anatomical damage due to barotrauma. However, it is unknown if a Yelloweye Rockfish remains a viable member of the population and reproduces in subsequent years following a barotrauma event and recompression with a DRM. The objectives of my study were to: 1) determine if Yelloweye Rockfish were able to reproduce one to two years following known forced decompression and recompression event(s); and 2) evaluate if barotrauma and recompression affected the quality of developing embryos. In 2010, Yelloweye Rockfish were sampled from an isolated reef in Prince William Sound, Alaska. Fifteen females tagged in 2008 and 2009 were recaptured in 2010, and reproductive status was identified by visual observation of the gonads and hematological sampling (i.e., vitellogenin and calcium^{2+} plasma concentrations). Oil globule volume, percent lipid, and caloric content were also measured for the embryos from seven of these females and these values were compared to embryos from 13 females with no previously documented barotrauma and recompression events. These results showed that all 15 Yelloweye Rockfish recaptured in 2010 were gravid (with eggs) or spent (having released eggs). In addition, there were no differences in median oil globule volume, caloric content, and percent lipid between individual embryos from new captures and recaptures. Results indicated that there is no evidence that reproduction and embryo quality of Yelloweye Rockfish is adversely affected one to two years following forced decompression and recompression with a DRM at the depths sampled in this study. This research provides information on the utility of DRMS as a tool for rockfish conservation and supports the importance of utilizing these devices by sport anglers.

Table of Contents

List of Figures

List of Tables

List of Appendices

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General Introduction[1]

Life history

Rockfishes *Sebastes* spp. are globally distributed, with 107 currently described extant species. Along the Pacific Coast of North America, at least 65 species have been identified and over 30 species inhabit the Gulf of Alaska (Love et al. 2002; Mecklenburg et al. 2002). Rockfishes are long lived, reaching ages in excess of 100 years, and typically have a small home range and high site fidelity. Species in this family exhibit a bet-hedging reproductive strategy and have high fecundity, with some species producing up to 1.7 million eggs per female (Love et al. 2002). During sexual maturation, female rockfish undergo vitellogenesis in which vitellogenin, a blood-plasma protein, circulates at high concentrations in the blood stream (Heppell and Sullivan 2000; Martin-Mills 2007). During this process, maturing oocytes in the ovary accumulate yolk after which the eggs are internally fertilized (Love et al. 2002). Rockfish next undergo a one- to two-month gestation period as embryos develop into larvae, followed by parturition, which involves the release of larvae (Love et al. 2002). In Alaska, parturition typically occurs during spring or summer months (O'Connell and Funk 1987; Yoklavich et al. 1996; Love et al. 2002).

Rockfishes are matrotrophically viviparous meaning that, in addition to energy in the yolk, energy is transferred directly from the mother to the embryos (Love et al. 2002). The energy contribution from the mother (parental female) post-fertilization can vary substantially based on the mechanism of embryonic nutrition (range, 3% to 92%; Dygert and Gunderson 1988; Love et al. 2002; Boehlert et al. 2006). Previous research on rockfishes and other marine fish species has shown that the maternal contribution to offspring development is a key factor in egg and larval survival (Hislop 1988; Trippel et al. 1997; Berkeley et al. 2004). Maternal effects, such as age and size, are also important determinants of embryo and larval quality. For example, Berkeley et al. (2004) found that larvae from older female Black Rockfish *S. melanops* had growth rates three times greater than larvae from younger females. Further,

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[1] This introduction is formatted following American Fisheries Society style guidelines.

larvae from older females survived twice as long as larvae from younger females when subjected to a 30-d starvation experiment.

Swimbladder function and barotrauma

Rockfishes have a physoclistous swimbladder which is closed from external sources of air (Love et al. 2002). Unlike physostomous fishes, there is no direct connection between the swimbladder and the esophagus for the uptake and release of gases; instead, these fishes secrete gases through the rete mirabile into the swimbladder for inflation and expel gases through the resorption chamber for deflation (Alexander 1966; Evans 1998; Pribyl 2010). Capillaries are found on the swimbladder of physoclistous fishes in areas where the membrane is thin, and these high gas pressure areas are the rete mirabile. Gas exchange occurs through both arterial and venous capillaries arranged in a counter-current system, and the rate of gas removal is dependent on the passive diffusion of gas into the blood and the rate of blood flow (Parker et al. 2006).

Fishes that have a physoclisticous swimbladder commonly suffer from barotrauma when they are rapidly forced to the water surface from deepwater habitats. Damage to body tissue results from the expansion of gases in the swimbladder (barotrauma) and the inability for those gases to be expelled when the fish is brought rapidly to the surface (Harden Jones 1952). Barotrauma can lead to bloating, swimbladder rupture, crushed and hemorrhaged organs, eversion of the esophagus, exophthalmia (popeye), and excessive buoyancy as decompressed gases escape into the visceral and cranial cavities (Parker et al. 2006; Hannah and Matteson 2007; Hannah et al. 2008; Jarvis and Lowe 2008).

Rockfishes have the ability to secrete and resorb gases at different rates. For example, Parker et al. (2006) examined differences in gas secretion and resorption between a semi-pelagic species (Black Rockfish) and a solitary, non-pelagic species (China Rockfish *S. nebulosus).* Their study showed that Black Rockfish were faster at gas resorption and secretion, which is most likely because this species schools and frequently changes depths. Compared to China Rockfish, the rete mirabile of Black Rockfish is larger, more developed, and the red blood cell count is higher, all important physiological advantages to meet the needs of this more active species. The authors concluded that swimbladder physiology is highly variable among rockfishes and is largely tied to activity level and depth occupancy.

Differences in how gases escape the swimbladder have been identified among species, which translate to variations in barotrauma injuries. These differences, in turn, affect the likelihood that a fish can submerge unassisted and exhibit normal behaviors (Hannah et al. 2008). For example, Hochhalter and Reed (2011) examined Yelloweye Rockfish *S. ruberrimus* and found that this species exhibited a high occurrence of esophageal eversion (stomach protrusion) post-capture, suggesting that these fish were positively buoyant at release because excess gases were unable to escape the body cavity. However, Hannah et al. (2008) examined Yellowtail Rockfish *S. flavidus* and found a low incidence of esophageal eversion and high submergence success at depths ranging from 10 to 51 m. Swimbladder gas expulsion rate and injury occurrence between these two species can likely be attributed to life-history differences; Yellowtail Rockfish are a semi-pelagic species, while Yelloweye Rockfish are demersal. In addition, Yellowtail Rockfish have been observed releasing swimbladder gases when brought to the surface (Pearcy 1992). Hannah et al. (2008) found that Quillback Rockfish *S. maliger*, also a demersal species with high submergence success, released gases when brought to the surface. Based on these and other studies, barotrauma signs, the ability of a fish to alleviate positive buoyancy, and the potential for the type of injury are highly variable among rockfishes.

Pribyl (2010) examined barotrauma injuries in multiple rockfish species at both macroscopic and microscopic levels. The author found that rockfishes are not completely immune to the effects of barotrauma at the tissue level and that the heart ventricle, rete mirabile, and head kidney can be injured from decompression. These injuries tended to be species specific; for example, Yelloweye Rockfish exhibited the highest proportion of granulomas in the head kidney.

Deepwater release and venting

Recompression using venting (fizzing) and deepwater release are two methods that have been investigated to increase the submergence success and alleviate barotrauma symptoms of fish subjected to decompression (Pribyl 2010; Butcher et al. 2012). The venting method uses a hypodermic needle (or other tool) to puncture the swimbladder through the body wall so gases can escape and a fish can resume neutral buoyancy. While this method has demonstrated short-term survival of fish subjected to forced decompression, many concerns arise when making a puncture into the abdomen of a fish (Butcher et al. 2012). For example, previous studies have identified that infection is probable (Butcher et al. 2012). Further, buoyancy control can be affected, causing excess energy exertion and increased vulnerability to predation (Rummer and Bennett 2005; Butcher et al. 2012). However, Butcher et al. (2012) examined recompression and venting and found that both methods increased short-term survival relative to no treatment at all for snapper *Pagrus auratus*.

Deepwater release is the recommended method for releasing and recompressing rockfishes in Alaska by the Alaska Department of Fish and Game, Division of Sport Fish. Their website provides anglers with information regarding the use of these devices: http://www.adfg.alaska.gov/index.cfm?adfg=fishingsportfishinginfo.rockfishconservation. This technique, when used by sport anglers, provides assistance to a fish suffering from barotrauma by returning the fish quickly to its capture depth. Deepwater-release mechanisms come in the form of weighted hooks or lines (as used in this study) or cage-like devices. Both methods have the same intent, which is to alleviate barotrauma injuries and assist fish subjected to forced decompression by giving them an opportunity to regain neutral buoyancy and increase the likelihood of survival post-decompression. In this thesis, the focus of this research is on the examination of fish that were recompressed with deepwater-release mechanisms.

Recompression study results

Recompression with a deepwater-release mechanism (DRM) is one method that has been examined by fisheries managers in North America and Australia as an approach to reduce discard mortality of angled fishes and reverse barotrauma injuries such as swimbladder damage. Many studies have addressed the effects of recompression. For example, Jarvis and Lowe (2008) determined that recompression of rockfish after capture reversed or alleviated external signs of barotrauma by recompressing expanded gases, reducing buoyancy, and increasing the solubility of gases in body fluids. Their findings showed >75% post-capture survival after recompression, regardless of the observed external barotrauma signs. Parker et al. (2006) conducted a simulated lab capture and recompression experiment and found that 77% of Black Rockfish swimbladders had partially repaired themselves within 21 d post-treatment and only three of the 90 test subjects died during the study. Although individuals in their study had at least partially healed swimbladders, some of the survivors had organ displacement. In addition, these authors found that Olive Rockfish *S. serranoides* had a higher occurrence of swimbladder tears relative to other rockfishes, which they attributed to the thinner swimbladder for this species. While many barotrauma studies have been conducted in cages or laboratory aquaria, these studies have shown promising results that rockfish can resume normal post-recompression behaviors (Parker et al. 2006; Hannah and Matteson 2007; Jarvis and Lowe 2008).

Studies on the Pacific coast of North America have also addressed short-term survival of recompressed rockfish. In Oregon, Parker et al. (2006) found that Black Rockfish had a low rate of mortality (<4%) after 21 d during simulated capture and recompression in a barometric chamber. In contrast, Jarvis and Lowe (2008) found that two-d, post-recompression survival in cages ranged from 36 to 82% for five rockfish species in California. Hochhalter and Reed (2011) found that Yelloweye Rockfish in Prince William Sound (PWS), Alaska, had a survival probability of 99% (95% CI = 0.478 – 0.999) when individuals were released at the depth of capture. In contrast, individuals released at the surface had a survival probability of 22% (95% CI = 0.149 – 0.315). Hannah and Matteson (2007) observed that there was a high degree of variability in the condition of different species of rockfish after being subjected to forced decompression. However, the condition of an individual fish condition at the surface was not a good predictor of survival after an individual had been recompressed.

Justification for DRM use on yelloweye rockfish in PWS

Deepwater-release mechanisms come in a variety of forms, but each has the same function: to release fish at or near the depth of capture to reverse barotrauma effects and increase the likelihood of survival. Management agencies in California and Oregon have been promoting the use of DRMs for many years. With an increased interest in their use, the ADF&G initiated a pilot study on their effectiveness in PWS in 2008. This study confirmed that Yelloweye Rockfish could survive recompression following a barotrauma event using a DRM (Hochhalter and Reed 2011). In 2009, ADF&G further investigated the survival of Yelloweye Rockfish post-recompression and began a mark-recapture study which found that Yelloweye Rockfish had a 99% survival probability when released using a DRM (Hochhalter and Reed 2011).

While positive results using recompression devices or simulated recompression events have been documented for many rockfish species (Jarvis and Lowe 2008; Pribyl et al. 2009; Hochhalter and Reed 2011), the mandatory use of these devices has not been required at all locations where rockfish are caught. In Alaska, the only regulation to date that requires DRMs was adopted by the Alaska Board of Fisheries in January 2013 for charter operators in southeast Alaska. The ADF&G in southcentral Alaska encourages the use of DRMs by sport anglers through public outreach programs; however, no regulations are in place requiring the use of DRMs. Currently, no rockfish species in Alaska are considered to be a conservation concern. However, non-retention regulations exist in California, Oregon, and Washington, which warrants conservative management along the Pacific coast of North America to ensure sustainability of rockfish species.

Conservative management of rockfishes is necessary to maintain healthy stocks given their unique physiology, long life span, and highly variable recruitment. Seven species of rockfish from California to Washington have been classified as depleted, and non-retention of captured fish has become a management strategy for rebuilding overfished rockfish stocks (PFMC 2008). Yelloweye Rockfish are one species that has been overfished from California to Washington, but there are currently no retention regulations for this species in these states (PFMC and NMFS 2009). Stocks of this species have also been listed as federally threatened under the Endangered Species Act from Puget Sound, Washington, to the Georgia Basin, British Columbia (NMFS 2010). Yelloweye Rockfish are an important species in Alaska because they are one of the most frequently captured rockfishes and are often caught as bycatch in sport fisheries within PWS (Hochhalter et al. 2011). For example, over 65% of demersal-shelf rockfish species caught annually by sport angling in southeast Alaska are Yelloweye Rockfish (McCurdy et al. 2008).

In Alaska, sport-caught rockfish catches have doubled since 1996, with over 40% of fish being released after they are brought to the surface (Hochhalter et al. 2011). Although rockfish status in Alaskan waters appears to be healthy, sustainable harvest levels are not known because no fishery-independent stock assessments have been conducted in areas such as PWS (Hochhalter et al. 2011). To account for this lack of assessment, low daily bag limits have been set since 1989 to protect demersal species, such as Yelloweye Rockfish, which are likely to suffer angling-induced barotrauma and are often caught incidentally in recreational fisheries for Pacific Halibut *Hippoglossus stenolepis* and Lingcod *Ophiodon elongatus* (Hochhalter et al. 2011). Current regulations allow the daily harvest of four rockfish per day, with only two rockfish allowed to be a demersal species. Although these regulations are believed to be conservative, boat angler effort has increased in PWS since the opening of the Anton Anderson Memorial Tunnel in Whittier in 2000. The tunnel provided convenient vehicle access to PWS. From 2001 to 2007, angler effort (both boat and shoreline) in western PWS increased from 35,281 to 96,209 angler days. Since 2007, angler effort has declined, but not to the levels observed prior to the opening of the tunnel (see Hochhalter et al. 2011 for a review). For example, the ADF&G Statewide Harvest Survey in 2012 showed an excess of 50,000 angler days in western PWS (Romberg et al., *In preparation*). This increase in angler effort increases the likelihood of rockfish encounters, which could result in a greater frequency of angling-induced barotrauma events.

Previous studies have documented an increase in rockfish survival post-recompression; however, their reproductive potential after being subjected to forced recompression is unknown. Although fish survival is clearly important for conservation and sustainability, it is important to understand the long-term effects of deepwater release on rockfish reproductive viability. One effect of catch-and-release fishing is an increase in fish stress hormones, which can constrain or limit growth and reproductive output (Wedemeyer 1996; Meka and McCormick 2005; Davie and Kopf 2006). For example, an individual may alter tissue or organ function to cope with stress (Schreck 2009). Lowe and Kelley (2004) found that California Sheephead *Semicossyphus pulcher* exposed to high levels of stress from capture, handling, and captivity had a temporary elevation in adrenalin followed by a surge of cortisol levels which increased the level of insulin-like growth factor-binding protein (IGFBP). Elevated levels of IGFBP can adversely impact egg production and may delay sexual maturity by affecting lipid content and the proximate composition of eggs (Eldridge et al. 1992; Schreck 2009). Energy may also be diverted due to such anabolic activities. Long-term exposure to stress hormones can reduce reproductive success, which at the population level could reduce recruitment and abundance (Barton 1997).

Lipids are a component of the egg yolk and a major source of nutrition and energy for embryos during development. For some species, lipid reserves are found in the oil globule, which is a proxy for energy reserves and is used to identify the energetic health of embryos among developmental stages (Weigand 1996; Berkeley et al. 2004; Sogard et al. 2008; Sewall and Rodgveller 2008). When larvae are extruded at parturition, the yolk has been absorbed. However, the oil globule remains and is a source of nutrition through the transition to exogenous feeding. The endogenous energy and triacylglycerol (TAG) is found in the oil globule. The amount of TAG in the oil globule increases with maternal age and is important for metabolism and growth (Weigand 1996; Norton et al. 2001; Berkeley et al. 2004). This oil globule is important because it serves as an energy reserve if there is limited food available during the initial stages of exogenous feeding or may enhance growth rates if consumed rapidly when food is available (Eldridge et al. 1992; Weigand 1996).

The availability of adequate lipid and protein is important for embryonic development and strongly influences larval survival by minimizing the potential for starvation (Rainuzzo et al. 1997;

MacFarland and Bowers 1995). For example, Berkeley et al. (2004) found that the volume of endogenous TAG found in the oil globule was the main correlate of larval Black Rockfish survival. Sewall and Rodgveller (2008) found a strong correlation between oil globule volume and lipid levels of Quillback Rockfish embryos and pre-parturition larvae. These studies demonstrate the importance of energy reserves when the transition to exogenous feeding begins and the necessity to evaluate potential factors that can influence the proximate composition of rockfish embryos. The energetic status of embryos is important because a critical survival period (and subsequently high mortality) occurs during the transition from endogenous to exogenous feeding. If nutrition is inadequate when exogenous feeding begins, the availability of energy reserves in the oil globule can increase the likelihood of larval survival and prevent potential effects to recruitment. Larvae are dependent on endogenous energy reserves, and the quality of maternal nutrition has a direct influence on their development and subsequent recruitment (Rainuzzo et al. 1997).

The overall goal of this research was to determine whether forced decompression (a barotrauma event) and subsequent recompression with a DRM affected the ability of a female Yelloweye Rockfish to successfully reproduce and contribute energy to embryos. Given the interest using DRMs, it is critical that studies consider effects on life history as well as variability between species to ensure the conservation of these long-lived fishes. Although survival is essential, reproduction must also occur following the use of DRMs and embryos must survive if populations are to persist. If this does not occur, other methods for reducing discard mortality may need to be identified to prevent rockfish populations from being depleted as catch-and-release practices continue to increase throughout their geographic distribution, such as PWS.

This thesis focuses two chapters on these considerations. For chapter 1, female Yelloweye Rockfish were examined to determine if they were able to reproduce following recompression. To accomplish this objective, Yelloweye Rockfish were captured and subjected to forced decompression (a barotrauma event), tagged, and released with a DRM in 2008 and/or 2009 at the same reef in PWS, Alaska, used by Hochhalter and Reed (2011). In subsequent years, tagged fish were recaptured and their reproductive status and embryos were examined using visual protocols. If it was not possible to visually observe gonads, histological sampling was used as an alternative method for determining whether fish were gravid. Vitellogenin and calcium^{2+} levels were also measured in previously captured fish, as well as fish with no known capture history, to allow for comparisons of these two hematological variables.

In chapter 2, the study objective was to identify if barotrauma and recompression event(s) affected the quality of Yelloweye Rockfish embryos. Embryos were examined at different stages of development from both recaptured females that had undergone previous forced decompression and recompression with a DRM and females with no known capture history. A random sample of embryos from each female was collected, and oil globule volume, caloric content, and percent lipid were quantified to determine embryo quality. Proximate composition values between embryos were compared from newly captured and recaptured females to identify if differences existed between these two groups. Differences between recaptured and newly captured fish will serve as an indicator that recompression with DRMs improves female fitness which increases nutrition quality and quantity available for embryonic development.

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Chapter 1 - Utilization of blood plasma for identifying sex and reproductive status of Yelloweye Rockfish subjected to barotrauma and recompression events[2]

Abstract

Discard mortality of rockfishes *Sebastes* spp. is a management concern along the west coast of North America. Although many rockfish species have exhibited the potential to survive following barotrauma injuries caused by forced decompression, it is unclear if barotrauma affects reproductive success. This study assessed whether Yelloweye Rockfish could experience a reproductive event following recompression and, in addition, could non-lethal blood plasma indicators (i.e., vitellogenin and calcium^{2+}) be used as a potential tool to identify sex, maturity, and reproductive viability of Yelloweye Rockfish *S. ruberrimus* up to two years following one or more barotrauma events and recompression with a deepwater-release mechanism (DRM). Results from hematological sampling demonstrated that mature females can be distinguished from males and immature individuals during oocyte development and immediately following parturition. However, it was not possible to discern mature females whose oocyctes were developing from post-parturition fish. All mature female Yelloweye Rockfish captured (*n* = 15) in 2008 or 2009 were gravid or spent in 2010. These results indicate that there is no evidence that reproduction of Yelloweye Rockfish is affected one to two years following forced decompression and recompression with a DRM.

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[2] This chapter is formatted following American Fisheries Society style guidelines.

1.1 Introduction

Rockfishes *Sebastes* spp. are long-lived marine species that are targeted by sport anglers and are also incidentally caught by anglers fishing for Pacific Halibut *Hippoglossus stenolepis* and Lingcod *Ophiodon elongates* (Hochhalter and Reed 2011). However, sustainable levels of rockfish harvest are unknown within many areas of Alaska, including Prince William Sound (PWS). Catch data have shown an increase in catch and harvest of rockfishes in recent years (Hochhalter et al. 2011). For example, the Alaska Department of Fish and Game (ADF&G) Statewide Harvest Survey (SWHS) estimated that total rockfish sport catch statewide in 2009, regardless of species, was 357,617 individuals, with 42% reported as released (most likely released at the surface). Further, the five-year (2006 – 2010) average from the SWHS showed that approximately 45% of rockfishes in Alaskan waters were released in PWS. To minimize adverse impacts on rockfish populations from angling, conservative management is necessary. To address this concern, current sport fishing regulations in PWS require that the first two demersal rockfishes caught by sport anglers must be retained, regardless of size, and all additional demersal rockfishes caught by sport angling must be released. This regulation was adopted in 1999 by the Alaska Board of Fisheries to reduce bycatch waste during other recreational fisheries in PWS (Hochhalter et al. 2011).

When sport-caught rockfishes are released at the surface, their survival probability is low due to their physiology and anatomy (Hannah et al. 2008; Hochhalter and Reed 2011). Unlike fishes with physostomous swimbladders that have a pneumatic duct to allow rapid release of gases, rockfishes have a physoclistous swimbladder which has no direct connection to the esophagus. Therefore, when a rockfish is caught by a sport angler and brought rapidly to the surface, they become positively buoyant and may also experience tissue damage (i.e., barotrauma). During rapid decompression, tissue damage can result from gases escaping the swimbladder into the body cavity, which also has the potential to rupture the swimbladder and cause external and internal injury (Parker et al. 2006; Hannah and Matteson 2007;

Pribyl 2010). External signs of barotrauma include esophageal eversion, exophthalmia (popeye), corneal emphysemas, bulging of the branchiostegal membrane, and a tight abdomen (Hannah et al. 2008; Jarvis and Lowe 2008). Pribyl (2010) conducted tissue-level examinations for rockfish subjected to forced decompression and found parasitic and bacterial infections as well as emphysemas in the heart ventricle and emboli in the head kidney and rete mirabile. Consequently, forced decompression has the potential to adversely affect rockfish tissues. As a result of injuries associated with forced decompression and the inability of physoclistous fishes to submerge when released at the surface, there is a lower likelihood of survival for individuals discarded at the surface (Diamond and Campbell 2009; Hochhalter and Reed 2011).

Recompression of captured rockfishes has been evaluated as an alternative management strategy instead of surface release to reverse barotrauma injuries in California, Oregon, and, more recently, in Alaska. The ADF&G, Division of Sport Fish, currently promotes the use of a deepwater-release mechanism (DRM) for releasing sport-caught rockfish that cannot be retained in an angler's bag limit (D. Bosch, ADF&G, personal communication). Deepwater release allows the fish to be submerged back to its depth of capture to reverse injuries associated with barotrauma by allowing expanded gases to recompress so that the fish can regain neutral buoyancy. Studies on the west coast of North America have addressed the short-term survival of recompressed rockfishes (Parker et al. 2006; Jarvis and Lowe 2008). In Oregon, Parker et al. (2006) found that Black Rockfish *S. melanops* had a low rate of mortality (<4%) after 21 d during simulated capture and recompression in a barometric chamber. In contrast, Jarvis and Lowe (2008) found that 2-d, post-recompression survival in cages ranged from 36 to 82% for five different rockfish species in California. Hochhalter and Reed (2011) found that Yelloweye Rockfish in PWS had a survival probability of 0.99 (95% CI = 0.48 – 1.0) when individuals were released at their depth of capture. In contrast, fish released at the surface had a survival probability of 0.22 (95% CI = 0.15 - 0.32). Hannah and Matteson (2007) observed that there was a high degree of variability in the condition of different species of rockfish after being subjected to forced decompression. However, the condition of a fish at the surface was not a good indicator of survival once an individual had been recompressed.

Decompression has the potential to disrupt a fish's behavior and physiology, including its reproductive viability. Rockfishes are matrotrophically viviparous (Love et al. 2002), meaning that they fertilize eggs internally, and females provide nutrition to the embryos directly and bear live young. Energy used during the reproductive cycle has the potential to be jeopardized and reallocated when disrupted by a stressful event such as being forced to surface. To assess the potential effects of forced decompression and subsequent recompression, it is important to understand how rockfish reproduction occurs. The reproductive cycle of female rockfish can be classified into five stages: 1) ovarian development and maturation (termed vitellogenesis); 2) copulation and fertilization; 3) gestation; 4) parturition; and 5) a resting period (Love et al. 2002). The maturation process includes the production of vitellogenin (VTG), which is an egg-yolk precursor protein found at high concentrations in the blood of vitellogenic females prior to spawning (Martin-Mills 2007). After vitellogenesis and fertilization, the gestation period lasts for one to two months and includes the development of embryos, followed by parturition, and the release of larvae. Parturition has been poorly documented in rockfishes, but has been observed to last a few hours for Yellowtail Rockfish *S. flavidus* but could be longer or shorter for other rockfish species (Love et al. 2002).

Vitellogenin concentration can be quantified by measuring proteins in the blood plasma, and has been used to determine sex and female stage of maturity in other fishes (Heppell and Sullivan 1999; Martin-Mills 2007). Vitellogenin levels have been found to increase during the primary yolk-globule stage, and concentrations remain elevated through secondary and tertiary yolk-globule stages. Levels of VTG begin to decline during gestation as embryos develop and individuals in a state of resting exhibit low VTG levels (Mori et al. 2003; Martin-Mills 2007). Hinck et al. (2011) showed that increases in VTG were associated with the spawning period in female Razorback Sucker *Xyrauchen texanus,* but remained at low concentration for males throughout their study.

Non-lethal methods for determining sex and status of maturation status by quantifying VTG as well as calcium (Ca^{2+}) have been used for many fish species (Heppell and Sullivan 1999; Gillespie and de Peyster 2004; Webb et al. 2002; Martin-Mills 2007; Hinck et al. 2011). One non-lethal method of detecting the sex and stage of maturation is an enzyme-linked immunosorbent assay (ELISA), which has been used to identify proteins such as VTG in the blood (Heppell and Sullivan 1999; Martin-Mills 2007). Martin-Mills (2007) concluded that blood plasma analyses using VTG and the sex hormone estradiol (E_2) were reliable measurements for distinguishing male Black Rockfish from females during the spawning period and, to a lesser extent, the stage of maturity among females. In addition to direct measurement of VTG, calcium (Ca^{2+}) is a secondary measure that has been used as an index of VTG to identify sex and maturation status of female fish. Calcium^{2+} is attracted to the carrier protein VTG, and concentrations also increase during gestation (Webb et al. 2002). Investigations for quantifying Ca^{2+} concentrations are beneficial as this measure is less expensive and a simpler alternative to measuring VTG concentrations. For example, Webb et al. (2002) examined sex and maturity stage of White Sturgeon *Acipenser transmontanus* and found that blood plasma indicators allowed accurate differentiation of sex for mature fish. Biochemical assays can be a valuable, non-lethal tool in fisheries management when there is a need to identify sex, maturity schedules, and reproductive timing of the species of interest. Such methods are desirable for examining populations of concern as it reduces the need to euthanize individuals to assess sex and female maturation status (Heppell and Sullivan 2000).

The objectives of this research were to identify if Yelloweye Rockfish could experience a reproductive event following recompression and use blood plasma VTG and calcium^{2+} concentration as a non-lethal means of sex and maturation status determination for Yelloweye Rockfish. To accomplish these objectives, visual observation and hematological tests were used to identify sex and reproductive status of captured Yelloweye Rockfish. Samples included fish that had been subjected to one or more decompression events in previous years as well as individuals that had no previously known decompression event history. This study also examined the use of VTG and calcium^{2+} as alternatives to histological analysis of gonads to identify sex and maturation status of Yelloweye Rockfish. These results will provide valuable information on the ability of Yelloweye Rockfish to reproduce one to two years following a barotrauma event and recompression with DRMs. Further, this study will also evaluate different methods for identifying Yelloweye Rockfish reproductive status. The insight gained in this study will provide guidance for managers on the potential value of DRMS as a viable approach for rockfish conservation.

1.2 Methods

Sample collection

Yelloweye Rockfish were collected by hook-and-line sampling aboard an ADF&G, Division of Sport Fish research vessel. The sampling site was a small reef at the head of Olsen Bay in Port Gravina, PWS, Alaska, which is located 47 km from Cordova and 97 km from Valdez (Figure 1.1). Barotrauma was previously induced during sampling and tagging of rockfish from May through September 2008 and May through August 2009 by ADF&G during a pilot evaluation and a separate mark-recapture study (Hochhalter and Reed 2011). In 2010, gross visual observation and histological sampling to assess the effects of forced decompression and barotrauma on reproductive viability for this project began on May 19 and continued through June 30. Sampling was designed to consist of five sampling events that were each six days in duration, with five days separating each event.

During all sampling events from 2008 through 2010, depth and time of capture observed on the boat's depth sounder, total time the fish was on deck, and the time the fish was released back at depth were recorded for all Yelloweye Rockfish. Captured fish were measured for total length (TL) to the nearest 1 mm, and visually observed for the presence of an individually numbered T-bar anchor tag (Model #FD-94; Floy Tag & Mfg., Inc., Seattle, Washington). Following visual observation, all fish were scanned for the presence of a passive integrated transponder (PIT) tag (Biomark Inc., Boise, Idaho) with a Biomark Pocket Reader PIT tag scanner. If no tag was detected (indicating that it had not been previously captured) and the fish was to be released, a PIT tag was inserted ventral and anterior to the insertion of the pectoral fin and a T-bar anchor tag was inserted adjacent to the dorsal fin. Parker and Rankin (2003) determined this location to be an ideal insertion site for PIT tags because retention was high (100% over 49 weeks) for Black Rockfish. Studies on Muskellunge *Esox masquinongy* determined that PIT tags are best suited for long-term studies because tag loss is higher for T-bar anchor tags (Rude et al. 2011). Although T-bar anchor tags do have utility as a secondary external mark, other studies have shown a reduction in retention over time for some fishes (Walsh and Winkelman 2002; Rude et al. 2011). The PIT tags used in this study were 12.5 mm in length (TX1411SST; 134.2 kHz), and were implanted using a plastic syringe-style implanter with a 3.2-cm non-replaceable 12-gauge needle (MK7 implanter; Biomark Inc.).

If a PIT or T-bar anchor tag was detected or observed, respectively, for Yelloweye Rockfish caught in 2010, the tag number was recorded for that fish. All fish that were captured were visually observed for external signs of barotrauma, which included recording if the fish had a tight abdomen (TA) and/or exhibited exophthalmia (EX), stomach eversion (EV), corneal emphysemas (bubbles in the eye; CB), or branchial protrusions (BP; Jarvis and Lowe 2008). Categorical assignments of the severity of these signs was recorded on a scale of zero to two, with zero being absent, one being moderate or present, and two being severe (Table 1.1). All captured fish were also visually examined for the presence of embryos (females) or the presence of the urogenital papilla or milt (males). If a captured fish was identified as being gravid (determined by the presence of an enlarged abdomen or signs of embryos) or spent (flabby abdomen) and had been tagged, it was euthanized by cranial contusion for subsequent laboratory analyses (see chapter 2).

Internal evaluations of euthanized fish were used to determine the effects of barotrauma on the swimbladder. Although swimbladder damage incurred in previous years may not affect current reproductive viability, it could serve as an indicator of damage resulting from capture events in previous years. Damage to the swimbladder was rated at three levels: 1) not ruptured; 2) partially ruptured; or 3) fully ruptured (Table 1.1).

Blood collection

A blood sample was collected from all Yelloweye Rockfish greater than 400-mm TL within five minutes of capture. One- to two-ml of blood was collected from the caudal vein of each fish with a 3-ml heparinized syringe fitted with a 21-gauge, 38-mm needle. Blood was transferred to 4-ml sodium-heparin BD vacutainer venous blood collection tubes and centrifuged for 10 minutes at 3,000 RPM. Following centrifugation, the plasma, which was approximately 50% of the total blood volume, was transferred via pipette into two separate 2-ml cryovials and stored in a liquid nitrogen cryoshipper. The cryoshipper maintained a temperature at or below -80ºC until samples could be transferred to a -80ºC deep freezer for storage until samples could be analyzed for VTG and calcium concentrations.

Gonad maturity assessment

Microscopic evaluation was used to identify sex and female gonad development stage (Table 1.2). The criteria used included five stages of female gonad development that were similar to those used by Sewall and Rodgveller (2008). Stage 0 represented a recruit spawner (sub adult), and was identified as a smaller individual that had a low percentage of immature eggs. Love et al. (2002) stated that subadult female rockfish often exhibited these characteristics in the year prior to their first spawning event. Stages 1, 2, and 3 were assigned to females with embryos in early, mid, and late stage of development, respectively. Stage-1 embryos were in very early stages of development, with no eye or body development and the presence of yolk. Stage-2 females had embryos where optic cups had formed with minimal retinal pigmentation and body development, while stage-3 females had embryos with darker retinal pigmentation, a developing body structure, and possibly a notochord indicated by the formation of spots. Stage-4 fish were identified as spent, post-parturition females and were observed having a sagging abdomen and some remnants of larvae or a spent gonad upon dissection (Table 1.2).

Plasma lab assessment

To quantify Ca^{2+} plasma concentrations, an Abaxis VetScan ® Classic (Abaxis, Inc. Union City, California) was used with an Avian/Reptilian Profile Plus rotor following the manufacturer's instructions. Individual rotors were inserted into the Vetscan which conducted multi-chemistry blood analysis in approximately 15 minutes with each reagent containing a diluent. This study focused solely on plasma Ca^{2+} concentrations produced by the Vetscan. After individual Ca^{2+} values were determined, the results were used to determine a range of concentrations that could be used to distinguish mature female Yelloweye Rockfish from males and immature fish. In addition, this evaluation was used to determine if the gonad development stages could be identified and distinguished among females.

To quantify plasma VTG, the methods of Martin-Mills (2007) were followed which were developed by Heppell and Sullivan (1999). To begin, purified VTG used by Martin-Mills (2007) from Black Rockfish was obtained to develop standards and was used in conjunction with an Enzyme-Linked Immunosorbent Assay (ELISA). To complete the assay, the next steps included the development of standards by serially diluting stock solutions of purified VTG in PBST (Phosphate Buffered Saline ([0.01 M $NaPO_4$, 0.15 M NaCl], and 0.05% Tween-20, pH 7.4) containing 2.5% normal goat serum (PBST-NGS). Following these methods (which were similar to Martin-Mills 2007), VTG standard concentrations were developed that ranged from 3.9 to 2,000 ug/ml. Four consecutive steps were next followed during the laboratory optimization phase: 1) microplate coating with antigen-primary antibody (purified VTG from black rockfish); 2) competition reaction; 3) incubation with a secondary antibody (goat anti-rabbit peroxidase conjugated antibody); and 4) enzyme substrate degradation. Assays were run one time and inter-assay variability was measured following the methods of Martin-Mills (2007) using pooled plasma samples. Following the laboratory optimization phase, the assay was used to determine plasma VTG concentrations for all plasma samples. Dilutions were made ranging from 1:10 to 1:2000 to determine variability that was anticipated for female plasma VTG concentrations based on results from Martin-Mills (2007). Standard curve samples were plotted on a log plot of OD 450 versus the sample concentration. A range of VTG values was identified for all individuals of known sex and stage of maturation that was available from microscopic evaluation. It was assumed that using vitellogenin from Black Rockfish would be effective as a surrogate (VTG from Yelloweye Rockfish was not available) and results from the ELISA would be similar to results from Martin-Mills (2007; S. Heppell, Oregon State University, personal communication).

Statistical analyses

The frequency of previous barotrauma signs observed in Yelloweye Rockfish captured at depths ≤4 atm and those individuals captured at >4 atm depths was examined to determine the effects of depth of fish capture. For blood analyses, values of VTG and Ca^{2+} were identified that corresponded with sex and gonad development stage for females based on microscopic evaluations. These results were applied to fish that did not have a necropsy. A one-way Analysis of Variance (ANOVA) was conducted to identify differences in mean Ca^{2+} concentrations among sex and stage of female maturity groupings. An ANOVA was also used to test for differences in plasma VTG concentrations among the aforementioned groups. For both ANOVAs, separate pairwise comparisons were completed using a Tukey's Honestly Significant Difference (HSD) post-hoc tests to determine which means were significantly different from each other. Relationships between plasma VTG and Ca^{2+} concentrations were identified for both sexes, as well as for females separately, using linear regression. All analyses were conducted in R statistical package (R Core Development Team 2012), and were considered significant at $\alpha = 0.05$.

1.3 Results

A total of 28 Yelloweye Rockfish were caught in 2010 that were originally captured and tagged in 2008 (*n* = 5) or 2009 (*n* = 23). All recaptured females (*n* = 15) contained embryos at some stage of development (*n* = 8) or had already gone through parturition and released larvae (*n* = 7). Five of these females were recaptured more than one time during 2009, indicating that they had been subjected to three or more decompression events. A total of nine males were recaptured but reproductive status could not be determined for these individuals.

The most common external barotrauma sign observed in recaptured Yelloweye Rockfish was EV, with 63% of recaptures exhibiting this barotrauma injury during one or more capture events (Table 1.3). Females captured exhibited EV 50% of the time regardless of capture depth, while males exhibited EV 90% of the time. At deeper depths, the presence of EV increased for males and immature fish of unknown sex, while only 50% of females exhibited EV regardless of capture depth. Females did not always exhibit EV and, unexpectedly, three of the gravid females did not exhibit EV at any capture event. One of these three fish was captured in 2008 and could have exhibited EV, but no barotrauma injuries were documented for this individual. One female classified as a recruit spawner was captured three times in 2009 and did not exhibit EV or any other specific barotrauma sign. However, this individual was observed with “fizzing” around its pectoral fins during its initial capture events in 2009 and its recapture in 2010.

Necropsies of females recaptured in 2010 showed that these fish did not have ruptured swimbladders. However, they did have a ruptured tunica externus which was categorized as a partially ruptured swimbladder. These necropsy examinations showed that no mature recaptured fish had a fully ruptured swimbladder and only one immature newly captured individual had a swimbladder that could be considered fully ruptured.

During 2008 and 2009, a PC was observed in 42% of the recaptured females, although this barotrauma injury was not consistently observed and reported in all years. As a result, the percentage of PC may have been higher than reported in this study (Table 1.3). Exophthalmia was observed for two recaptured fish (a male and an immature individual) during initial capture, but these fish did not exhibit EX when recaptured in 2010. Corneal gas bubbles were documented only once during this study which was observed for the same male that exhibited EX. Branchial protrusions were not well documented during this study. Protrusions were not always readily visible, and there may have been some observer error in identifying the protrusions. With the exception of EV for females (which was 50%), barotrauma signs increased with depth of capture (Table 1.3).

Blood was extracted from 61 Yelloweye Rockfish, with 28 of these individuals previously captured in 2008 and 2009 (46%; Table 1.4). Twenty-two fish were positively identified as males by visual observation (36%), with nine individuals (41%) captured in 2008 or 2009. Five fish were classified as being immature (18%), and four of these individuals were recaptures (80%). Twenty-eight fish (46%) were positively identified as females by visual observation and fifteen (54%) of these fish were recaptures (seven fish were released alive). Based on visual observation, seven of the recaptured females were spent (46%), seven were gravid (46%), and one individual was classified as a recruit spawner post-dissection (8%).

Calcium plasma concentrations ranged from 13.1 to 20.0 mg/dL for all mature female fish, while males, immature individuals, and recruit spawner females had plasma Ca^{2+} concentrations levels at or below 13.1 mg/dL (Figure 1.2). There were significant differences in mean plasma Ca^{2+} concentrations among sex and gonad development stage categories for all individuals ($F = 19.56$, $P < 0.0001$). Specifically, Ca^{2+} plasma concentrations of stage-2 fish were significantly different from males, stage-0 (recruits), and immature fish. While stage-2 fish were also the only stage where mean Ca^{2+} was greater than the other female gonad development stages, it was not significantly different from the other stages.

Mean plasma Ca^{2+} concentrations of females (excluding recruit spawners) were significantly different from males and immature fish.

Female VTG concentrations ranged from 13 to 425 µg/ml, while males were below 11 µg/ml or had undetectable VTG levels (Figure 1.3). There was a significant difference in plasma VTG concentrations among groups ($F = 14.39$, $P < 0.0001$), with mean plasma VTG concentration for females (excluding recruit spawners) greater than males. With the exception of stage-0 females whose VTG was significantly lower than other females, there were no significant differences among female stages of gonad development.

There was a positive significant relationship between plasma VTG and Ca^{2+} when male and female blood samples were combined ($r^2 = 0.59$, $p < 0.0001$; Figure 1.4). However, this relationship was highly variable. A positive relationship between VTG and Ca^{2+} was also observed among female stages of development, although this relationship was also highly variable ($r^2 = 0.24$, $p = 0.002$).

1.4 Discussion

Barotrauma and reproduction

Barotrauma injuries were observed less frequently in the current study than other decompression studies (Hannah and Matteson 2007; Pribyl 2010). Pribyl (2010) estimated that approximately 80% of Black Rockfish in their study experienced EV, while Yelloweye Rockfish in the current study only exhibited this barotrauma injury 63% of the time. However, Pribyl (2010) studied a pelagic species (Black Rockfish) in a simulated laboratory setting and at shallower depths than in the current study. Although EV was the most common barotrauma injury observed in the current study, gravid female Yelloweye Rockfish did not exhibit signs of EV as frequently as males. This result was unexpected considering the large volume of space occupied by developing gonads in the body cavity. Gases were likely escaping the body cavity, which prevented EV or swimbladder rupture to occur in female

Yelloweye Rockfish. While recompression of rockfishes has been found to reverse injuries due to barotrauma (Parker et al. 2006; Jarvis and Lowe 2008; Pribyl 2010), developing gonads can occupy much of the abdominal cavity which could have the potential to complicate recovery. In this study, gonad volume did not appear to increase barotrauma injuries or affect recovery.

Few individuals in the current study exhibited EX; however, fish that are captured from depths greater than this study (>7 atm) typically have more severe barotrauma injuries, such as EX (Hannah and Matteson 2007; Jarvis and Lowe 2008; Rogers et al. 2011). Rogers et al. (2011) examined EX in Rosy Rockfish *S. rosaceus* captured between 63 and 110 m in length and found that even though barotrauma injury can disrupt visual performance, recompression can quickly alleviate the symptoms of EX and allow fish to recover and resume normal visual capabilities. If Yelloweye Rockfish in the current study were unable to resume normal vision or physical functions, they may have difficulty finding prey which could affect fitness and energy allocations needed for reproduction. Based on the survival study results of Yelloweye Rockfish captured and released with a DRM at this same study site (Hochhalter and Reed 2011), it is assumed that fish with barotrauma injuries such as EX or EV were able to recover and resume normal physiological functions.

No recaptured Yelloweye Rockfish appeared to be unhealthy based on visual observation and no recaptured fish had a fully ruptured swimbladder. Although recaptured individuals were able to heal swimbladder damage after initial capture in 2008 or 2009, it is unknown how long it took for that process to occur. Parker et al. (2006) found that 77% of Black Rockfish could repair their swimbladder within 21 d. Pribyl (2010) reported similar results for Black Rockfish, but found that the tunica externus that surrounds the swimbladder took longer to heal. At the end of a 31-d recovery period, the author found that the tunica externus had not healed in 80% of fish. The inability to heal a ruptured swimbladder can affect the ability of an individual to maintain neutral buoyancy and has the potential to affect the ability to find prey (Pribyl 2010). The immediate effects of swimbladder injury post-recompression for Yelloweye

Rockfish are unknown; however, survival and future reproduction of Yelloweye Rockfish in this study lead researchers to believe that the immediate effects are minimal at this study site with the use of a DRM.

Blood analyses

The use of blood-plasma measurements for identifying sex and maturation stage has been successfully used for many fish species (Heppell and Sullivan 1999; Webb et al. 2002; Gillespie and de Peyster 2004; Martin-Mills 2007). In this study, non-lethal hematological analyses using blood plasma were useful for distinguishing adult female Yelloweye Rockfish from other individuals. However, this approach was not a useful tool for distinguishing among stages of female gonad development. While all female Yelloweye Rockfish were collected during oocyte development or post-parturition, no individuals from the early or mid-phase of the vitellogenic period were collected during this study. As a result, there was minimal contrast among reproductive developmental stages. Stage-2 individuals had the highest levels of VTG, but the degree of variability among gonad development stages precluded using this measure as a reliable diagnostic tool for individual stages of gonad development. Three males were observed with small quantities of VTG. It is unknown why these values were observed; however, as with any lab work there is the potential for cross contamination when working with many samples.

Calcium^{2+} plasma concentrations could also be used to differentiate male from female Yelloweye Rockfish that were undergoing oocyte development and post-parturition in the current study and as identified in studies on other fish species (Webb et al 2002; Jamalzadeh et al. 2012). Unlike VTG, females in stage 2 of gonad development could be identified from the other stages because these fish exhibited significantly higher values of Ca^{2+}. Quantifying Ca^{2+} levels was much less lab intensive for identifying sex and gonad development stage than using VTG. As a result, this could be considered a much simpler and also more reliable tool for evaluating the reproductive status of individual female Yelloweye Rockfish as it has also been used in other fish species and the values identified in this study were similar to those identified in other studies (Webb et al. 2002; Jamalzadeh et al. 2012). In addition, this method may potentially be used to determine the timing of the reproductive cycle for the target species, but it does not appear to be useful as a means to determine specific stages of gonad development.

Research on other fishes has been able to include females from the early and mid-phases of the vitellogenic period which provided better contrast between female stages of reproductive development. Heppell and Sullivan (1999) examined the maturity of Gag *Mycteroperca microlepis* and identified that VTG reached peak levels during late vitellogenesis and declined only slightly during oocyte maturation or ovulation. These authors reported that VTG could not be detected in the plasma for male, immature, or pre-vitellogenic females. Martin-Mills (2007) reported similar results for VTG and estradiol (E_2), and concluded that these blood-plasma measures can be used to identify mature female Black Rockfish from other individuals during the spawning period. Although female gonad development stage was examined throughout the entire year, pre- and early vitellogenic females did not have higher concentrations of VTG than males. Because female Yelloweye Rockfish were not captured during the early period of vitellogenesis in this study, it is not known if males in the current study could be differentiated from females during this period of development. Based on previous studies, it is unlikely that Yelloweye Rockfish VTG concentrations would be significantly different between males and females during early vitellogenesis (Heppell and Sullivan 1999; Martin-Mills 2007).

Study caveats

One caveat of this study was due to the use of the Vetscan®. Plasma Ca^{2+}concentrations for gravid females often reached the maximum detection levels for this equipment. This outcome resulted in what appeared to be maximum values that could be reached and the appearance of categorical values at higher Ca^{2+} levels instead of continuous values of Ca^{2+}. However, it is unknown how these values would have differed using different approach such as a flame atomic absorption (AA) spectrometer (Gillespie and de Peyster 2003). By utilizing an alternative approach, future evaluations may have the ability to more reliably identify developmental stages which may assist distinguishing spent fish from females close to releasing larvae.

An additional study caveat was that injuries due to barotrauma were not recorded during all years for all fish. In the 2008 feasibility study, researchers did not document BP or PC throughout the entire study period. As a result, the data collected were only representative of those individuals where full observations of an individual fish were made. When observations were not made, fish were not included in the results. This does not mean that no barotrauma signs occurred, it just means no observations were made and recorded. Therefore, fewer individuals were used in the data set for determining how often barotrauma injuries were observed.

Future research/knowledge gaps

Additional research is warranted on other rockfish species to determine if previous decompression events affect future reproductive viability. Barotrauma studies have demonstrated that there are species-specific responses to barotrauma injuries (Hannah and Matteson 2007; Hannah et al. 2008; Pribyl 2010), and rockfishes could exhibit a species-level differences in reproductive viability after being subjected to decompression. Previous studies have shown that barotrauma injuries are reversible and future reproduction is not negatively affected (Pribyl 2010; Rogers et al. 2011). Although it appears that rockfishes can heal from barotrauma injuries, it is unknown how barotrauma may affect an individual during the gestation period. It is also unclear if gravid females will abort and fail to complete gestation and parturition during the same year that they are subjected to angling-induced barotrauma and recompression event. Based on the results of Pribyl (2010), fish may have reduced fitness that not only affects parental spawners, but could also impact embryo development if parental female fitness is reduced during gestation by recovery from barotrauma injuries. In the current study, the goal was only to sample fish near or during the gestational period. Therefore, Yelloweye Rockfish were not collected during pre- or mid-vitellogenic stages during recapture events. Research on other rockfish species in which samples are collected throughout the year would provide a better representation of reproductive timing and blood-plasma concentrations for all stages of maturation. Future research of VTG and Ca^{2+} from Yelloweye Rockfish in PWS should include a larger timeframe to compare all plasma concentration levels.

Conclusion

The results from this study will not only provide managers with information on the reproductive viability of Yelloweye Rockfish following an angling-induced barotrauma event and recompression with a DRM, but will also contribute valuable information on the time period that female Yelloweye Rockfish are gravid in PWS. If the use of a DRM is going to be considered as a management tool, these results could be used for implementing more specific regulations such as requiring the use of DRMs during time periods when females are visually gravid. While recruitment is a key component in fisheries management for maintaining productivity, the dynamics and strategies of reproduction are also important (Lowerre-Barbieri et al. 2011). Deepwater release is a viable conservation tool for all rockfish populations, especially Yelloweye Rockfish as shown here with the ability of fish to reproduce one to two years following recompression events.

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Table 1.1.– External and internal barotrauma injury observations, ratings, and description for Yelloweye Rockfish.

Type	Rating	Description
Esophageal eversion (EV)	0	None observed
	1	Stomach everted into the pharynx only
	2	Stomach everted beyond the buccal cavity
Prolapsed cloaca (PC)	0	None observed
	1	Herniations: Disruptions of the body wall in the pericloacal area through which the gut protruded
	2	Intussusceptions: Actual eversion of the terminal portion of the intestine through its own lumen
Exophthalmia (EX)	0	None observed
	1	One eye protruding outward from orbit
	2	Both eyes protruding outward from orbit
Branchial protrusions (BP)	0	No protrusions observed
	1	Portions of the gut protruded through the brachial opening
Tight abdomen (TA)	0	No tightening of the abdomen
	1	Abdomen swollen, tight to touch
Corneal gas bubbles (CB)	0	None observed
	1	Corneal gas bubbles present in the eyes
Swimbladder tearing or rupturing	0	Fully intact
	1	Partially ruptured: Ruptured tunica externa
	2	As indicated by a visible tear in both layers or by the swimbladder holding no gas or collapsing under light finger pressure

Table 1.2.–Description of sex and gonad stages identified for Yelloweye Rockfish.

Stage #	ID	Description
Stage 0	Recruit	Female with gonad and a small amount of eggs that were not mature
Stage 1	Early	Female gonad with embryos exhibiting yolk, but no eye or body development
Stage 2	Mid	Female gonad with embryos exhibiting optic cups, early retinal pigmentation, and some body development
Stage 3	Late	Female gonad with embryos exhibiting mid-dark retinal pigmentation, developing body structure, and possibly the formation of spots
Stage 4	Spent	Female with a sagging belly and residual larvae or spent gonad
Stage 5	Male	Male identified by urogenital papilla and/or presence of milt in gonads
Stage 6	Immature	Individual with gonads that are small, translucent, stringy, pink, yellow or amber in color

Table 1.3.–Number of Yelloweye Rockfish observed with the corresponding barotrauma sign (*N* = 27 recaptures) at different atmospheres (2008 and 2009).

	Captured at 2 – 4 atm			Captured at >4 atm		
Barotrauma sign	Female	Male	Immature	Female	Male	Immature
Total captures (N)	8	8	3	4	2	2
Esophageal eversion	4	7	1	2	2	1
Exophthalmia	0	0	0	0	1	1
Tight abdomen	3	5	3	2	2	2
Prolapsed cloaca	3	na	0	2	na	1

*Prolapsed cloaca was only recorded for female fish therefore it was not applicable (na) to males.

Table 1.4.–Life history stage and sample size collected for hematological sampling for Yelloweye Rockfish.

Stage	Description	Number of new captures	Number of recaptures
0	Recruit	1	1
1	Early	4	2
2	Mid	4	3
3	Late	7	2
4	Spent	3	7
5	Male	13	9
6	Immature	1	4

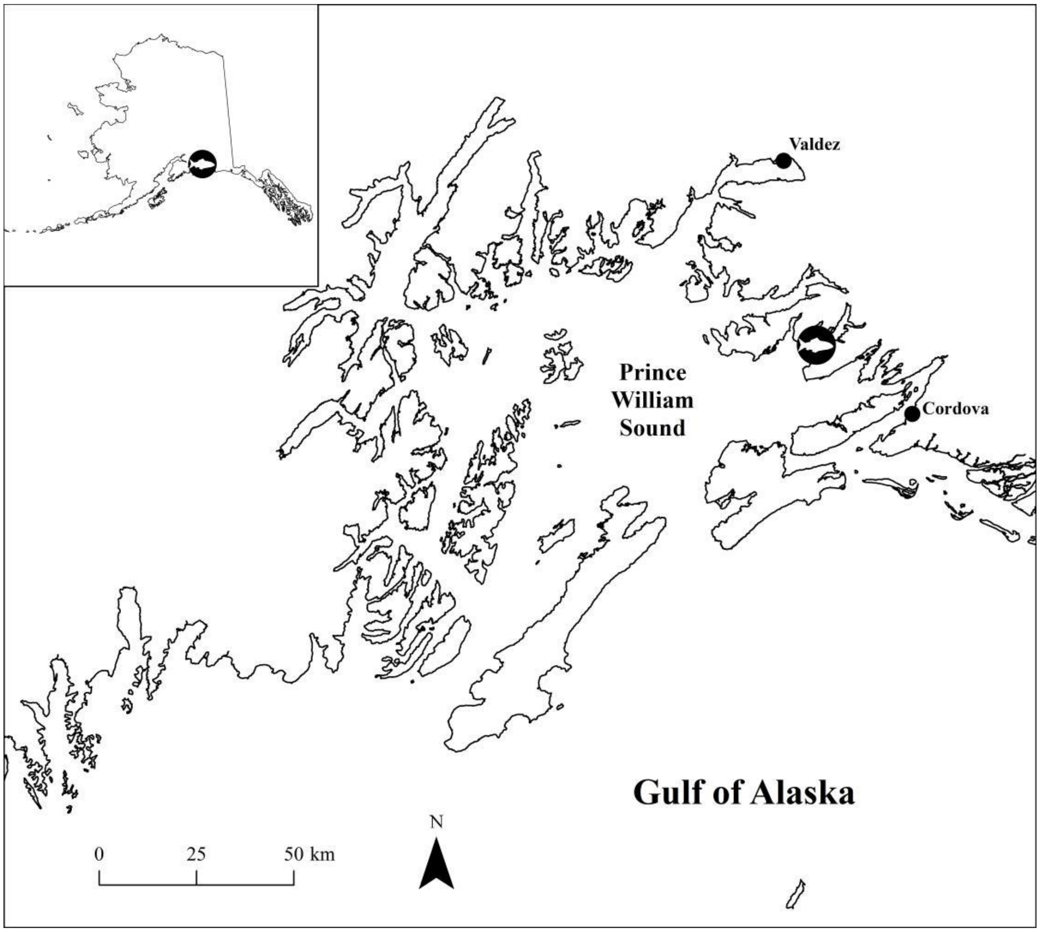

Figure 1.1.–Map showing sampling site (white fish on black circle) near Port Gravina, Prince William Sound, Alaska (Map courtesy of M. Albert, ADF&G).

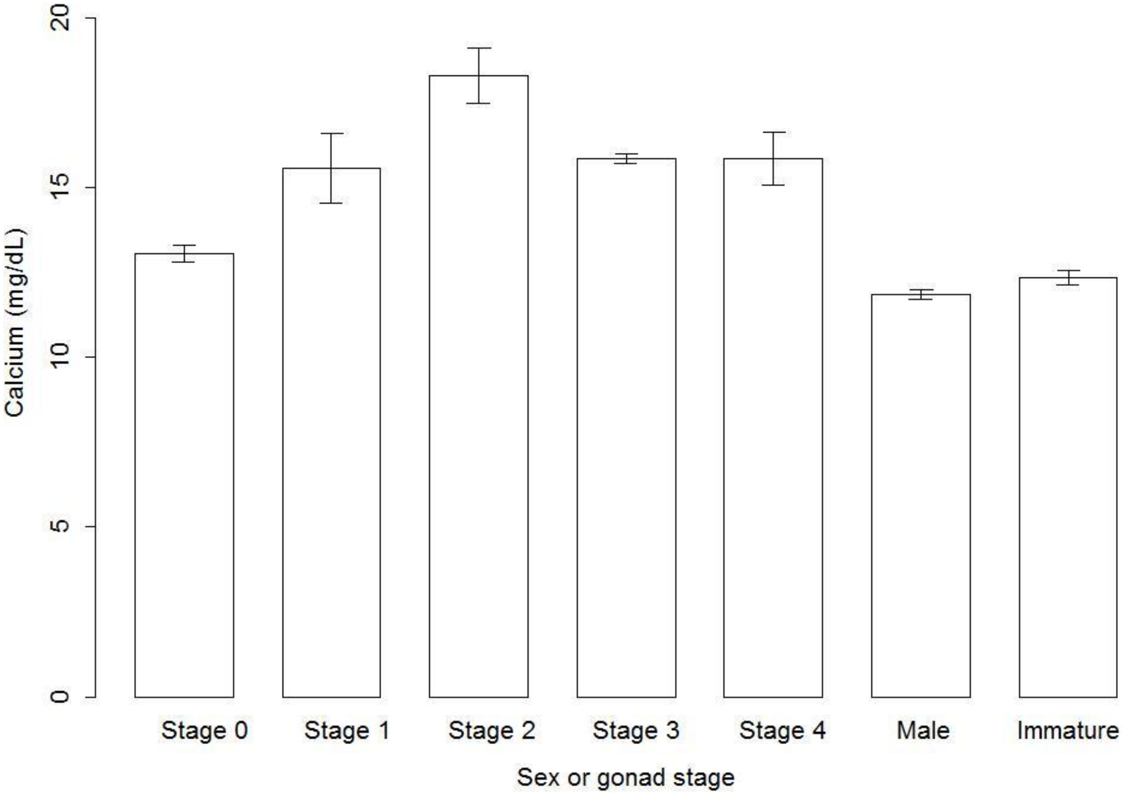

Figure 1.2.–Mean (±SE) calcium plasma concentrations observed for Yelloweye Rockfish. Stages 0 – 4 represent females.

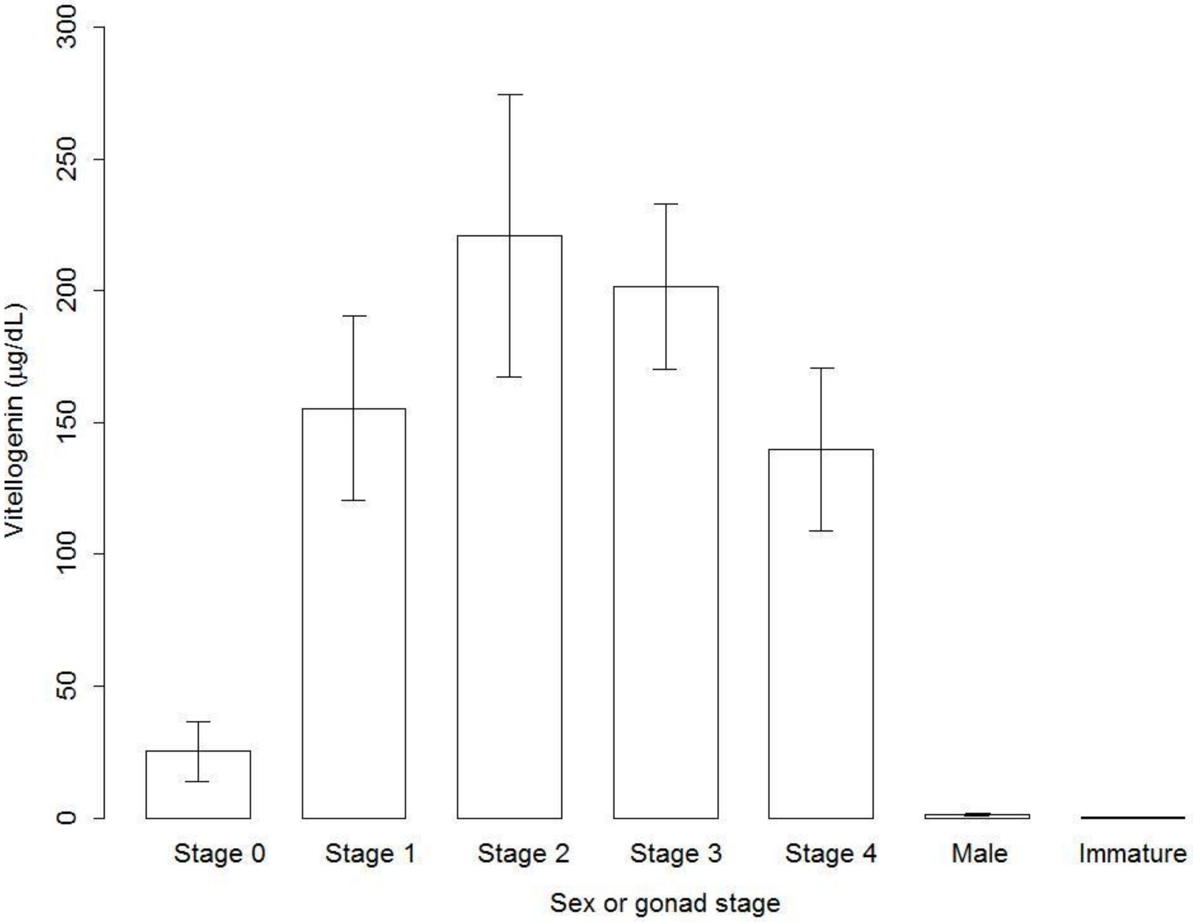

Figure 1.3.–Mean (±SE) vitellogenin plasma concentrations observed for Yelloweye Rockfish. Stages 0 – 4 represent females.

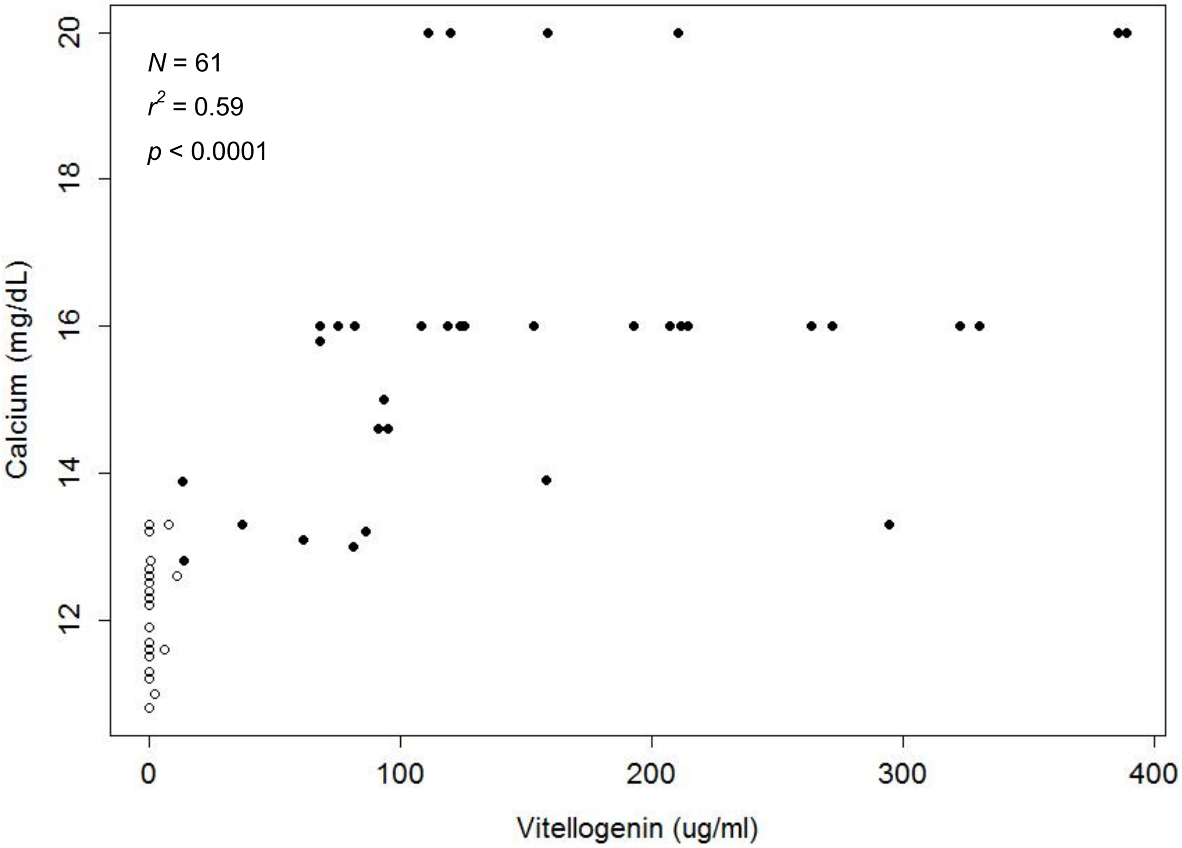

Figure 1.4.–Relationship between calcium and vitellogenin plasma concentrations for Yelloweye Rockfish. Filled circles represent mature females, while open circles represent male and immature fish.

Chapter 2 – The effects of forced decompression and recompression on the quality of Yelloweye Rockfish embryos[3]

Abstract

Catch-and-release fishing has the potential to adversely affect reproduction for rockfishes *Sebastes* spp. due to angling-induced barotrauma caused by forced decompression. Although Yelloweye Rockfish *Sebastes ruberrimus* have been found to be reproductively viable following a forced decompression and subsequent recompression event, the energetic cost of recovery from such an event may negatively affect reproductive fitness through reduced embryo quality. During this study, seven female Yelloweye Rockfish were captured one to two years after initial capture and recompression with a deepwater-release mechanism (DRM). Embryo quality from each female was examined and compared with a sample of embryos from 13 females with no previously known capture events to identify differences in embryo oil globule volume, caloric content, and percent lipid. There were no significant differences identified in these proximate composition measures, parent age and length, and depth at first capture between recaptured females and individuals with no previously known capture events. This study provides baseline information demonstrating that forced decompression and recompression with a DRM does not appear to negatively affect embryo quality one to two years following a recompression event and contributes to the importance of the use of DRMs by sport anglers for rockfish conservation.

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[3] This chapter is formatted following American Fisheries Society style guidelines.

2.1 Introduction

Rockfishes *Sebastes* spp. are iteroparous, have internal fertilization, and bear live young. Previous research has determined that for some rockfish species, energy is transferred from the mother to the embryo directly in addition to energy that is also derived from the yolk (i.e., matrotrophically viviparous; Love et al. 2002). Although many rockfishes are matrotrophically viviparous, there is substantial variability among species, with 3 to 92% of the energy contribution directly from the mother post-fertilization (Love et al. 2002). For example, Dygert and Gunderson (1988) found that 11.5% of energy utilized by Copper Rockfish *S. caurinus* embryos during gestation had been contributed post-fertilization by the mother. Alternatively, Boehlert and Yoklavich (1984) found that Black Rockfish *S. melanops* had a maternal contribution of 70%. These authors found that total nitrogen remained nearly constant during gestation and, as a result, the maternal energy contribution was likely nitrogenous, coming from amino acids or peptides. Endogenous energy for embryos and larvae is also found in the oil globule and is composed primarily of triacylglycerol (TAG), which provides energy for metabolism and growth (Norton et al. 2001). During the transition to exogenous feeding, it is likely that the oil globule is an important source of energy for rockfish larvae as they can become susceptible to starvation if maternal nutrition is inadequate (Boehlert and Yoklavich 1984; Fisher et al. 2007).

Previous research on rockfishes, as well as other fish species, has shown that the maternal contribution to development is a key factor in egg and larval survival (Hislop 1988; Trippel et al. 1997; Berkeley et al. 2004). Maternal effects, such as age, size, and proximate composition, can also play a role in embryo quality and the timing of parturition for species that have long reproductive life spans (Sogard et al. 2008). Specifically, older, larger females often produce larger, higher quality embryos relative to younger individuals; however, this is not universal and may be attributed to older females having more experience with reproduction (Trippel 1998; Sogard et al. 2008). Berkeley et al. (2004) observed that older Black Rockfish larvae had larger oil globules than younger fish which translated to a higher probability of survival for older individuals. Sogard et al. (2008) observed differences in the timing of larval release in four of six rockfish species and found that larger females released larvae earlier. This study concluded that maternal effects such as age and size, although variable among rockfish species, were reflected in larval quantity, quality, or extrusion time.

Other physical or environmental stressors can affect a fishes' ability to conduct life functions, such as reproduction (Schreck 2009). Catch-and-release fishing of rockfish can be stressful and presents problems due to physiological constraints associated with having a physoclistic swimbladder. When individuals are forced rapidly to the surface during capture, the expansion of gases in the swimbladder causes damage to tissues (i.e., barotrauma) which is often observed by external and internal signs of injury (Hannah and Matteson 2007; Hannah et al. 2008; Pribyl 2010). Forced decompression can also cause rockfishes to become positively buoyant and, as a result, they are often unable to submerge unassisted (Hannah et al. 2008; Hochhalter 2012). Recent research has found that Yelloweye Rockfish *S. rubberimus* can survive forced decompression when recompressed with a deepwater-release mechanism (DRM; Jarvis and Lowe 2008; Hochhalter and Reed 2011). A recompression event is an assisted release that involves descending a captured individual back to a depth at or near original capture. However, it is unknown how forced decompression and recompression with a DRM will affect the fitness of parental females and how this effect could be transferred to offspring in subsequent years.

Yelloweye Rockfish are frequently captured in the Prince William Sound (PWS) sport fishery and throughout Alaska (Hochhalter et al. 2011). This species was the focus of this study because of their importance in the sport fishery, their proven high site fidelity in previous years, and the ability to recapture them following release with a DRM. In Chapter 1, it was shown that female Yelloweye Rockfish can successfully reproduce one to two years following a recompression event using a DRM.

However, it is not known if decompression and recompression had an effect on the quality of embryos from those same fish.

The objective of this research was to identify Yelloweye Rockfish embryo quality by examining oil globule volume (OGV), caloric content, and percent lipid from mature females and then compare between individuals that were subjected to one or more angling-induced decompression events and recompressed with a DRM (recaptures) and individuals that had not been subjected to a previously known decompression event (new captures). The results from this study will provide valuable information on Yelloweye Rockfish embryo quality during development as well as baseline data on the potential effects of angling-induced barotrauma and recompression with DRMs on recruitment and year-class strength.

2.2 Methods

Sample collection

Yelloweye Rockfish were collected by hook-and-line sampling aboard an Alaska Department of Fish and Game (ADF&G), Division of Sport Fish research vessel. The sampling site was a small reef at the head of Olsen Bay in Port Gravina, PWS, Alaska, which is located approximately 47 km from Cordova and 97 km from Valdez (Figure 2.1). Barotrauma was previously induced during sampling and tagging of rockfish from May through September 2008 and May through August 2009 by ADF&G during a pilot evaluation and a separate mark-recapture study. Yelloweye Rockfish were targeted and captured opportunistically during 22 d of sampling in May and June 2010 when females had developing embryos.

The depth observed on the boats depth sounder and time of capture was recorded upon hooking a Yelloweye Rockfish. Captured fish were measured for total length (TL) to the nearest 1 mm and visually observed for the presence of an individually numbered T-bar anchor tag (Model #FD-94; Floy Tag & Mfg., Inc., Seattle, Washington). Following visual observation, all fish were scanned for the presence of a passive integrated transponder (PIT) tag (Biomark Inc., Boise, Idaho) with a Biomark Pocket Reader

PIT tag scanner. If a PIT or T-bar anchor tag was detected or observed (which noted a recaptured rockfish), the tag number was recorded for that fish. All recaptured and newly captured fish were visually observed for external barotrauma signs, which included assessment of whether the fish had a tight abdomen (TA), exophthalmia (EX), stomach eversion (EV), corneal emphysemas (bubbles in the eye; CB), or branchial protrusions (BP) (Jarvis and Lowe 2008). Categorical assignments of the severity of these signs was recorded on a scale of 0 to 2, with zero being absent, one being moderate or present, and two being severe (Table 2.1).

All captured fish were visually examined to identify if embryos (females) or milt (males) were present. If a captured fish appeared to be a gravid female and had been previously tagged, it was sacrificed by cranial contusion for future laboratory analyses. A random sample of untagged, gravid females were also captured at random, euthanized by cranial contusion, and weighed to the nearest 0.01 kg. A necropsy of these fish was conducted by making a longitudinal incision from the anus to just below the pericardium. For internal observations, the methods of Jarvis and Lowe (2008) were followed to identify damage or rupturing of the swimbladder (Table 2.1). Although swimbladder damage at the time of capture in 2010 would not have an effect on embryo quality in 2010, it provides information on what likely occurred during the recapture event in previous years. Damage to the swimbladder was identified at three levels: 1) not ruptured; 2) partially ruptured; or 3) fully ruptured (Table 2.1). The presence of organ torsion was also recorded, as well as any other abnormalities. Ovaries were removed from euthanized individuals and frozen for laboratory analyses.

Sagittal otoliths were removed from euthanized fish by using a knife to expose the otic capsule where the otolith was located. After the otic cup was opened, the otoliths were removed with forceps, cleaned of the otolithic membrane, and stored dry in vials for later age estimation. Ages were estimated using the break and burn technique (Chilton and Beamish 1982) by the Alaska Department of Fish and

Game (ADF&G), Homer office. Annuli on otoliths were identified by two separate observers, and any discrepancies were resolved using a concert read.

Laboratory analyses

Microscopic evaluation was used to identify stages of embryo development. The stage of Yelloweye Rockfish embryo development was determined using a modified version of the criteria developed by Yamada and Kusakari (1991) for Korean rockfish *S. schlegeli* and used by Sewall and Rodgveller (2008) and Rodgveller et al. (2011) for Black Rockfish. Based on Sewall and Rodgveller (2008), the criteria were modified to only include three levels for embryo stage of development: stage 1 (early), stage 2 (mid), or stage 3 (late) to account for developmental differences (Table 2.2). Stage-1 embryos were in the early stages of development, with no eye or body development and the presence of yolk. Stage-2 females had embryos where optic cups had formed with minimal retinal pigmentation and body development, while stage-3 females had embryos with darker retinal pigmentation, a developing body structure, and the formation of spots along the notochord (Figure 2.2).

Two samples of embryos (0.5 to 1 g each) from each individual fish were counted separately using a dissecting scope under 4X magnification to determine the average wet mass per individual embryo from each female. Averages of the two samples determined the mean wet mass per individual embryo from each female. Oil globule volume (OGV) of developing embryos was determined by sampling 20 embryos from each individual female following the methods of Sewall and Rodgveller (2008). These methods included photographing individual embryos using a Leica DM 1000 microscope (Leica, Wetzlar, Germany) and a Leica DFC420C digital color camera at 4X magnification. After images were taken, two perpendicular measurements of the oil globule diameters were made using the Leica Application Suite V3.3.0 and recorded in micrometers (μm). Using each diameter measurement, OGV was calculated as the volume of a sphere ($V = (\pi * d^3)/6$), which provided a volume estimate for each recorded diameter measurement. As with other studies, it was assumed that oil globules were spherical

(Berkley et al. 2004; Sewall and Rodgveller 2008). The estimates were averaged for each embryo, and the final mean OGV for each female was determined by calculating the average of the 20 embryo OGV measurements.

For caloric content and percent lipid analyses, a random sample of each female's ovaries were selected, freeze dried at -58ºC for 60 hours, and stored dry. A Parr Model 1281 Automatic Bomb Calorimeter (Parr Instrument Company, Moline, Illinois) was used to measure the energy content of developing embryos. Two freeze-dried ovarian samples from each female (between 0.5 and 1 g per sample) were combusted in a high-pressure oxygen atmosphere in a metal pressure chamber. Total energy content (calories) for each sample was converted to kilocalories (kcals). Lipids were extracted from freeze-dried embryos using a modified Soxhlet procedure following the procedures of Schlechtriem et al. (2003). Following these methods, two replicates of freeze-dried embryos were measured at approximately 0.5 g. Prior to lipid extraction, a thimble was weighed and tared, and the sample was transferred to the thimble and weighed to the nearest 0.0001 g. The thimble was placed in a Soxtec extractor and processing began by heating a 2:1 chloroform methanol solution. Lipids were extracted by passing the 2:1 chloroform methanol solution through a Soxhlet apparatus for 18 hours, which remained at room temperature under a fume hood for 30 minutes following extraction to allow the remaining solvent to evaporate. Samples were subsequently dried in a drying oven at 50ºC for 30 minutes and the lipid extracted sample was weighed and recorded to the nearest 0.0001 g. Percent lipid was calculated by subtracting sample dry mass from the initial wet mass to determine the amount of lipid removed.

Statistical analyses

A Kruskal-Wallis rank sum test was used to determine if the depth of first capture, parent age, and weight were significantly different for recaptured and newly captured female Yelloweye Rockfish. A Kruskal-Wallis rank sum test was also used to determine if embryo proximate-composition variables (i.e., OGV, caloric content, percent lipid) were significantly different between recaptured and newly captured individuals. The Kruskal-Wallis rank sum test was also used to determine if OGV, caloric content, percent lipid, and parental female age were significantly different among stages of embryo development, regardless of capture history. All analyses were conducted in R statistical package (www.r-project.org), and were considered significant at $\alpha = 0.05$.

2.3 Results

Twenty female Yelloweye Rockfish were captured for this study. Thirteen of these fish were new captures, and the other seven individuals were first captured in 2008 or 2009 (Table 2.3). Three of the recaptured fish were caught for a third time in 2010 and had been subjected to known forced decompression and angling-induced barotrauma events on three separate occasions, which included the 2010 capture and recompression with a DRM during the first two capture events. The remaining four recaptures were captured for a second time in 2010 and had only been subjected to one previously known angling-induced barotrauma event and one recompression with a DRM. One of the seven recaptured females had small gonads, which only allowed for evaluation of OGV.

Newly captured fish were caught at depths ranging from 36 to 74 m, whereas recaptured individuals were caught at depths between 34 and 65 m in 2010. In 2008 and 2009, recaptured fish were caught at depths between 28 and 65 m in depth. The depth of first capture did not differ significantly between recaptures and new captures ($H = 0.38$, $p = 0.54$). The length and age of new captures ranged from 395 to 635 mm and 15 to 36 years, respectively, while these estimates for recaptures ranged from 365 to 510 mm and 17 to 34 years, respectively. There were no significant differences in length and age between new captures and recaptures (length: $H = 3.05$, $p = 0.08$; age: $H = 0.19$, $p = 0.66$).

Developmental stages of embryos from each female used for analyses consisted of five fish at stage one, seven fish at stage two, and eight fish at stage three (Table 2.3). Parental female age for each stage of embryo development was similar, with females carrying stage-1 embryos having a median age of

22 (range,16 to 33), while females with stage-2 and -3 embryos had a median age of 26 (range, 17 to 36) and 27 (range, 15 to 34), respectively (Table 2.4). There were no significant differences in parental female age among stages of embryo development ($H = 0.65$, $p = 0.72$; Figure 2.3).

Embryo quality

Median OGV, caloric content, and percent lipid for all Yelloweye Rockfish embryos combined (new captures and recaptures) was 0.015 mm^3 (range, 0.006 to 0.035 mm^3), 6.06 kcal (range, 5.59 to 6.29 kcal), and 26.95% (range, 20.7% to 33.7%), respectively (Table 2.4). Median OGV, caloric content, and percent lipid for new captures was 0.014 mm^3, 6.06 kcal, and 28.53%, respectively, while recaptures had a median of 0.015 mm^3, 6.05 kcal, and 25.92%, respectively (Table 2.5). There was no significant difference in the OGV ($H = 0.025$, $p = 0.87$), caloric content ($H < 0.002$, $p = 0.97$), or percent lipid ($H = 0.031$, $p = 0.86$) between recaptures and new captures (Table 2.6, Figure 2.4). For all sampled fish combined (recaptures and new captures), OGV and caloric content were significantly different among embryo developmental stages ($H = 13.74$, $p = 0.001$; $H = 12.002$, $p < 0.003$, respectively; Table 2.4; Figure 2.4), exhibiting a decline from early to later stages of embryo development. In contrast, there was no significant difference in percent lipid, among embryo developmental stages ($H = 0.61$, $p = 0.73$).

External and internal barotrauma observations

Two of the three Yelloweye Rockfish subjected to angling-induced barotrauma events during two previous capture events exhibited a PC and a TA during their first capture in 2008 or 2009 (Table 2.7). No data were recorded on the third individual, so it may have exhibited TA and PC. All three individuals were captured at a depth greater than 33 m during their third capture event. One individual (age 16) that was recaptured three times had developing embryos, and TA and PC were observed upon first capture in June 2009 at a depth of 34 m. In August 2009, this fish was captured for a second time at 29 m and had EX in one eye and was bleeding from the location where it was hooked. When this individual was captured for the third time in May 2010, it was gravid and only had TA. The individual without PC or

TA that had been captured three times had slight EV at first capture and severe EV at second capture. Because this individual was captured in August 2008 and 2009, information on reproduction was unknown. In 2010, this fish was captured in late June, was gravid, and close to parturition. It should be noted that the swimbladder for these three fish had not fully ruptured at the time of their sacrifice in 2010.

Four Yelloweye Rockfish in this study were subjected to one previously known decompression event and recompression with a DRM. Two of these four fish were identified with PC and TA at first capture. No barotrauma signs were recorded for the other two individuals at first capture due to observer's inconsistently recording barotrauma signs in 2008. Each of these fish were captured at 36 m or deeper at first capture. The individual at liberty the longest (729 d) did not have external barotrauma signs recorded at first capture, but exhibited EV and PC at the second capture event. One fish that was at liberty for 702 d also did not have initial external barotrauma symptoms recorded, but was observed with EX in one eye and PC at its second capture in 2010. Gross internal examinations found that each of the fish recaptured in 2010 had a partially ruptured swimbladder. Only two of the fish in this study had fully ruptured swimbladders, and both individuals were first time captures.

2.4 Discussion

To maintain high levels of productivity, fisheries managers must consider harvest levels that allow for a sufficient reproductive stock. For many species, there has been an increased understanding of reproductive dynamics and strategies (Lowerre-Barbieri et al. 2011); specifically, that reproductive performance has the potential to affect recruitment. For example, the production of eggs that are able to survive to older life stages is important to ensure recruitment success; however, this is not always considered when managing stocks (Lowerre-Barbieri et al. 2011). Recruitment variability is common for rockfishes and the potential for overfishing is a concern for managers not only in PWS, but throughout the west coast of North America (Hochhalter et al. 2011). Recruitment success has the potential to be jeopardized if the potential effects of barotrauma and deepwater release are not examined to determine their effects on reproductive success.

Embryo quality

This study did not identify differences between individual proximate composition variables for newly captured and recaptured fish, indicating that a previous barotrauma event(s) does not affect the quality of developing Yelloweye Rockfish embryos. Lipid content and composition of fish eggs can change during development due to physiological events and the amount of energy demands of the eggs (Weigand 1996). Fisher et al. (2007) found that larvae with larger oil globules have an enhanced resistance to starvation because they have greater endogenous lipid reserves. The oil globule acts as a caloric reserve during the early stages of larval feeding and is an important resource during times of inadequate food supply following parturition (Eldridge et al. 1992).

There was no relationship among combined Yelloweye Rockfish embryo samples for percent lipid, OGV, and caloric content. However, OGV and caloric values declined during development, which would indicate that they were being utilized by embryos. Although Sewall and Rodgveller (2008) noted that lipid and protein of developing quillback rockfish *S. maliger* embryos were consumed in significant amounts, lipid was lost at a greater rate than protein. Berkeley et al. (2004) found that greater larval survival from older female Black Rockfish could be attributed to larger oil globules and a greater contribution of lipid to the egg. However, no relationship was detected between total body lipid content in rockfish larvae and oil globule volume. In contrast, Sewall and Rodgveller (2008) found that oil globule volume was highly correlated to lipid content in quillback rockfish. In the current study on Yelloweye Rockfish, no relationships between lipid, OGV, and caloric content could be detected, most likely due to small sample sizes.

External and internal barotrauma observations

Yelloweye Rockfish in this study exhibited external barotrauma injuries such as EV, TA, and less frequently EX, CB, and a PC. Fish exhibited these symptoms because gases escape the swimbladder into the body cavity and move in an anterio-dorsal direction. Although previous studies have found that rockfish are capable of reversing the injuries due to forced decompression (Hannah et al. 2008; Pribyl 2010; Hochhalter and Reed 2011), there is a finite amount of energy available to repair these injuries. This energy could have been reallocated to reproductive development, thereby making it less available for embryo nutrition or parental fitness during the gestation period. Decreased embryo quality was not observed during the current study, suggesting that the effects of previous decompression events were not transferred to embryo quality.

Study caveats

Although Yelloweye Rockfish can live in excess of 100 years, this study only identified a snapshot of ages (15 to 36 years; Love et al. 2002). A broader representation of the age structure, obtained through the collection of a larger sample size, would provide additional information on the effects of decompression and subsequent recompression on older fish. However, the actual age structure at this particular location in PWS is unknown, so the fish collected in this study may be representative of the current population. It is not known if new captures in this study had been subjected to angling-induced barotrauma outside of the study area. While the study site was remote, anglers have been observed fishing in this area at the same reef. A study conducted in PWS at a nearby reef identified that 22% of Yelloweye Rockfish released at the surface were capable of re-submerging unassisted (Hochhalter and Reed 2011). As a result, if fish had been previously captured, there is the potential they could have re-submerged unassisted without our knowledge.

Examination of embryos from the same females over multiple reproductive events and throughout the entire reproductive cycle would provide valuable information on the long-term and repeated effects of forced decompression and subsequent recompression. In this study, sample sizes were small and stage assignments for stage-2 and -3 embryos were difficult to distinguish. Error in these assignments could have led to the lack of variability observed between these two embryo stages. Additional research is necessary to not only evaluate the survival of rockfish species subjected to angling-induced barotrauma, but to also examine the effects of maternal condition on the energetic contribution to offspring.

Conclusion

This study found no detectable differences in embryo quality for female Yelloweye Rockfish subjected to angling-induced barotrauma and recompression with a DRM relative to individuals with no previously known barotrauma history. Oil globule volume and caloric content results were similar to Black Rockfish study results (Sewall and Rodgveller 2008). Further, there were no differences in embryo quality between post-decompression females and females with no known capture history one to two years after subjecting females to forced decompression and subsequent release with a DRM. Although sample sizes were small, deepwater release does not appear to negatively affect female parent fitness. Females not only survived deepwater release, but were capable of reproducing and providing energy to embryos in subsequent years. This study shows that DRMs do not have negative effects on Yelloweye Rockfish and should be considered as a valuable tool for the conservation of this long-lived species.

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Table 2.1.–External and internal barotrauma injury observations, ratings, and descriptions for Yelloweye Rockfish.

Type	Rating	Description
Esophageal eversion (EV)	0	None observed
	1	Stomach everted into the pharynx only
	2	Stomach everted beyond the buccal cavity
Prolapsed cloaca (PC)	0	None observed
	1	Herniations: Disruptions of the body wall in the pericloacal area through which the gut protruded
	2	Intussusceptions: Actual eversion of the terminal portion of the intestine through its own lumen
Exophthalmia (EX)	0	None observed
	1	One eye protruding outward from orbit
	2	Both eyes protruding outward from orbit
Branchial protrusions (BP)	0	No protrusions observed
	1	Portions of the gut protruded through the brachial opening
Tight abdomen (TA)	0	No tightening of the abdomen
	1	Abdomen swollen, tight to touch
Corneal gas bubbles (CB)	0	None observed
	1	Corneal gas bubbles present in the eyes
Swim bladder tearing or rupturing	0	Fully intact
	1	Partially ruptured: Ruptured tunica externa
	2	As indicated by a visible tear in both layers or by the swimbladder holding no gas or collapsing under light finger pressure

Table 2.2.–Modified stage of embryonic development used for Yelloweye Rockfish.

Stage	Description
1	Embryo with yolk, no eye or body development
2	Optic cups, early retinal pigmentation, some body development
3	Mid-dark retinal pigmentation, developing body structure, possibly spots forming

Table 2.3.–Sample sizes of newly captured and recaptured Yelloweye Rockfish for each stage of embryo development.

Stage of embryo	Total captures	Recaptures	New captures	Recaptures from 2008	Recaptures from 2009	Recaptured once in 2008 and once in 2009	Recaptured twice in 2009
1	6	2	4	1	0	0	1
2	7*	3*	4	0	2*	0	1
3	8	2	6	1	0	1	0

*Stage 2 had one additional recaptured sample for oil globule volume.

Table 2.4.–Sample size, median age, and length of adult female's sampled and proximate composition for corresponding embryos of all sampled Yelloweye Rockfish, separated by stage of embryo development.

	Stage 1 (Early)	Stage 2 (Mid)	Stage 3 (Late)
Adult female			
Sample size (n)	6	6*	8
Age (years)	22	26	27
Length (mm)	502.5	501	484
Embryo			
Wet mass per individual (μg)	660.75	761.51	824.65
Moisture (%)	82.32	85.60	85.79
Dry mass per individual (μg)	121.32	109.04	110.42
Oil globule volume (mm^3)	0.023	0.016	0.012
Lipid (% dry mass)	28.23	24.68	27.53
Caloric content (kcal)	6.23	6.06	6.00

*Sample size for stage 2 was 7 fish for oil globule volume which added a third recapture to this sample. The ovaries from this fish were too small to conduct other sampling because it was one of the smallest fish sampled and the smallest recapture individual.

Table 2.5.–Median (ranges in parentheses) oil globule volume, caloric content, and lipid for each stage of embryo development, separated by recaptures and new captures.

	New capture	Recapture
Oil globule volume (mm^3)	0.014 (0.007 – 0.035)	0.015 (0.006 – 0.024)
Lipid (% dry mass)	28.53 (20.65 – 33.23)	25.92 (23.07 – 33.65)
Caloric content (kcal)	6.06 (5.59 – 6.29)	6.05 (5.64 – 6.36)

Table 2.6.–Median oil globule volume (OGV), caloric content, and lipid for each stage of embryo development, separated by fish that were captured one time (*n* = 14) and only having one barotrauma event and fish that were recaptured two or more times and subjected to barotrauma on two or more occasions and released with a DRM (*n* = 6; sample size of recaptures for OGV = 7).

	New capture			Recapture		
Stage of embryo	1	2	3	1	2	3
Sample size (n)	3	4	6	2	2*	2
OGV (mm^3)	0.22	0.017	0.012	0.022	0.015	0.009
Caloric content (kcal/g)	6.26	6.09	5.99	6.25	6.03	5.84
Lipid (%)	29.80	24.68	27.53	25.92	28.36	29.12

*Sample size for OGV was three fish for stage-2 recaptures.

Table 2.7.–Barotrauma signs and sample size for all captured individuals separated by new captures (2010) and recaptures (2008/2009 and 2010). EV = esophageal eversion (stomach protrusion), PC = prolapsed cloaca, BP = branchial protrusions, EX = exophthalmia, TA = tight abdomen, CB = corneal gas bubbles.

	n	EV	PC	BP	EX	TA	CB
New captures (2010)	14	5 (36%)	2 (14%)	1 (7%)	4 (29%)	12 (86%)	1 (7%)
Recaptures (2010)	5	1 (20%)	2 (40%)	0 (0%)	1 (20%)	4 (80%)	0 (0%)
Recaptures (2009)	5	2 (40%)	3 (60%)	0 (0%)	1 (20%)	5 (100%)	0 (0%)

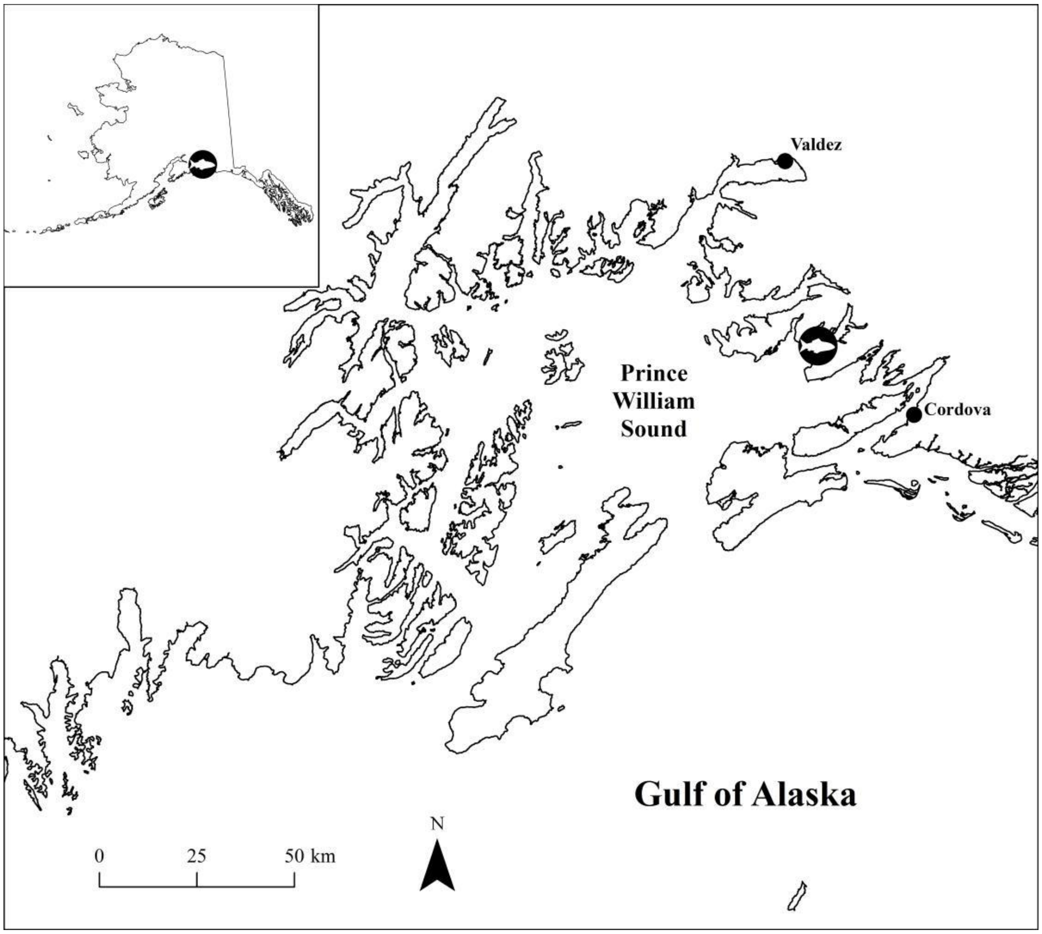

Figure 2.1.–Map showing sampling site (white fish on black circle) near Port Gravina, Prince William Sound, Alaska (Map courtesy of M. Albert, ADF&G).

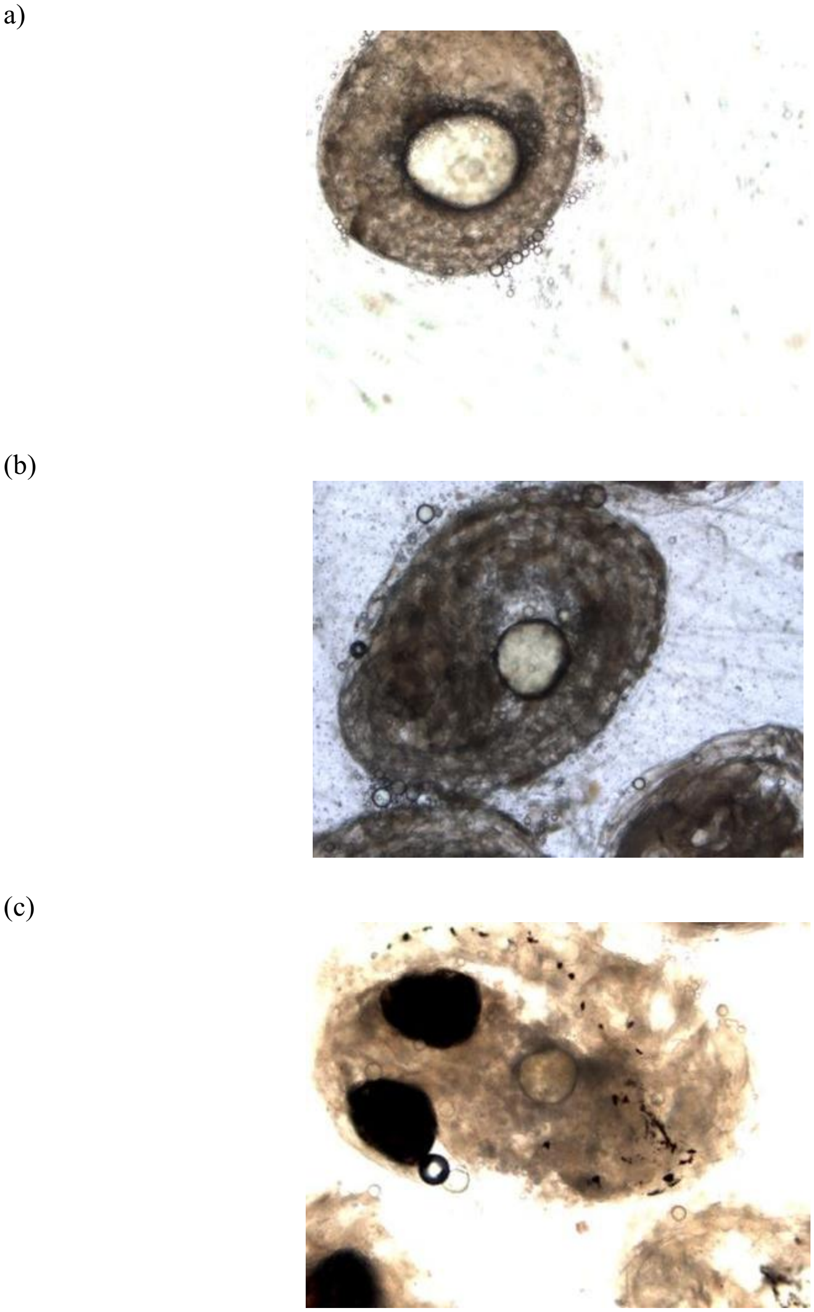

Figure 2.2.–Yelloweye Rockfish embryos and their stages of development: (a) 1 (early); (b) 2 (mid); and (c) 3 (late), modified from Sewall and Rodgveller (2008).

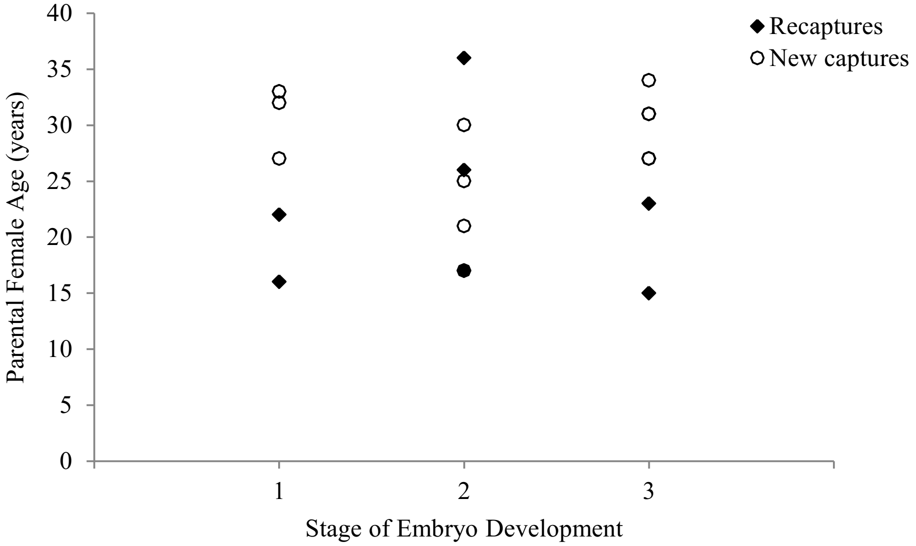

Figure 2.3.–Adult female Yelloweye Rockfish age (years) and stage of embryo development.

Note: Date of capture varied as individual fish were captured over a six-week period.

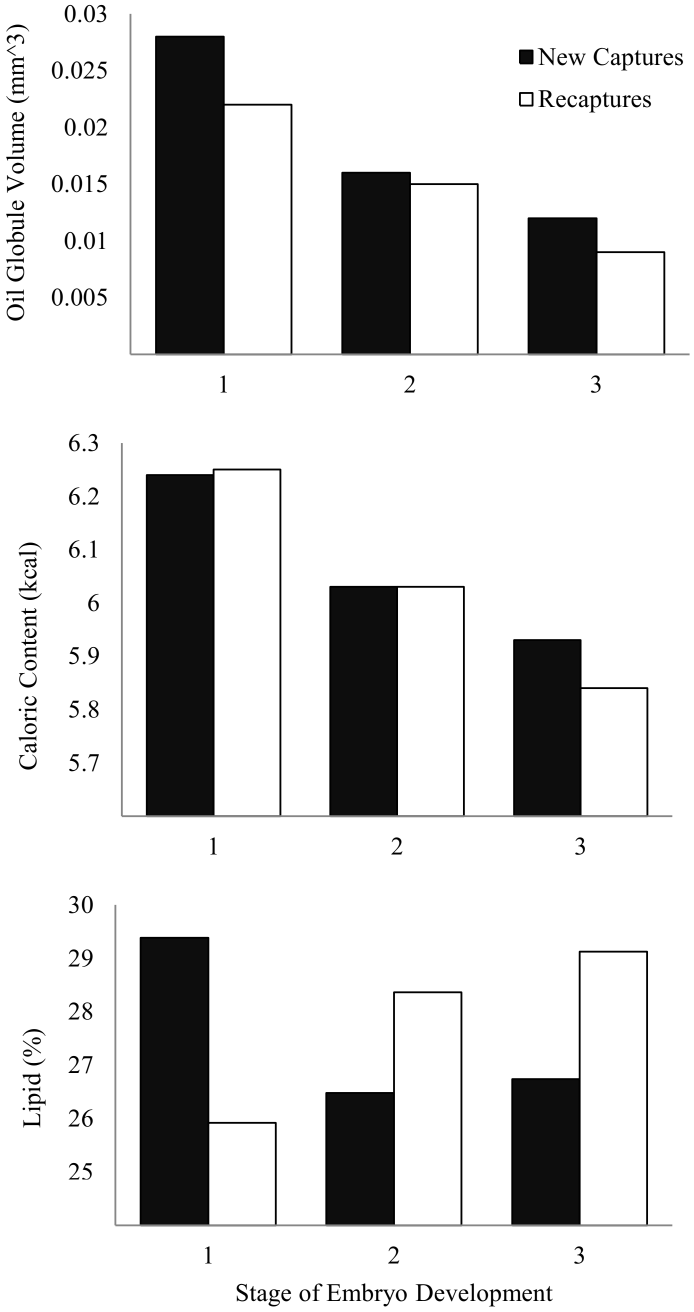

Figure 2.4.–Median oil globule volume (OGV), caloric content, and percent lipid for each stage of Yelloweye Rockfish embryo development separated by recaptures and new captures.

Conclusion and Recommendations

This study provides valuable information on the utility of deepwater-release mechanisms (DRMs) for rockfish *Sebastes spp* conservation. Results showed that Yelloweye Rockfish *S. rubberimus* reproduction and embryos appear to be unaffected by angling-induced barotrauma and recompression with a DRM. This is important to note because a previous study showed that the use of DRMs increases survival of Yelloweye Rockfish in Prince William Sound (PWS; Hochhalter and Reed 2011). These authors sampled the same reef used in the current study, and found high survival (99%) of released Yelloweye Rockfish when using a DRM. The current study found that these same fish reproduce 1 to 2 years following one or more decompression and recompression events (Chapter 1) and that embryo quality is similar to fish with no known decompression-recompression history (Chapter 2).

Although this study provided valuable information on Yelloweye Rockfish viability and embryo quality, it was difficult to collect a large sample size. Weather impacted data collection as did mechanical problems with equipment operation. Future research should be conducted over multiple years and year round. Such research would provide a thorough assessment of the reproductive cycle and the probability of reproduction in future years after angling-induced barotrauma and recompression with DRMs. Over the three years of sampling by ADF&G staff, nearly 65% of the Yelloweye Rockfish tagged at the study site were recaptured at least one additional time. For future studies there is a need to increase the sample size and extend this research to other rockfish species.

The use of DRMs is currently being promoted in California and Oregon for rockfishes and in Australia and other parts of the United States for other fish species (Theberge and Parker 2007; Brown et al. 2008; Johnson et al. 2008). The ADF&G, Division of Sport Fish, has also begun to promote the use of DRMs in Alaska waters and, in January 2013, the Alaska Board of Fisheries (BOF) implemented regulations specific to southeastern Alaska making it mandatory for charter boats to use DRMs and release all sport-caught, non-pelagic rockfish (Chadwick and Miller 2013). While no rockfish species in Alaska have zero-retention regulations, bag limits for Yelloweye Rockfish in southeastern Alaska are lower than those in PWS, and allocations between user groups are in place. A proposal accepted by the BOF (to require the release of non-pelagic rockfish) was submitted by the southeast Alaska charter industry to the BOF 2012 in response to study results on the effectiveness of DRMs (Jarvis and Lowe 2008; Hochhalter and Reed 2011). It is likely that similar proposals will be submitted to the Alaska BOF for rockfish at future BOF meetings. Although mandatory DRM use may be difficult to enforce, it is anticipated that anglers will learn how to properly use these devices. Yelloweye Rockfish that are subjected to forced decompression are an example of a species that is capable of reproducing post-capture and recompression; however, this may not be the case with all rockfish species, and additional research is necessary over the long term for different species in different areas.

The current regulation requiring the retention of the first two demersal (non-pelagic) species in PWS does not allow anglers to choose when and what fish to release. An alternative to consider is the mandatory release of visually gravid females. A second alternative is to evaluate the size and age structure of all rockfish species harvested and released most frequently throughout Alaska as determined from ADF&G port sampling surveys. Based on these data, managers could determine female rockfish size that allows for one or more reproductive events and require the release of smaller (younger) rockfish to allow for a reproductive event(s) to occur. Additional data should also be collected to determine if differences exist for older rockfish embryo quality relative to younger fish. This evaluation could be used to determine the parental female size and age that allows for the greatest release of the most viable rockfish embryos and larvae. For example, Yelloweye Rockfish in this study were gravid at lengths as short as 395 mm. While not part of this study, a Yelloweye Rockfish captured near the sample site had a length of 675 mm and was 93 years of age. This individual was spent, likely just having completed a successful reproductive event. Therefore, consideration of a slot limit that requires the release of Yelloweye Rockfish between 400 and 600 mm, in particular during the gestation period, might reduce the impact of harvest on reproductively viable individuals and those that have not had the opportunity for a reproductive event. Hochhalter and Reed (2011) urged caution when considering the implementation of size limits for Yelloweye Rockfish because the benefits of using DRMs is likely size dependent and more data is needed. However, not having a slot or size limit requires that juveniles that have not reproduced must be retained by anglers as well as older, “trophy” fish that have the potential to release a greater number of more viable larvae (Berkeley et al. 2004). Inclusion of a slot limit during the gestation period may reduce the number of females harvested, but still allow anglers to harvest larger fish thus not deterring sport anglers away from this fishery altogether. However, a management strategy such as this has the potential to affect the age structure and should be further researched before considering a regulation of this type. A third alternative to consider if the catchabilty of rockfish is high during the reproductive period would be to not allow the retention of any rockfish species during May and June. This would cover the vast majority of the spawning season and anglers could be required to use DRMs when releasing fish.

Collecting additional information on other rockfish species and their ability to reproduce following deepwater release would be important prior to implementing a regulation that includes all species. However, a blanket regulation for all rockfishes could reduce concerns associated with the inability of anglers to correctly identify rockfish species, sex or reproductive status. If the current regulation (the retention of the first two demersal species caught) continues in PWS, managers should consider recommendations from anglers that would make DRM use mandatory for all rockfish caught once bag limits are reached, regardless of time of year or reproductive status. Currently, mortality of released rockfish is considered to be nearly 100%. However, Hochhalter and Reed (2011) estimated the release mortality of PWS Yelloweye Rockfish at 22% when released at the surface without a DRM and 99% when released in deepwater with a DRM.

Angler education is always going to be the key to sharing information regarding the success of deepwater release use for rockfish. As a result, educating anglers on research that provides results demonstrating successful rockfish conservation techniques is important. Currently, the ADF&G provides information for anglers on rockfish conservation via their website as well as through additional outreach opportunities. Presentations have also been provided at advisory committee meetings as well as other events where sport anglers are present.

Continued research on all rockfish species in Alaskan waters, their survival probabilities, and their ability to reproduce following forced decompression will be beneficial for fisheries managers. A long-term understanding of recovery following barotrauma injuries and reproductive capabilities is essential to increase our understanding of rockfish population viability. In conclusion, angler education about DRMs and considerations of proposals to the BOF requiring the use of DRMs should be considered as both education and appropriate regulations are valuable conservation tools in Alaskan water for catch-and-release fishing for rockfish.

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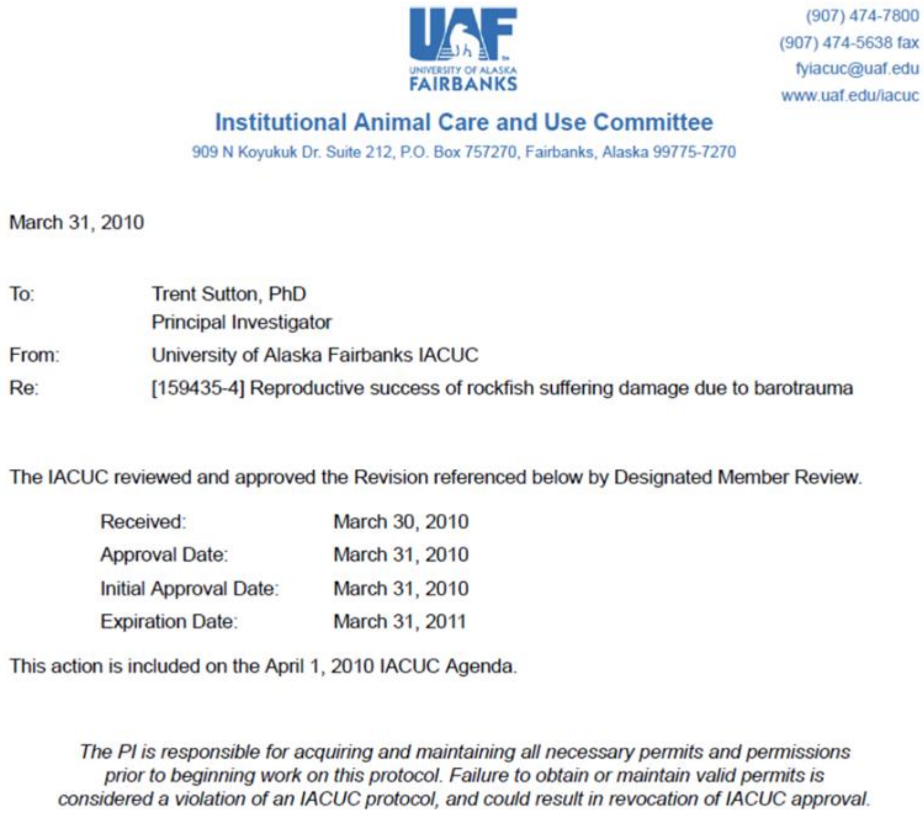
Appendix A
IACUC Approval



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March 31, 2010

To:	Trent Sutton, PhD Principal Investigator
From:	University of Alaska Fairbanks IACUC
Re:	[159435-4] Reproductive success of rockfish suffering damage due to barotrauma

The IACUC reviewed and approved the Revision referenced below by Designated Member Review.

Received:	March 30, 2010
Approval Date:	March 31, 2010
Initial Approval Date:	March 31, 2010
Expiration Date:	March 31, 2011

This action is included on the April 1, 2010 IACUC Agenda.

The PI is responsible for acquiring and maintaining all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol, and could result in revocation of IACUC approval.