THE INSULIN-LIKE GROWTH FACTOR-1 (IGF1) SYSTEM AS A POTENTIAL BIOMARKER FOR NUTRITIONAL STATUS AND GROWTH RATE IN PACIFIC ROCKFISH (SEBASTES SPP.)

A Thesis
presented to
the Faculty of California Polytechnic State University,
San Luis Obispo

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Biological Sciences

by Nicole Leslie Hack
March 2018
COMMITTEE MEMBERSHIP

TITLE: The insulin-like growth factor-1 (Igf1) system as a potential biomarker for nutritional status and growth rate in Pacific rockfish (Sebastes spp.)

AUTHOR: Nicole Leslie Hack

DATE SUBMITTED: March 2018

COMMITTEE CHAIR: Sean C. Lema, Ph.D.
Associate Professor, Biological Sciences

COMMITTEE MEMBER: Dean E. Wendt, Ph.D.
Professor and Dean, College of Science and Mathematics

COMMITTEE MEMBER: Brian R. Beckman, Ph.D.
Research Fisheries Biologist at NOAA Northwest Fisheries Science Center
ABSTRACT

The insulin-like growth factor-1 (Igf1) system as a potential biomarker for nutritional status and growth rate in Pacific rockfish (*Sebastes spp.)*

Nicole Leslie Hack

Growth performance in vertebrates is regulated by environmental factors including the quality and quantity of food, which influences growth via endocrine pathways such as the growth hormone (GH) / insulin-like growth factor somatotropic axis. In several teleost fishes, circulating concentrations of insulin-like growth factor-1 (Igf1) correlate positively with growth rate, and it has been proposed that plasma Igf1 levels may serve as an indicator of growth variation for fisheries and aquaculture applications. Here, I tested whether plasma Igf1 concentrations might serve as an indicator of somatic growth in olive rockfish (*Sebastes serranoides*), one species among dozens of rockfishes important to commercial and recreational fisheries in the Northern Pacific Ocean. I reared juvenile olive rockfish under food ration treatments of 1% or 4% wet mass per d for 98 d to experimentally generate variation in growth. Juvenile rockfish in the 4% ration grew 60% more quickly in mass and 22% faster in length than fish in 1% ration. Plasma Igf1 levels were elevated in rockfish under the 4% ration, and individual Igf1 levels correlated positively with growth rate, as well as with individual variation in hepatic *igf1* mRNA levels. These data in olive rockfish support the possible use of plasma Igf1 as a positive indicator of growth rate variation in rockfishes. Using my findings from this experiment, I further investigated the use of this biomarker in wild rockfish by examining patterns of Igf1 variation in blue rockfish (*Sebastes mystinus*) caught within and outside of two Marine Protected Areas (MPAs) along California’s coast: Piedras Blancas MPA and Point Buchon MPA. Individual Igf1 levels correlated positively with increasing size as seen in laboratory reared fish. After correcting plasma Igf1 values for body size, circulating Igf1 was observed to be higher in blue rockfish within the boundaries of the Piedras Blancas MPA compared to fish from an adjacent site with no fishing restrictions. Igf1 levels in blue rockfish caught within the Point Buchon MPA, however, were similar to those outside of that MPA. These results suggest that blue rockfish within the Piedras Blancas MPA may experience enhanced growth relative to conspecifics outside of that MPA’s boundaries, and that such growth increases may be specific to MPA locations. My findings support previous studies that Igf1 is a positive indicator for growth in teleost fish and can be used as a tractable biomarker in wild rockfish which could enhance management efforts of fish stocks within marine protected areas.
ACKNOWLEDGMENTS

The authors thank Rob Brewster for assistance with tank construction, Tom Moylan and Jason Felton for logistical assistance throughout the experiment, and Gianna Milano, Meredith Beeson, Kasey Cordova, Frances Glaser, Dante Delaney, Lizzie de Luca, Thomas Eldib, Ella Abelli-Amen, Christa Lam, Laura Manning, Niki Monjazeb, Haley Perkins, Rachel Roorda, Emily Sroczynski, Nicholas Wall, Krista Lai, Mel Kasai, Haley Gonzales, Marie Solis, Jade Wacker, Natalie Miller-Binkley, Sam Farrow, Peter VanderBloomer, Miguel Jimenez for assistance with animal care. We acknowledge support from the NOAA IOOS program through CeNCOOS for the ambient temperature data collected at the Cal Poly Pier (award to R. Walter and D. Wendt, Cal Poly).

This research supported by funding from California SeaGrant (R/HCME-15). Additional support was provided to N. Hack by California State University’s (CSU) Council on Ocean Affairs, Science & Technology (COAST), by the Baker-Koob Endowment, and by College Based Fee support from California Polytechnic State University. Fish were collected under Scientific Collecting Permit # SC-4793 from the California Department of Fish and Wildlife.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF TABLES</th>
<th>ix</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
</tbody>
</table>

CHAPTER 1 - Insulin-like growth factor I (IGF-I) as a physiological biomarker for growth rate in juvenile Sebastes rockfishes

1.1. Abstract ................................. 6

1.2. Introduction ................................ 8

1.3. Materials and Methods ................................ 11

1.3.1. Animal collection and husbandry .......................... 11

1.3.2. Species identification .................................. 11

1.3.3. Sequencing of partial cDNAs linked to IGF signaling from olive rockfish ........................................... 12

1.3.4. Food ration treatments ...................................... 14

1.3.5. Plasma Igf1 quantification .................................. 15

1.3.6. Quantification of Igf system gene transcripts ................. 15

1.3.7. Statistical Analyses ........................................ 17

1.4. Results ............................................ 18

1.4.1. Identification of partial cDNAs from olive rockfish .......... 18

1.4.2. Ration differences generate variation in growth rate .......... 19

1.4.3. Effect of ration amount on plasma Igf1 concentrations ........ 19

1.4.4. Regulation of mRNAs encoding Igfs and Igfbps in liver ....... 20
1.4.5. Ration effects on Igf system-associated mRNAs in skeletal muscle ................................................................. 21

1.5. Discussion ....................................................................................................................................................... 22

1.5.1. Variation in growth related to plasma Igf1 ............................................................ 23

1.5.2. Growth-related variation in hepatic and muscle Igf1 and Igf2 gene expression .............................................................. 25

1.5.3. Responses of Igfbp and Igf1 receptor mRNA abundance to ration amount .......................................................... 28

1.6. Conclusions ..................................................................................................................................................... 34

1.7. Tables .............................................................................................................................................................. 35

1.8. Figures ............................................................................................................................................................ 40

CHAPTER 2 - Spatial and temporal variation in plasma insulin-like growth factor-1 (Igf1) in blue rockfish (Sebastes mystinus) in MPAs ................................................................. 50

2.1. Abstract .......................................................................................................................................................... 50

2.2. Introduction ................................................................................................................................................... 52

2.3. Methods ........................................................................................................................................................ 55

2.3.1. Animals .................................................................................................................................................... 55

2.3.2. Study Sites ............................................................................................................................................... 56

2.3.3. Plasma Igf1 Quantification ......................................................................................................................... 58

2.3.4. Statistical Analyses ................................................................................................................................ 59

2.4. Results ........................................................................................................................................................ 60

2.4.1. Body size .................................................................................................................................................. 60

2.4.2. Plasma Igf1 standardization ....................................................................................................................... 61

2.4.3. Plasma Igf1 variation with habitat protection status ..................................................................................... 62

2.4.4. Environmental influences on plasma Igf1 ............................................................................................... 63
2.5. Discussion ......................................................................................................................... 63

2.5.1. Plasma Igf1 variation with habitat protection status................................. 64

2.5.2. Environmental influences on plasma Igf1 ............................................... 66

2.6. Conclusion .................................................................................................................... 68

2.7. Tables ........................................................................................................................... 69

2.8. Figures .......................................................................................................................... 70

References ......................................................................................................................... 85
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Degenerate primers used for amplification and sequencing of partial</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>cDNAs from olive rockfish, <em>Sebastes serranoides</em></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>Gene-specific primers for SYBR green quantitative PCR in olive</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>rockfish</td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>Mass and length-specific somatic growth rates (SGR) calculated across</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>different time intervals</td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>Liver mRNA gene correlation matrix. Correlations are Pearson’s</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>product-moment correlations. Bolded and stared values indicate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>significant correlations (FDR corrected P &gt; 0.05)</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>Muscle mRNA gene correlation matrix. Correlations are Pearson’s</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>product-moment correlations. Bolded and stared values indicate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>significant correlations (FDR corrected P &gt; 0.05)</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>Principal component loadings. Variable in order are wind speed, swell</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>height, wave height, wave cardinal direction, secchi depth, surface</td>
<td></td>
</tr>
<tr>
<td></td>
<td>temperature, and depth</td>
<td></td>
</tr>
</tbody>
</table>
1.1. Mean (±SEM) values of (A) mass, (B) standard length, and (C) condition factor for rockfish reared under high ration (4% wet wt.) or low ration (1% wet wt.) conditions. Mass and body condition factor differed between treatments beginning on day 24, while length differed beginning on day 48.................................................................40

1.2. High (4%) ration fish (dark gray bars, n = 25) showed a greater (A) mass-specific and (B) length-specific somatic growth rate (SGR) than low (1%) ration fish (light gray bars, n = 26). Bars represent group means (±SEM) of percent change per day with p-values from student t-tests..........................41

1.3. Mass and standard length (SL) relations separated by treatment before and after experimental manipulation. Initial measures prior to the experiment (day 0 - triangles) are lower and have shallower slopes than final measurements (day 98 – circles) ...........................................................................................................42

1.4. Comparison of mean (±SEM) plasma total Igf1 concentrations between high ration (4% wet wt.) and low ration (1% wet wt.) treatments. Rockfish in the high ration treatment (n = 24) had significantly higher plasma Igf1 than low ration fish (n = 26) .............................................................................................................43

1.5. Individual (A) mass-specific specific growth rate (SGR) and (B) length-specific SGR both correlated positively to plasma Igf1 concentration. SGR values shown are calculated from the body size change across the entire experimental period (day 0 to 98). Lines represent Pearson correlation relationships for fish from both ration treatments combined (n = 50).............................................................................................................44

1.6. Relative hepatic mRNA levels from the high and low ration treatments. (A) Levels of igf1 and igf2 did not differ between treatments. (B) Transcripts encoding Igf binding proteins igfbp1a and igfbp1b were expressed at higher relative levels in rockfish reared under the low (1%) ration treatment. Data are shown as mean (±SEM) values. Lines indicate significant differences between treatments (Student’s t-test: *P < 0.05, **P < 0.01, ***P < 0.0001).............................................................................................................45

1.7. Individual variation in liver igf1 mRNA levels correlated positively with plasma Igf1 concentrations. Pearson’s product-moment correlation line shows the relationship for all fish combined (n = 50). Ration treatments are shown for reference .............................................................................................................46
1.8. Individual variation in liver $igf1$ mRNA levels correlated positively with plasma Igf1 concentrations within both (A) high ($n = 24$) and (B) low ($n = 19$) treatments. Pearson’s product-moment correlation line shows the relationship for fish in each treatment .................................................................47

1.9. Relative mRNA levels for $igf1$, $igf2$, and several genes encoding Igfbps in skeletal muscle of rockfish from the high (4%) and low (1%) ration treatments. (A) Transcript abundance for $igf1$, but not $igf2$, was higher in fish from the high ration treatment. (B) Only $igfbp5a$ and $igfbp5b$ mRNA levels in muscle differed between the two ration treatment groups. (C) There were no differences between treatments for both Igf receptors. Data are plotted as mean ($\pm$SEM) values, and lines indicate significant differences between treatments (Student’s t-test: *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$).................................................................48

1.10. Correlation between plasma Igf1 concentrations and relative levels of mRNAs encoding $igf1$ in skeletal muscle for (A) all fish combined ($n = 50$), (B) high treatment, and (C) low treatment fish. The line represents a significant Pearson correlation relationship. for Treatments shown for reference in all fish combined.................................................................49

2.1. Map showing sampling locations in Central California with Marine Protected Areas (MPA) shown in blue. Sampled 500 m$^2$ cells were fished within (1) Piedras Blancas and (2) Point Buchon MPAs and also in associated reference sites (REF) ...........................................................................................................70

2.2. Mean ($\pm$SEM) values of total lengths for blue rockfish caught at Piedras Blancas (PBL) and Point Buchon (PBN) within the marine protected area (MPA) and at adjacent reference sites (REF). Fish caught at Piedras Blancas were significantly larger than those caught at Point Buchon. Number of samples are indicated inside each bar. Stars indicate significant differences between treatments (Student’s t-test: NS - not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$).................................................................71

2.3. Mean ($\pm$SEM) values of total lengths for blue rockfish caught at (A) Piedras Blancas and (B) Point Buchon within the marine protected area (MPA) and at adjacent reference sites (REF) separated by sampling date. Number of samples are indicated inside each bar. Stars indicate significant differences between treatments (Student’s t-test: NS - not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$).................................................................72

2.4. Linear regression ($r^2 = 0.066$) of total lengths by plasma Igf1 concentrations for all fish combined. Regression and standard error (gray) include both locations and all sampled cells. Protection status only shown for reference...........................................................................................................73

2.5. Linear regression ($r^2 = -0.004$) of total lengths by length standardized
plasma Igf1 concentrations for all fish combined. Protection status only shown for reference.........................................................74

2.6. Mean (±SEM) values of length standardize plasma Igf1 for blue rockfish caught at (A) Piedras Blancas and (B) Point Buchon within the marine protected area (MPA) and at adjacent reference sites (REF). Stars indicate significant differences between treatments (Student’s t-test: NS - not significant, *P < 0.05, **P < 0.01, ***P < 0.0001)..................................................75

2.7. Mean (±SEM) values of length standardize plasma Igf1 for blue rockfish caught at (A) Piedras Blancas and (B) Point Buchon within the marine protected area (MPA) and at adjacent reference sites (REF) separated by sampling date. Number of samples are indicated inside each bar. Stars indicate significant differences between treatments (Student’s t-test: NS - not significant, *P < 0.05, **P < 0.01, ***P < 0.0001).............................76

2.8. Kernel densities for length standardized plasma Igf1 concentration at Piedras Blancas. Igf1 concentrations are standardized to regional lengths on paired sampling dates. All dates are shown ..........................................................77

2.9. Kernel densities for length standardized plasma Igf1 concentration at Point Buchon. Igf1 concentrations are standardized to regional lengths on paired sampling dates. All dates are shown ..........................................................78

2.10. Individual plasma Igf1 values standardized to total length for all fish caught in Piedras Blancas (PBL) and Point Buchon (PBN) marine protected areas (MPA) and at adjacent reference sites (REF) separated by sampling date ..................................................................................79

2.11. Principal components analysis showing relation of highest components in relation to Location. Piedras Blancas (PBL, green) exhibits higher wave action (wave height and secchi turbidity) while Point Buchon (PBN, yellow) has higher temperatures.........................................................80

2.12. Principal components analysis showing relation of highest components in relation to protection status. Marine Protected Areas (MPA) had higher swell and wind speed while reference sites (REF) has higher turbidity (secchi) ..................................................................................81

2.13. Principal components analysis showing relation of two highest components in relation to sampling date. August 17, 2016 has notably high wave height and cloud cover .................................................................................82

2.14. Principal components analysis showing relation of two highest components in relation to total length standardized Igf1. Green are higher than expected Igf1 levels for their length and redder points are lower than expected Igf1 levels ..................................................................................83
2.15. Principal components analysis showing relation of highest components in relation to protection status. Marine Protected Areas (MPA) had higher swell and wind speed while reference sites (REF) has higher turbidity (secchi) ................................................................. 84
INTRODUCTION

Over-harvesting of marine species occurs globally and is considered one of the most detrimental influences of humans on marine ecosystems especially for coastal regions of California (Gray, 1997; Halpern et al., 2008). Such overexploitation of marine fish and invertebrates not only leads to collapses in populations of these harvested species, but can also undermine entire marine ecosystems by changing trophic cascades and triggering ecological phase shifts in marine communities (e.g. Mumby et al., 2006; Ling et al., 2009). Additional stressors of variable temperatures, upwelling, and food availability each can impact fish population dynamics such as recruitment success, reproductive output, and density-dependent growth (Caselle et al., 2010; VenTresca et al., 1996). Nutritional stress in the form of reduced food quantity or quality, in particular, severely affects growth performance in fish thus hampering reproduction and survival. For populations of marine fishes, the lack of noninvasive metrics for determining spatial and temporal variation in growth performance has limited the ability to predict variation in fish stock reproduction and recruitment, along with links to natural changes in prey availability, temperature, or other environmental factors.

While somatic growth serves as a reliable indicator of individual and population fitness, this has historically been an evasive measurement in fishes in the wild. Currently, commonly used methods for quantifying individual growth rates of wild fishes require terminal sampling (otolith analysis), time consuming tagging (mark-recapture), or have limited value as indicators of somatic growth (RNA:DNA ratios) (Andrews et al., 2011). The development of rapid, non-lethal methods for quantifying growth rates is needed to provide data necessary for informed fisheries management.
External environmental influences are translated into changes in growth in part via the altered secretion of stimulatory or inhibitory hormones within the somatotropic endocrine axis. Blood hormone concentrations have shown to be easily accessible indicators of growth regulation and metabolism (Möstl and Palme, 2002). The growth hormone (GH)/insulin-like growth factor (Igf) system, specifically, has been shown to be a key regulator of changes in somatic growth with variation in nutritional status (Fuentes et al., 2013; Picha et al., 2008a; Reindl and Sheridan, 2012). Changes in nutritional conditions have been shown to alter liver production of the somatomedin hormone Igf1 in fishes. Given that relationship between Igf1 and nutritional status, it has been proposed that circulating concentrations of Igf1 may serve as a reliable indicator of recent growth rate in fishes (Beckman, 2011; Picha et al., 2008a; Reinecke, 2010). In most vertebrates, insulin-like growth factor-1 (Igf1) is the predominant mediator of growth following activation of the GH/Igf axis (e.g. Duan, 1997; Perez-Sanchez and Le Bail, 1999; Reinecke et al., 2005). In brief, GH secreted from the anterior pituitary binds the GH receptor to stimulate hepatic production of Igf1, which regulates somatic tissue growth by binding Igf1 receptors in target tissues while exerting negative feedback on pituitary GH production (Duan et al., 2010; Fuentes et al., 2013; Le Roith et al., 2001; Wood et al., 2005). Conserved amongst vertebrates, Igf1 regulates growth by promoting cell proliferation, cartilage growth, and skeletal elongation (Reinecke et al., 2005; McCormick et al., 1992; Duan, 1997; Chen et al., 2000; Wood et al., 2005). The function of Igf1 is controlled in part by Igf binding proteins (Igfbps) which modulate the activity of Igf1 and Igf2 by binding to the hormones thus limiting the amount of Igf hormone available to activate receptors, effectively inhibiting or prompting Igf action.
Only a handful of studies have examined the relationship between Igf1 and growth variation in fishes (Beckman, 2011; Picha et al., 2008a). Variations in growth caused by manipulated food quantities has been attributed to differences in Igf1 concentrations (e.g., Kelley et al., 2001; Picha et al., 2008a; Reinecke, 2010; Shimizu and Dickhoff, 2017). It is thought that these changes in plasma Igf1 with food ration result from GH resistance in the liver, as GH levels tend to be inversely related to Igf1 levels (e.g., Fox et al., 2006). Positive correlations between plasma Igf1 and growth rate have been seen in tilapia (Uchida et al., 2003), coho salmon (Beckman et al., 2004a,b), and chum salmon (Oncorhynchus keta, Kaneko et al., 2015).

Individual variation in hepatic igf1 mRNA levels were correlated with plasma Igf1 (Gabillard et al., 2003; Pierce et al., 2005) and over all liver igf1 mRNAs have been shown to decrease in relative abundance in fish experiencing restricted food rations or fasting conditions in a variety of taxa (Pierce et al., 2005; Vera Cruz et al., 2006; Monserrat et al., 2007; Kawanago et al., 2014). Although the liver is commonly accepted as the main tissue of Igf1 synthesis, extrahepatic Igf1 production also appears important for regulating growth of some tissues via autocrine or paracrine effects (e.g., Firth and Baxter, 2002; Franz et al., 2016), and can be regulated in a tissue-specific pattern (Eppler et al., 2010; Fox et al., 2010). Just as in the liver, food restriction has been observed to alter extrahepatic igf1 mRNA levels in select tissues of fish (Fox et al., 2010; Norbeck et al., 2007, Peterson and Waldbieser, 2009; Terova et al., 2007). Both Igf1 and Igf2 have been demonstrated to increase igf1 transcription in cultured myocytes from gilthead seabream (Azizi et al., 2016). Supporting this idea, muscle igf1 transcription has also been
shown to respond to acute changes in food intake in Atlantic salmon, rainbow trout, and tilapia; in these species, igf1 is downregulated by fasting and upregulated immediately following refeeding (Breves et al., 2016; Bower et al., 2008; Fox et al., 2010; Gabillard et al., 2006; Monserrat et al., 2007; Picha et al., 2008b).

Igfbps are critical for modulating the effects of Igf hormones, and the ratio of Igf:Igfbp in circulation plays a critical role in regulating Igf1 availability for receptor binding (Clemmons, 2016). Igfbp1 proteins – which have been duplicated in teleosts – are thought to be among of the highest concentration binding proteins in circulation in fishes, and Igfbp1 has been shown to bind Igf1 with high affinity in several teleost species (Shimizu and Dickhoff, 2017). The expression of both Igfbp1a (regarded as the 28-32 kDa Igfbp protein in fishes) and Igfbp1b (regarded as the 20-25kDa Igfbp) has been shown to be altered by nutritional status, sometimes in patterns that appear more responsive to feeding than even Igf1 in some teleosts (Shimizu et al., 2006; Picha et al., 2008a). Generally, both Igfbp1a and 1b show elevated hepatic and plasma expression under nutritional restriction (Shimizu and Dickhoff, 2017). Overexpression of hepatic Igfbps is generally thought to increase relative Igf1 binding in circulation, thereby reducing Igf1 stimulation of growth in peripheral tissues (Clemmons, 2016).

Previous experimental studies in juvenile Sebastes rockfishes have shown that individual plasma Igf1 concentrations correlate positively with individual SGR variation (Chapter 1; Hack et al., unpub. results), indicating that concentrations of Igf1 can provide an instantaneous picture of an individual’s relative growth rate in these fishes. Ecological factors such as water temperature, upwelling intensity, and turbidity influence quantity of food resources available to coastal marine species thus indirectly affecting reproductive
output, recruitment success, and density-dependent growth (e.g., Frank et al., 2007; Frederiksen et al., 2006; Harley et al., 2006; Hunt and McKinnell 2006; Caselle et al., 2010; VenTresca et al., 1996; White and Caselle, 2008). Hormonal biomarkers are currently mostly used within the aquaculture industry to monitor growth and health of farmed stocks. By expanding the assessment to recreationally and commercially important species, this tool could be used to measure the regional variation in population production and size, as well as how these correlate to ecosystem processes, fishing pressures, and decadal oscillations.

In the present study, we assessed the relationship between circulating Igf1 and growth rate in olive rockfish, one of several Sebastes rockfishes important as recreational and commercial groundfish fisheries in the Northern Pacific Ocean. By feeding groups of juvenile olive rockfish two different ration amounts (1% or 4% wet mass per d) for 98 d, we intentionally generated differences in SGR, with rockfish given the 4% ration growing 60% faster in mass and 22% more rapidly in length per day compared to fish given the 1% ration. We then used these ration-induced differences in growth to evaluate how growth variation links to changes in circulating Igf1, as well as relative mRNA levels of igf1 and several other genes encoding proteins involved in the somatotropic endocrine axis. Finally, we tested the use of this biomarker in the field by measuring plasma Igf1 concentrations in blue rockfish from within and outside of MPAs along the central coast of California.
CHAPTER 1

Insulin-like growth factor I (IGF-I) as a physiological biomarker for growth rate in juvenile Sebastes rockfishes

1.1. Abstract

Currently, commonly used methods for quantifying individual growth rates of wild fishes require terminal sampling (otolith analysis), time consuming tagging (mark-recapture), or have limited value as indicators of somatic growth (RNA:DNA ratios). The development of rapid, non-lethal methods for quantifying growth rates is needed to provide data necessary for informed fisheries management. Blood hormone concentrations have shown to be accessible indicators of growth regulation and metabolism. Specifically, insulin like growth factor-I (Igf1) has a low clearance rate and robust relationship to somatic growth in several fishes, making it a potential endocrine biomarker of specific growth rate for fisheries applications. Here, we tested whether plasma Igf1 concentrations could be used as a tractable indicator of somatic growth and nutritional status in Sebastes rockfishes, a group of species important to commercial and recreational fisheries on the Pacific coast of N. America. To test associations between Igf1 and growth rate, we collected juvenile olive rockfish (Sebastes serranoides) from central California, USA, and reared them in captivity under food rations of 1% (n=27) or 4% (n=26) wet mass per day to experimentally generate growth variation. Fish raised under higher rations exhibited high plasma total Igf1 concentrations as well as lower hepatic mRNA levels for Igf binding protein-1a (igfbp1a), Igf binding protein-1b
(igfbp1b) and higher skeletal muscle mRNA levels of Igf binding protein-5a (igfbp5a) and Igf binding protein-5b (igfbp5b).
1.2. Introduction

Nutritional stress in the form of reduced food quantity or quality affects growth performance in fish, which in turn can impact population-level processes such as recruitment success or reproductive output (Caselle et al., 2010; VenTresca et al., 1996). For populations of marine fishes, the lack of noninvasive metrics for determining spatial and temporal variation in growth performance has limited the ability to predict variation in fish stock reproduction and recruitment. The effects of nutritional stress on somatic growth in fishes, however, are regulated in part via changes in a variety of endocrine pathways including those involved in the somatotropic, thyroid, and glucocorticoid endocrine axes (Picha et al., 2008a; Power et al., 2001; Sadoul and Vijayan, 2016; Won and Borski, 2013), and indices of these endocrine pathways may be useful as biomarkers for assessing growth variation in fish culture as well as in wild fish stocks.

The growth hormone (GH)/insulin-like growth factor (Igf) system, in particular, has been shown to be a key regulator of changes in somatic growth with variation in nutritional status (Fuentes et al., 2013; Picha et al., 2008a; Reindl and Sheridan, 2012). In most vertebrates, insulin-like growth factor-1 (Igf1) is the predominant mediator of growth following activation of the GH/Igf axis (e.g. Duan, 1997; Pérez-Sánchez and Le Bail, 1999; Reinecke et al., 2005). In short, GH secreted from the anterior pituitary binds the GH receptor to stimulate hepatic production of Igf1, which regulates somatic tissue growth by binding Igf1 receptors in target tissues while exerting negative feedback on pituitary GH production (Duan et al., 2010; Fuentes et al., 2013; Le Roith et al., 2001; Wood et al., 2005).
Teleost fishes have also evolved at least six types of Igf binding proteins (Igfbps), which themselves evolved into multiple isoforms following gene duplication events in some teleost lineages (Daza et al., 2011; Shimizu and Dickhoff, 2017). Igfbps modulate the activity of Igf1 and Igf2 by binding the hormones and regulating the amount of Igf hormone available to activate receptors, effectively inhibiting or prompting Igf action. There is also evidence, however, that some Igfbps have their own biological activity and can activate Igf1 receptors or other cell-surface or intranuclear proteins (e.g., transforming growth factor-β5 receptor, peroxisome proliferator-activated receptor γ) independent of Igf hormone binding (Baxter, 2015; Chan et al., 2009; Clemmons, 2007, 2016; Duan and Xu, 2005; Huang et al., 2003; Jogic-Brahim et al., 2009).

For fish experiencing food limitation or deprivation, circulating concentrations of Igf1 decrease while some Igfbps increase, ultimately contributing to reduced muscle and skeletal growth (e.g., Kelley et al., 2001; Picha et al., 2008a; Reinecke, 2010; Shimizu and Dickhoff, 2017). Such declines in circulating Igf1 with food restriction have been observed in a wide variety of fishes, including Mozambique tilapia (Oreochromis mossambicus, Breves et al., 2014; Uchida et al., 2003), Arctic charr (Salvelinus alpinus, Cameron et al., 2007), gilthead sea bream (Sparus aurata, Pérez-Sánchez et al., 1995), and several species of salmonids (Beckman et al., 2004a,b; Bower et al., 2008; Breves et al., 2016; Kaneko et al., 2015; Pierce et al., 2005; Wilkinson et al., 2006). Igfbp expression in fishes has also been found to be responsive to variation in food consumption. Although the number of Igfbp isoforms that have evolved appear to differ across teleost fish taxa, variation in food availability has been shown to influence expression of the type 1 form of Igfbp (Igfbp1) across several taxa. For instance, Igfbp1
protein levels in blood circulation have been observed to decline in post-smolt coho salmon, *Oncorhynchus kisutch*, in the hours after feeding (Shimizu et al., 2009), and fasted coho salmon were found to have higher plasma Igfbp1 levels than fish not experiencing food restriction (Shimizu et al., 2006). Mechanistically, at least some of these nutrition-associated changes in Igfbp expression appear to be caused by inhibition of Igfbp1 gene expression. This is supported by the observation of elevated mRNAs encoding the type 1 *igfbp* gene in the liver of Atlantic salmon, *Salmo salar*, smolts under food restriction (Hevrøy et al., 2011). Breves et al. (2016) likewise observed increased hepatic *igfbp1a1* mRNA levels in Atlantic salmon parr fasted for 3 to 10 d.

In this study, the relationships between growth rate and the Igf1 system were examined in juvenile olive rockfish (*Sebastes serranoides*) by creating a range of positive growth rates and exploring differences between experimental treatments as well as variation that occurred among individuals within treatments. Rockfishes of genus *Sebastes* are an important component of commercial and recreational groundfish fisheries in the northern Pacific Ocean (e.g., Miller et al., 2014; Parker et al., 2000), and several species are being explored for their economic viability and best rearing practices in mariculture (e.g., Lee, 2001; Son et al., 2014). Juvenile olive rockfish were reared for 98 d under differing ration levels, and then examined for differences in growth rate, plasma Igf1 concentration, and the relative abundance of gene transcripts encoding *igf1*, *igf2*, as well as isoforms of type 1, 2 and 5 *igfbps* in the liver and skeletal muscle. The link between somatic growth and transcript expression levels was also examined for Igf1 receptors a (*igf1ra*) and b (*igf1rb*) in skeletal muscle, to assess how variation in nutritional status and growth alters components of Igf axis signaling in this target tissue.
1.3. Materials and Methods

1.3.1. Animal collection and husbandry

Young-of-the-year juvenile (3-10 mo) olive rockfish (*S. serranoides*) were collected from San Luis Bay in Central California, USA between 5 May and 10 July 2016. All fish were collected using a Standard Monitoring Unit for the Recruitment of Fishes (SMURF) (Ammann, 2004), which was placed under California Polytechnic State University’s Center for Coastal Marine Sciences pier facility (35°10'12.3''N 120°44'27.2''W). The SMURF was deployed approximately 1 to 3 m below the surface for durations varying between 3 to 11 (4.35 ± 1.66) days.

Upon collection, juvenile rockfish were transferred to flow-through 340 L tanks where they were maintained in captivity under ambient salinity (33‰), temperature (range: 12.4 – 18.9°C), and photoperiod conditions. Fish were fed *ad libitum* daily with commercial fish pellet feed (BioPro2 pellets, 1.5 mm, BioOregon, Longview, WA, USA) for at least 3 weeks prior to the start of the experiment. All procedures were approved by the Institutional Animal Care and Use Committee of California Polytechnic State University (Protocol # 1504).

1.3.2. Species identification

Juvenile *S. serranoides* can be difficult to identify to the species level using only morphological traits, especially from the sympatric congener yellowtail rockfish, *S. flavidus*. Therefore a ~369 bp region of the mitochondrial DNA D-loop control region (*S. serranoides*, DQ678575 and *S. flavidus*, DQ678548) was amplified and sequenced for each fish used in the experiment to confirm species identity. PCR was performed using
degenerate primers to the mtDNA D-loop region developed by Hyde and Vetter (2007). Nucleotide sequences for those primers were as follows: (forward) 5’-
CCTGAAAATAGGAACCAAATGCCAG-3’, and (reverse) 5’-
GAGGAYAAAGCAGTTGAGC-3’. Genomic DNA was isolated from skeletal muscle of each fish using the DNeasy® Cell and Tissue Kit (Qiagen, Valencia, CA, USA), and the resulting genomic DNA was amplified in 50 µl PCR reactions containing 25 µl of GoTaq® Colorless Master Mix (Promega Corp., Madison, WI, USA), 18 µl nuclease-free H₂O, 1 µl each of forward and reverse primer (10 mM), and 5 µl of genomic DNA (69.5-154 ng/µl). All reactions were conducted using a thermal profile of 95°C for 5 min followed by 38 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min, and then a 2 min final extension at 72°C. The resulting PCR products were examined on 1.2% EtBr gels before being cleaned (QIAquick® PCR Purification Kit, Qiagen) and Sanger sequenced (Molecular Cloning Labs, South San Francisco, CA, USA). The resulting sequences were then aligned using Sequencher v5.1 software (GeneCodes Corp., Ann Arbor, MI, USA) against existing mtDNA D-loop sequences from GenBank to confirm species identity.

1.3.3. Sequencing of partial cDNAs linked to IGF signaling from olive rockfish

Total RNA was extracted from the liver and skeletal muscle tissues of an olive rockfish (86.8 mm standard length [SL], 12.77 g body mass) using TriReagent® (Molecular Research Center, Inc., Cincinnati, OH, USA) and bromochloropropane for phase separation. Extracted RNA was then quantified by spectrophotometry (260:280 =
First strand cDNA was generated by reverse transcription (GoScript™ Reverse Transcription System, Promega) in 20 µl reactions containing 4.88 µg total RNA template (8 µl), 4 µl 5X buffer, 3 µl MgCl₂, 1 µl random primers, 1 µl dNTPs, 0.5 µl RNase inhibitor, 1 µl reverse transcriptase, and 1.5 µl nuclease-free water. Reactions were incubated at 25 °C for 5 min, 42 °C for 1 h, followed by 70 °C for 15 min to inactivate the reverse transcriptase enzyme.

Degenerate primers were used to perform PCR to amplify partial cDNAs encoding igf1, igf2, and the Igf1 receptors igf1ra and igf1rb as well as select igfbps from olive rockfish (Table 1). Degenerate primers were designed from consensus regions of these genes identified by BLAST search of the genome assemblies for flag rockfish (Sebastes rubrivinctus, GCA_000475215) and tiger rockfish (Sebastes nigrocinctus, GCA_000475235), which were the only Sebastes taxa with genomes available at the time of primer design. Partial cDNAs encoding igf1 (accession no. AF481856), elongation factor 1-alpha (ef1α, KF430623), and ribosomal protein L17 (rpl17, KF430620) from Schlegel’s black rockfish (Sebastes schlegelii), and igf2 (Y16643) from shorthorn sculpin (Myoxocephalus scorpius) were also used as part of the alignments for primer design. The resulting partial sequences were aligned using Sequencher v5.1 software (GeneCodes Corp.) to find consensus nucleotide regions, and degenerate primers were synthesized by Eurofins Genomics (Louisville, KY, USA).

PCR was performed with degenerate primers in 50 µl reactions containing 25 µl GoTaq® Polymerase Colorless Master Mix (Promega), 2 µl cDNA, 1 µl each of forward
and reverse primers (10-50 mM), and 21 µl nuclease-free H₂O. Reactions were amplified under a thermal profile of 95 °C for 2 min, 37 cycles of 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min and 20 s, succeeded by 2 min of 72 °C. PCR products were then tested by gel electrophoresis on 1.2% agarose gels. Any products that were of expected size were cleaned (QIAQuick PCR Kit, Qiagen) and Sanger sequenced (Molecular Cloning Labs).

1.3.4. Food ration treatments

Each olive rockfish was implanted intraperitoneally with a passive integrated transponder (PIT) tag (7 mm, Loligo Systems, Inc., Viborg, Denmark) for individual identification, which allowed for repeated measurements of standard length (SL, mm) and body mass (g) from the same fish. Fish were then systematically assigned to one of six 340 L tanks (0.97 m diameter x 0.48 m depth) to ensure each tank had the same average wet body mass (2.95 ± 0.12 g, F₅₄₆ = 0.0827, p = 0.9946) prior to commencing food ration treatments. Tanks were randomly assigned to one of the following two food rations: 1) high feed (4% mass of feed per fish wet mass; n = 25 fish), or 2) low feed (1% mass of feed per fish wet mass; n = 27 fish) with three replicate tanks per treatment group. Each tank contained 7 to 10 fish to allow for social interactions, and fish were fed 1.5 mm pellet feed (BioPro2 pellets, Bio-Oregon®) daily.

Fish standard lengths and weights were measured at day 0 (baseline) and then at time points of day 24, day 48, day 75, day 91, and day 98 of the experimental treatments to quantify body size and growth variation. Lengths (SL) and weights were used to quantify body condition factor (K), calculated as (mass/standard length³) * 100 (e.g.,
Ricker, 1975; Lambert and Dutil, 1997), as well as specific growth rate (SGR), calculated as follows (e.g., Lugert et al., 2014):

\[
SGR = \log\left(\frac{mass_{final} - mass_{initial}}{total\ days} \times 100\right)
\]

After 98 days of the experimental ration treatments, fish were euthanized (tricaine methanesulfonate, MS222, Argent Aquaculture, LLC, Redmond, WA, USA) and blood was collected by severing the caudal peduncle. Blood was centrifuged at 3,000 x g for 10 min at 4°C, and the resulting plasma was collected and stored at -80°C. Liver and skeletal muscle tissues were also dissected from each fish, frozen immediately in liquid N₂, and kept at -80°C until RNA extraction.

1.3.5. Plasma Igf1 quantification

Plasma total (combined bound and unbound to Igfbps) Igf1 concentrations were determined using a time-resolved fluoroimmunoassay (TR-FIA) with DELFIA assay reagents (Perkin-Elmer) and anti-Igf1 antiserum to barramundi (Lates calcarifer) (GroPep BioReagents, Ltd., Thebarton, SA, Australia). This TR-FIA and barramundi anti-IGF-I antiserum was previously validated for use in Sebastes rockfishes.

1.3.6. Quantification of Igf system gene transcripts

The relative abundance of gene transcripts for igf1 and igf2, Igf1 receptors a (igfra) and b (igfrb), and isoforms of Igf binding proteins type 1 (igfbp1a, and -1b), type 2 (igfbp2a, and -2b), and type 5 (igfbp5a, and -5b) were quantified in the liver and skeletal muscle using real-time quantitative reverse transcription PCR (qRT-PCR).
Total RNA was extracted from liver and muscle tissues using TriReagent® (Molecular Research Center, Inc.) and bromochloropropane. RNA was subsequently DNase treated (TURBO DNA-free kit, Life Technologies) and quantified by spectrophotometry (260:280 = 2.02, P300 NanoPhotometer, Implen, Inc.). RNA from each fish was then diluted to 68.6 ng·µl⁻¹ for liver and 20.8 ng·µl⁻¹ for skeletal muscle to standardize total RNA concentrations prior to reverse transcription. RNA was reverse transcribed in 24 µl reactions with 2.86 µg (liver) or 0.87 µg (muscle) DNase-treated RNA template (10 µl), 4.8 µl 5X buffer (GoScript™, Promega), 3.775 µl MgCl₂ (3.9 mM concentration), 1.2 µl dNTPs (0.5 mM each dNTP), 1.2 µl random primers, 0.125 µl RNase (Recombinant RNasin® Ribonuclease Inhibitor, Promega), 0.9 µl GoScript™ reverse transcriptase (Promega), and 2 µl nuclease-free water. Reverse transcription reactions were conducted at 25°C for 5 min, 42°C for 60 min, and then completed with a reverse transcriptase inactivation at 70°C for 15 min.

Primers for SYBR Green qRT-PCR were designed to the protein coding regions of each olive rockfish partial cDNA using the PrimerQuest tool of Integrated DNA Technologies (Coralville, IA, USA). Primers were also designed to amplify efla and rpl17 for use as reference genes. All primers were synthesized by Eurofins Genomics (Louisville, KY) and kept at -20°C until use.

Quantitative real-time PCR assays were conducted in 16 µl reactions with 8 µl iTaq™ Universal SYBR® Green Supermix (BioRad Laboratories, Inc., Hercules, CA, USA), 1 µl of both forward and reverse primers (10 mM), 4.5 µl nuclease-free H₂O, and 1.5 µl cDNA template. All SYBR Green qRT-PCR reactions were run on a CFX Connect™ Real-Time PCR System (Bio-Rad Laboratories, Inc.) under a thermal profile
of 95°C for 2 min, 42 cycles of 95 °C for 5 s and 60 °C for 30 s, followed by 65°C for 15 s. For each gene, a standard curve was made from a pool of RNA from samples comprised of fish from both ration treatments. This pooled sample was serially diluted and each standard concentration assayed in triplicate. Correlation coefficients (r²) for the standard curves were always greater than r² = 0.96. Melt curve analyses were also performed to confirm amplification of a single product and the absence of primer–dimers during each quantitative PCR run. PCR efficiencies for each gene were calculated using the equation: 

\[
\% \text{ efficiency} = \left[ 10^{-\frac{1}{\text{slope}}} - 1 \right] \times 100; \text{mean efficiencies are provided in Table 2.}
\]

For each gene, relative mRNA levels were calculated using the standard curve and normalized to the geometric mean of \textit{rpl8} and \textit{ef-1}α mRNA expression. Abundance values of each gene of interest were then expressed as a relative level by dividing the resulting values by the mean value of the low treatment group to obtain a value of 1. Specificity of the primer sets was also assessed by Sanger sequencing select PCR products for each gene.

### 1.3.7. Statistical Analyses

Repeated-measures ANOVA models were used to test for effects of ration treatment on body mass, length, and condition factor (K) over the 98 d experimental period. There were no within treatment tank effects, which was found by comparing ANOVA models with and without ‘tank’ as a fixed effect, so ‘tank’ was not considered in the analysis. Student t-tests were then used to test for differences in mass, length, and condition factor between the high and low food ration treatments at each measurement.
day. Pearson’s correlations were also used to examine relationships between Igf1 and both mass-specific SGR and length-specific SGR for all fish combined. Associations between plasma Igf1 concentrations and both hepatic and muscle igf1 mRNA levels for all fish, as well as within treatments, were tested using Pearson’s correlations. All statistics were conducted using R v3.3.2 through RStudio v1.0136.

1.4. RESULTS

1.4.1. Identification of partial cDNAs from olive rockfish

Degenerate primer PCR amplified partial cDNAs encoding igf1 (362 bp, GenBank accession no. MG366820) and igf2 (451 bp, MG366821) for olive rockfish, as well as cDNAs for the following Igfbps: igfbp1a (679 bp, MG366822), igfbp1b (702 bp, MG366823), igfbp2a (519 bp, MG366824), igfbp2b (678 bp, MG366825), igfbp5a (200 bp, MG366826), and igfbp5b (707 bp, MG366827). Partial cDNAs were also sequenced for the Igf1 receptors a (igf1ra, 426 bp, MG366828) and (igf1rb, 340 bp, MG366829). BLAST analyses using GenBank (https://www.ncbi.nlm.nih.gov/) combined with phylogenetic analysis of the deduced amino acid sequences for the partial cDNAs encoding Igfbps confirmed the identity of these cDNAs.

Partial sequences encoding 416 bp of the cDNA for elongation factor 1-α (ef1α) (MG366830) and 399 bp of a cDNA for 60S ribosomal protein L17 (rpl17) (MG366831) were also amplified and sequenced from olive rockfish for use as internal reference genes in real-time quantitative reverse-transcription PCR.
1.4.2. Ration differences generate variation in growth rate

There were no significant differences in body size parameters (mass, standard length, and condition factor) between rockfish in the two ration treatments at the start of the experiment (day 0, \( p > 0.24 \)), prior to commencing feeding. However, fish in the 4% ration treatment were significantly larger in mass (\( F_{1,50} = 58.12, p < 0.001 \)) and body condition factor (\( F_{1,50} = 28.65, p < 0.0001 \)) after 24 d, and were larger in SL (\( F_{1,50} = 27.46, p < 0.0001 \)) after 48 d (Fig. 1), compared to fish in the 1% ration treatment.

Rockfish in the 4% ration treatment remained larger in mass and length and also had a higher condition factor until the end of the experiment on day 98 (Fig. 1). As expected by those body size differences, fish in the 4% ration treatment ultimately exhibited greater mass-specific SGR (\( F_{1,50} = 146.77, p < 0.0001 \)) and length-specific SGR (\( F_{1,48} = 59.37, p < 0.0001 \)) compared to fish in the 1% ration treatment (Fig. 2).

Variances in mass were even across all tanks in each treatment prior to the experiment (\( F_{5,46} = 0.397, p = 0.8484 \)) as well as after 98 d of manipulated feed (\( F_{5,46} = 1.841, p = 0.1235 \)). Standard lengths similarly showed equal variances in initial (\( F_{5,46} = 0.3281, p = 0.8935 \)) and final measurements (\( F_{5,46} = 1.299, p = 0.281 \), Fig. 3).

1.4.3. Effect of ration amount on plasma Igf1 concentrations

Plasma Igf1 concentrations were significantly higher in rockfish from the high ration treatment (\( F_{1,48} = 9.509, p = 0.0034 \)) (Fig. 4). When looking at all fish mass and length specific SGR were both strongly correlated to plasma Igf1 levels (Fig. 5), whereas Igf1 concentrations showed no relationship with change in body condition factor (\( r = -0.042, p = 0.769 \)).
Mass-specific SGR amongst all fish was more strongly correlated than length-specific SGR but both correlated to plasma Igf1 concentrations for multiple time periods (Table 3). All time intervals for mass-specific SGR were correlated to plasma Igf1 except the last 7 days (day 91-98, Table 3). Excluding the last 7 days, SGR for mass became more strongly correlated to Igf1 at later time periods. Length-specific SGR was significantly correlated to Igf1 concentrations at every time point. There were no significant correlations within ration treatments.

1.4.4. Regulation of mRNAs encoding Igs and Igfbps in liver

There was no difference between treatments for transcripts of igf1 (F$_{1,45}$ = 1.663, p = 0.2038) or igf2 (F$_{1,45}$ = 1.366, p = 0.2487) in the liver (Fig. 6A). Of the binding proteins, igfbp1a (F$_{1,44}$ = 11.63, p = 0.0014) and igfbp1b (F$_{1,45}$ = 24.30, p < 0.0001) had the only differences between treatments with approximately 3 and 4-fold greater abundance, respectively, in fish from the low ration treatment (Fig. 6B). Liver igfbp2a, igfbp2b, igfbp5a and igfbp5b did not differ in relative mRNA abundance between ration treatments.

Plasma Igf1 and hepatic igf1 mRNA correlated strongly across all fish (r = 0.5447, p = 0.0001; Fig. 7). Within treatments, Igf1 also correlated to liver igf1 with the low ration having a stronger correlation (r = 0.7031, p = 0.0005; Fig. 8A) than the high ration (r = 0.5537, p = 0.0050; Fig. 8B).

No significant correlations were observed between plasma Igf1 and mRNA levels for igfbp1a (r = -0.119, p = 0.44), igfbp1b (r = -0.227, p = 0.14), or any other Igfbp gene transcript in the liver (p = 0.50-0.89). On the other hand, there were many correlations
amongst liver mRNA transcripts with the low treatment having more correlates than the high ration treatment (Table 4).

1.4.5. Ration effects on Igf system-associated mRNAs in skeletal muscle

Transcripts encoding igf1 were significantly more abundant in the skeletal muscle of rockfish in the high ration treatment than in the low ration (F_{1,44} = 30.50, p < 0.0001) but igf2 did not differ between treatments (F_{1,44} = 3.468, p = 0.0692; Fig. 9A). While igfbp1a, igfbp1b, igfbp2a and igfbp2b mRNA levels did not show any differences between treatments, igfbp5a (F_{1,44} = 5.963, p = 0.0187) and igfbp5b (F_{1,45} = 9.919, p = 0.0029) levels were significantly higher in the high ration treatment compared to respective low treatment fish (Fig. 9B). None of the examined Igf1 receptors showed any difference in mRNA abundance between treatments (Fig. 9C).

Muscle igf1 mRNA abundance correlated to plasma Igf1 when grouping all fish (r = 0.4880, p = 0.0007; Fig. 10A) but this relationship was driven by rockfish in the high ration treatment only (r = 0.4144, p = 0.0493; Fig. 10B), as the relationship was not significant when fish in the low treatment were analyzed separately (r = 0.1487, p = 0.509; Fig. 10C). Relatively few mRNA transcripts correlated amongst fish in the same treatment but similar to liver tissue, the low treatment had more correlates than the high ration fish (Table 5).
1.5. Discussion

Fish in the wild experience shifting conditions of food abundance or quality, temperature, photoperiod, and other environmental parameters that can affect rates of development and somatic growth. Such external environmental influences are translated into changes in growth in part via the altered secretion of stimulatory or inhibitory hormones within the somatotropic endocrine axis. In particular, changes in nutritional conditions have been shown to alter liver production of the somatomedin hormone Igf1 in fishes (Breves et al., 2014; Breves et al., 2016). Given that relationship between Igf1 and nutritional status, it has been proposed that circulating concentrations of Igf1 may serve as a reliable indicator of recent growth rate in fishes (Beckman, 2011; Picha et al., 2008a; Reinecke, 2010).

In the present study, the relationship between circulating Igf1 and growth rate was examined in olive rockfish, one of several Sebastes rockfishes important as recreational and commercial groundfish fisheries in the Northern Pacific Ocean. By feeding groups of juvenile olive rockfish two different ration amounts (1% or 4% wet mass per d) for 98 d, differences in SGR were intentionally generated, with rockfish given the 4% ration growing 60% faster in mass and 22% more rapidly in length per day compared to fish given the 1% ration. Ration-induced differences in growth were then used to evaluate how growth variation, both between treatments and among all fish, links to changes in circulating Igf1, as well as relative mRNA levels of igf1 and several other genes encoding proteins involved in the somatotropic endocrine axis.
1.5.1. Variation in growth related to plasma Igf1

Juvenile olive rockfish experiencing faster growth under the high ration treatment exhibited higher plasma Igf1 concentrations. Similar differences in circulating Igf1 concentrations linked to variation in food ration have been observed previously in other fishes including tilapia (Breves et al., 2014; Uchida et al., 2003), sea bream (Pérez-Sánchez et al., 1995), and salmon (e.g., Beckman et al., 2004a; Breves et al., 2016; Pierce et al., 2005). It is thought that these changes in plasma Igf1 with food ration result from GH resistance in the liver, as there are typically negative correlations between plasma GH and Igf1 levels in food restricted fish, and positive correlations in fed individuals (e.g., Fox et al., 2006). Supporting this idea, olive rockfish displayed a positive correlation between plasma Igf1 and hepatic igf1 mRNA levels, suggesting that the variation in plasma Igf1 observed was at least in part a result of variation in hepatic Igf1 production.

Individual variation in plasma Igf1 in olive rockfish correlated positively with individual variation in growth rate, supporting the previously proposed idea that variation in circulating Igf1 concentration may serve as a physiological indicator of growth rate variation across a variety of fishes (Beckman, 2011; Picha et al., 2008a). The strength of this correlation between plasma Igf1 and growth rate varied from approximately $r = 0.29$ to 0.43, depending on whether SGR was measured using body length or mass, and generally was observed as more robust with measurements of mass-specific SGR (Table 3).

To date, only a handful of studies have examined the relationship between Igf1 and growth variation in fishes (Beckman, 2011; Picha et al., 2008a). Igf1 was found to correlate positively with SGR in tilapia (Uchida et al., 2003), coho salmon (Beckman et
al., 2004a,b), and chum salmon (*Oncorhynchus keta*, Kaneko et al., 2015). Generally, studies that have examined associations between plasma Igf1 concentrations and SGR in teleost fishes have observed correlations with coefficients within the range of $r = 0.26$ to 0.76 (Beckman, 2011). While some studies have observed that circulating Igf1 correlates more robustly with length-specific SGR (Beckman et al., 2004a, see also Beckman, 2011), the data shown here indicates that circulating Igf1 may correlate more strongly to mass-specific SGR in olive rockfish. Conversely, Igf1 concentrations did not correlate well with condition factor which agrees with results seen in other teleost fish (Beckman et al., 2004a).

While the reason for the discrepancy in length- versus mass-specific SGR correlations is not entirely clear, it is possible that species-level variation in length-weight growth relationships may lead to differences in which SGR measurements exhibit a more robust relationship with plasma Igf1 variation. What is more, the relationships between nutritional status, plasma Igf1, and growth rate can be responsive to conditions such as day length, salinity, and water temperature (Beckman, 2011). Future studies seeking to evaluate Igf1 as a physiological indicator of growth variation in rockfishes should therefore examine both mass- and length-specific SGR in the context of variation in environmental parameters relevant to the ecological conditions experienced by these species in the wild.

Interestingly, plasma Igf1 concentrations in olive rockfish also correlated over the last 7 d of growth to length-specific SGR but not to mass-specific SGR, despite mass-specific SGR showing strongly correlative relationships at all other measured time intervals (Table 3). In contrast, other studies have observed Igf1 concentrations having
the strongest correlation over the most recent growth history (e.g., Beckman et al., 2004a). Again, given that somatic growth rates can vary due to a variety of ecological factors in addition to food ration amount, such as changes in life history stage, photoperiod, temperature, or toxicants—and that the relative effects of such factors on growth can vary among species (Beckman et al., 2011; Picha et al., 2008a)—any one of several factors may have contributed to the weaker statistical relationship between mass-specific SGR and plasma Igf1 during the last measurement time interval (day 91-98). Such effects are often linked to variation in feeding efficiency, which can lead to changes in growth even with equivalent diet composition and food consumption rates (Mingarro et al., 2002; Vera Cruz et al., 2006). In Pacific rockfishes, growth rate velocities have been demonstrated to change with development age (Tsang et al., 2007), and both temperature and body size can impact growth in these taxa (e.g., Boehlert and Yoklavich, 1983; Kamimura et al., 2012). Due to changes in water temperature, fish growth typically slows in the fall in conjunction with lowered plasma Igf1 levels (Larson et al., 2001; Mingarro et al., 2002). Given that the last sampling date (day 98) was 21 October 2016 – and that fish were reared under ambient photoperiod and ocean temperatures – the absence of a significant correlation between Igf1 and mass-specific SGR across the final 7 d of the experimental period may have resulted from a slowing of growth with the transition into the fall season.

1.5.2. Growth-related variation in hepatic and muscle Igf1 and Igf2 gene expression

Liver igf1 mRNAs have been shown to decrease in relative abundance in fish experiencing restricted food rations or fasting conditions in a variety of taxa, including
chum salmon (*O. tshawytscha*, Pierce et al., 2005), Nile tilapia (*O. niloticus*, Vera Cruz et al., 2006), rainbow trout (*O. mykiss*, Monserrat et al., 2007), and yellowtail (*Seriola quinqueradiata*, Kawanago et al., 2014). Studies in other fishes, such as Mozambique tilapia (Breves et al., 2014) and Atlantic salmon (Breves et al., 2016), however, did not observe any changes in hepatic *igf1* mRNAs when food was withheld. Hepatic *igf1* similarly failed to track changes in plasma Igf1 in masu salmon (*O. masu*) during compensatory growth (Kawaguchi et al., 2013). The present results with olive rockfish correspond with those later studies as there was no alteration in liver *igf1* mRNA levels with differences in ration amount or growth rate. However, individual variation in hepatic *igf1* mRNA levels were correlated with plasma Igf1 in olive rockfish, as seen in other fish species undergoing varied positive growth (Gabillard et al., 2003; Pierce et al., 2005).

Although the liver is commonly accepted as the main tissue of Igf1 synthesis, extrahepatic Igf1 production also appears important for regulating growth of some tissues via autocrine or paracrine effects (e.g., Firth and Baxter, 2002; Franz et al., 2016), and can be regulated in a tissue-specific pattern (Eppler et al., 2010; Fox et al., 2010). Just as in the liver, food restriction has been observed to alter extrahepatic *igf1* mRNA levels in select tissues of fish (Fox et al., 2010; Norbeck et al., 2007; Peterson and Waldbieser, 2009; Terova et al., 2007). In the current study, rockfish from 4% ration treatments had elevated relative levels of *igf1* mRNAs in skeletal muscle. Both Igf1 and Igf2 have demonstrated to increase *igf1* transcription in cultured myocytes from gilthead sea bream (Azizi et al., 2016), so the higher muscle *igf1* mRNA levels observed in rockfish from the 4% ration treatment may result directly from the higher circulating levels of Igf1 in these fish, and indirectly as a result of the elevated ration amount. Supporting this idea, muscle
*igf1* transcription has also been shown to respond to acute changes in food intake in Atlantic salmon, rainbow trout, and tilapia; in these species, *igf1* is downregulated by fasting and upregulated immediately following refeeding (Breves et al., 2016; Bower et al., 2008; Fox et al., 2010; Gabillard et al., 2006; Monserrat et al., 2007; Picha et al., 2008b).

Monserrat and colleagues (2007) hypothesized that fish muscle *igf1* gene transcription may exhibit a more rapid response capacity than *igf1* gene expression in liver tissue, which might be expected if changes in hepatic GH stimulation results in a rapid release of Igf1 or Igf2 in advance of any subsequent upregulation of gene expression for these hormones. Muscle *igf1* mRNA levels have been found to change within days of initiating fasting (Breves et al., 2016; Monserrat et al., 2007) and refeeding (Chauvigné et al, 2003; Fuentes et al., 2012; Gabillard et al., 2006), while liver *igf1* mRNAs may not change even after 12 weeks of restricted feed (Gabillard et al., 2003). Alternatively, it is possible that liver *igf1* transcription only responds strongly to severe nutritional stresses, such as complete fasting (Kawanago et al., 2014; Monserrat et al., 2007; Uchida et al., 2003). Regardless of response rate, locally produced Igf1 seems to play an important role in regulating growth, at least in vertebrates with a conserved Igf system given that liver Igf1-knockout mice exhibit normal growth (Le Roith et al., 2001).

While some studies in teleost fishes have observed notable responses of Igf2 signaling in reaction to nutritional deprivation (e.g., Gabillard et al., 2006), olive rockfish did not display any response in hepatic or muscle *igf2* mRNA levels when given a limited ration amount. Similarly, Monserrat and colleagues (2007) did not observe any changes
in *igf2* mRNA levels in the liver or muscle of rainbow trout under differing feeding regimes, although this study was conducted on older fish undergoing starvation and may not be comparable. The majority of studies that looked at *igf2* transcription response to nutritional status focused on testing the effects of extreme nutritional deprivation (i.e., fasting) followed by refeeding, and as such it not entirely comparable to the current study (Bower et al, 2008; Chauvigné et al, 2003; Peterson and Waldbieser, 2009). These previous findings along with the current findings imply that *igf2* gene expression may only be altered under severe nutritional stress but more research is needed in variation in positive growth rate.

**1.5.3. Responses of Igfbp and Igf1 receptor mRNA abundance to ration amount**

Igfbps are critical for modulating the effects of Igf hormones, and the ratio of Igf:Igfbp in circulation plays a critical role in regulating Igf1 availability for receptor binding (Clemmons, 2016). In this study only total Igf1 concentrations in plasma were measured, thus it is impossible to evaluate any changes in the Igf1 bound:unbound ratio that could result from changes in nutrition and growth rate. Even so, by examining the relative mRNA abundance of several *igfbps* in liver and muscle tissue, this study provides an initial picture of how Igfbp expression is impacted by variation in growth resulting from differences in food consumption

Igfbp1 proteins – which have been duplicated in teleosts – are thought to be among of the highest concentration binding proteins in circulation in fishes, and Igfbp1 has been shown to bind Igf1 with high affinity in several teleost species (Shimizu and Dickhoff, 2017). The expression of both Igfbp1a (regarded as the 28-32 kDa Igfbp
protein in fishes) and Igfbp1b (regarded as the 20-25kDa Igfbp) has demonstrated to be altered by nutritional status, sometimes in patterns that appear more responsive to feeding than even Igf1 in some teleosts (Shimizu et al., 2006; Picha et al., 2008a). Generally, both Igfbp1a and 1b show elevated hepatic and plasma expression under nutritional restriction (Shimizu and Dickhoff, 2017). Juvenile olive rockfish experiencing limited food seem to follow the same pattern as they had higher hepatic abundance of both igfbp1a and igfbp1b transcripts. These high levels of hepatic igfbp1a and 1b mRNAs under food restriction correspond to the findings of previous studies in fasted Atlantic salmon (Breves et al., 2016), masu salmon (Kawaguchi et al., 2013), and Mozambique tilapia (Breves et al., 2014).

Overexpression of hepatic Igfbps is generally thought to increase relative Igf1 binding in circulation, thereby reducing Igf1 stimulation of growth in peripheral tissues (Clemmons, 2016). Due to the lack of response in the olive rockfish liver from all other binding proteins, it is likely that igfbp1a and igfbp1b play a key role in modulating the amount of available Igf1 in circulation, ultimately contributing to the observed differences in growth. In juvenile salmon, for instance, circulating Igfbp1a and Igfbp1b levels both correlated inversely with growth rate (Kawaguchi et al., 2013). In light of that relationship, Kawaguchi and coworkers (2013) proposed that plasma Igf1 may serve as a reliable, positive indicator of growth, while plasma Igfbp1b may be a negative indicator. In olive rockfish, there were no significant correlations between individual hepatic igfbp1a or igfbp1b mRNA levels and individual growth rate. Nonetheless, given the magnitude of hepatic igfbp1 mRNA responses to food limitation, it is still possible that
*igfbp1a* and *igfbp1b* levels are more sensitive to shifts in food consumption than plasma Igf1 under some conditions of nutritional stress (Picha et al., 2008a).

Generally, transcripts encoding *igfbp1a* and *igfbp1b* are at lower abundance in skeletal muscle than in liver (Bower et al., 2008; Bower and Johnston, 2010; Breves et al., 2014; Fuentes et al., 2013; Safian et al., 2012). Because of this, *igfbp1* mRNAs are less frequently measured in muscle tissues from teleosts exposed to food manipulation experiments, and relatively little is known about the role of extrahepatic Igfbp1 production in regulating muscle growth. There were no observed changes in olive rockfish muscle *igfbp1a* or *igfbp1b* mRNA levels to differences in food ration, even though both transcripts were at detectable levels in this tissue. Notably, there are conflicting findings concerning the expressional regulation of *igfbp1* mRNAs in skeletal muscle in fishes, even across studies using the same species. In Atlantic salmon, for instance, some studies have been unable to detect *igfbp1* mRNAs in homogenized fast-twitch (white) muscle tissue or isolated, cultured myocytes (Bower et al., 2008; Bower and Johnston, 2010). Other work with this species, however, not only detected *igfbp1* transcript expression in this same tissue, but also observed expressional regulation in response to temperature (Hevroy et al., 2015). These incongruent findings suggest that muscle *igfbp1* expression may vary with several factors besides nutritional status, such as development age or ecological conditions, which may interact to obscure clear patterns of *igfbp1* regulation in muscle, depending on the experimental testing conditions.

Similar to other teleosts, olive rockfish possess mRNA encoding two distinct *igfbp2* mRNAs (*igfbp2a* and *igfbp2b*). In teleosts, *igfbp2* transcripts are found at the highest relative levels in liver and white muscle (e.g., Safian et al., 2012), with plasma
Igfbp2 functioning as the primary carrier of Igfs in blood circulation (Shimizu and Dickhoff, 2017). The role of Igfbp2 in teleost fishes represents a distinct change in the function of this protein compared to mammals where Igfbp3 is the main transporter of Igfs (Shimizu and Dickhoff, 2017). Surprisingly, overexpression of Igfbp2 inhibits cell proliferation and DNA synthesis in both cultured mammalian and zebrafish (Danio rerio) cells (Duan et al., 1999). Additionally, hepatic igfbp2 mRNA expression is elevated by GH and suppressed by prolonged food deprivation (Duan et al., 1999; Chen et al., 2014; Gabillard et al., 2006; Kelley et al., 2001; Safian et al., 2012). This implies that hepatic Igfbp2 production increases under GH stimulation to help dampen the concurrent release of Igfs from the liver but is downregulated to shift the bound:unbound ratio of Igfs in plasma under severe nutritional stresses.

In the rockfish studied here, liver and muscle igfbp2a and igfbp2b mRNA levels were unaffected by food ration. In studies using other teleosts, expressional regulation of hepatic igfbp2 transcripts have been observed largely in the context of fasting/refeeding experimental manipulations. Liver igfbp2 has shown to respond to fasting in several fishes (Chen et al., 2014; Duan et al., 1999; Safian et al., 2012), although not all species (Breves et al., 2014; Gabillard et al., 2006). Muscle igfbp2 has also been observed to be downregulated during fasting in fine flounder (Paralichthys adspersus, Safian et al., 2012) and rainbow trout (Gabillard et al., 2006), and then return to basal levels after refeeding. Given that the experimental treatments with rockfish involved differences in ration amount and not complete food deprivation/fasting, it appears that the severity of nutritional stress in the 1% ration treatment was insufficient to induce changes in Igfbp2 gene expression. Future studies, however, should examine whether a more severe fasting
stress would result in altered hepatic or muscle expression of Igfbp2 mRNAs in rockfish, as has been observed in other fishes.

Igfbp5 is expressed in many tissues and has been linked to bone growth (Duan et al., 2005), juvenile development (Salih et al., 2004), and skeletal muscle differentiation (Ren et al., 2008; Safian et al., 2012). Transcripts encoding igfbp5a and igfbp5b are present in the liver of many teleost fishes (Breves et al., 2014; Gabillard et al., 2006; Kamangar et al., 2006; Safian et al., 2012; Pedroso et al., 2009; Zheng et al., 2017), but have generally not been shown to be sensitive to variation in nutritional status in this tissue (e.g., Breves et al., 2014; Gabillard et al., 2006). In skeletal muscle, however, Igfbp5 gene transcription does appear to be regulated by nutrition (Bower et al., 2008; Bower & Johnston, 2010; Gabillard et al., 2006; MacQueen et al., 2011; Zheng et al., 2017; but see Amaral & Johnston, 2011). For instance, in fine flounder, muscle igfbp5 mRNAs were downregulated while fasting and upregulated during refeeding (Safian et al., 2012). Olive rockfish showed elevated mRNA levels for both igfbp5a and igfbp5b in muscle supporting those previously observed effects of nutritional variation on Igfbp5 expression in this tissue. While the function and mechanism of action for Igfbp5 regulation in muscle remains unclear, Bower and Johnston (2010) observed that amino acid addition alone led to an increase in igfbp5 expression in cultured myotube cells from Atlantic salmon, suggesting that specific composition of nutritional variation may in part influence the dynamics of muscle Igfbp5.

Partial cDNAs were amplified and sequenced encoding two forms of Igf1 receptors (igf1ra and igf1rb) from olive rockfish. Multiple Igf1 receptors have been likewise detected in other fish species (Azizi et al., 2016; Chan et al., 1997; Escobar et
al., 2011; Greene and Chen, 1999), and these different Igf1r forms have been found to exhibit dissimilar patterns of tissue expression in some contexts (e.g., Maures et al., 2002). In target tissues such as skeletal muscle, the effects of Igf1 on cell proliferation, differentiation, and migration occur via type 1 Igf receptors, which activate intracellular transduction cascades including the phosphatidylinositol 3-kinase (PI3K)-Akt pathway (Dupont and LeRoith, 2001).

Although juvenile rockfish under differing food rations did not alter Igf1r mRNA levels in liver or muscle tissue, based on prior studies in other fishes, the 1% ration treatment may not have been a sufficient enough reduction in food to induce transcriptional changes in these genes. In other studies of teleost fishes, extreme nutritional stresses such as fasting have been shown to influence Igf1r transcript levels. In Atlantic salmon, transcript abundance for igf1ra – but not for igf1rb – declined in white muscle over a period of 3 to 14 d when fish switched from fasting to satiation feeding (Bower et al., 2008). Similarly, in rainbow trout, fasting increased muscle igf1ra mRNA abundance which decreased during re-feeding while igf1rb was unresponsive (Chauvigné et al., 2003). Taken together, these findings point to functional specialization of the two teleost Igf1r types, at least in salmonids. Evidence for hormonal regulation of muscle Igf1 receptors supports such functional differentiation, as Azizi and coworkers (2016) recently found that Igf1 downregulated both igf1ra and igf1rb transcripts while Igf2 upregulated only igf1rb in culture myocytes from sea bream.
1.6. Conclusions

Individual variation in circulating Igf1 concentrations were positively correlated with individual variation in SGR in juvenile olive rockfish, supporting the possibility of using Igf1 levels as an index of growth variation in *Sebastes* rockfishes for aquaculture or fishery management applications. Correlation coefficients between Igf1 and SGR ranged from $r = 0.29$ to $0.43$, depending on the size measure (mass, length) used to calculate growth rate, as well as the time period evaluated. Correlations were generally found to be more robust when using mass-specific SGR measures than for length-specific SGR measures, except for the final several days. What is more, this study provides further evidence for induction of hepatic *igfbp1a* and *igfbp1b* transcription in teleost fishes experiencing food limitation. That finding reinforces the proposed functional role of these Igfbps in catabolism (e.g., Shimizu and Dickhoff, 2017), and supports the possibility of using Igfbp1a or Igfbp1b protein or mRNA measurements as negative endocrine indices of growth rate in teleost fishes.
1.7. Tables

Table 1.1. Degenerate primers used for amplification and sequencing of partial cDNAs from olive rockfish, *Sebastes serranoides*.

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Primer</th>
<th>Nucleotide sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin-like growth factor-1 (igf1)</td>
<td>igf1-for1</td>
<td>CGC AAT GGA ACA AAG TSG GAA TAT</td>
</tr>
<tr>
<td></td>
<td>igf1-for2</td>
<td>CGC TCT TTC CTT TCA GTG GCA T</td>
</tr>
<tr>
<td></td>
<td>igf1-for3</td>
<td>GCT GCG ATG TGY TGT ATC TCC T</td>
</tr>
<tr>
<td></td>
<td>igf1-rev3</td>
<td>CCT GGT TTA CTG AAA TAA AAG CCT CTC</td>
</tr>
<tr>
<td></td>
<td>igf1-rev2</td>
<td>GTG CTC TYG GCA TGT CTG TGT</td>
</tr>
<tr>
<td></td>
<td>igf1_rev1</td>
<td>CAA TTC CTA CAC AAA ATG TAA GMA GCT</td>
</tr>
<tr>
<td>Insulin-like growth factor-2 (igf2)</td>
<td>igf2-for1</td>
<td>GGA TAG CAG CAG AAT GAA GGT CAA G</td>
</tr>
<tr>
<td></td>
<td>igf2-for2</td>
<td>AGA TGT CTT CGT CCA GTC GTG C</td>
</tr>
<tr>
<td></td>
<td>igf2-rev2</td>
<td>CTG CCA CGC CTC GTA TTT GG</td>
</tr>
<tr>
<td></td>
<td>igf2-rev1</td>
<td>TAG TTG TCC GTG GCG AGC AAG A</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 1a (igfbp1a)</td>
<td>IGFBP1a-for1</td>
<td>GTG GTG CTG ACA GGG ACT CTG</td>
</tr>
<tr>
<td></td>
<td>IGFBP1a-for2</td>
<td>GTC CAG AGC TCA GTC GCT</td>
</tr>
<tr>
<td></td>
<td>IGFBP1a-rev2</td>
<td>ATC TTC TTG CCG TTC CAG GAG</td>
</tr>
<tr>
<td></td>
<td>IGFBP1a-rev1</td>
<td>TAA GGG CAC TCG GCG TCT G</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 1b (igfbp1b)</td>
<td>IGFBP1b-for1</td>
<td>ATG TCT GGA TTA CAT GAG AAG CTG A</td>
</tr>
<tr>
<td></td>
<td>IGFBP1b-for2</td>
<td>CAT CCG CTG TGC CGT CTG TA</td>
</tr>
<tr>
<td></td>
<td>IGFBP1b-rev2</td>
<td>GTC ACC GAA CAG GTY GCT CGA TC</td>
</tr>
<tr>
<td></td>
<td>IGFBP1b-rev1</td>
<td>GCG ACT TCT TGA TGA</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 2a (igfbp2a)</td>
<td>IGFBP2a-for1</td>
<td>CAG CTG GAT CCT CTC C</td>
</tr>
<tr>
<td></td>
<td>IGFBP2a-for2</td>
<td>GGT TAT CTC TGA AGG GCA T</td>
</tr>
<tr>
<td></td>
<td>IGFBP2b-for1</td>
<td>CTG TTT GCA TAC TTT CTG TCT</td>
</tr>
<tr>
<td></td>
<td>IGFBP2b-rev1</td>
<td>TTT AGG CTG TGC GGG AAT C</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 2b (igfbp2b)</td>
<td>IGFBP5a-for1</td>
<td>CAG TCT CCT CAT GAG AAG CTG A</td>
</tr>
<tr>
<td></td>
<td>IGFBP5a-for2</td>
<td>CAT CCG CTG TGC CGT CTG TA</td>
</tr>
<tr>
<td></td>
<td>IGFBP5a-rev2</td>
<td>GTC ACC GAA CAG GTY GCT CGA TC</td>
</tr>
<tr>
<td></td>
<td>IGFBP5a-rev1</td>
<td>GCT GCT TGA CAG TCT TGT TT</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 5a (igfbp5a)</td>
<td>IGFBP5b-for1</td>
<td>CGT GCT CCT CAT GAG AAG CTG A</td>
</tr>
<tr>
<td></td>
<td>IGFBP5b-rev</td>
<td>CAG CTT GCT GAT CCT CTC C</td>
</tr>
<tr>
<td></td>
<td>IGFBP5b-rev1</td>
<td>GGT TAT CTC TGA AGG GCA T</td>
</tr>
<tr>
<td></td>
<td>IGFBP5b-rev2</td>
<td>CTG TTT GCA TAC TTT CTG TCT</td>
</tr>
<tr>
<td></td>
<td>IGFBP5b-rev1</td>
<td>TTT AGG CTG TGC GGG AAT C</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 5b (igfbp5b)</td>
<td>EF1a-for1</td>
<td>CGG TGA GTT TGA GCC TGG TAT TCT</td>
</tr>
<tr>
<td></td>
<td>EF1a-for2</td>
<td>GGC TCT TGT GGG ATC AGT TTG AC</td>
</tr>
<tr>
<td></td>
<td>EF1a-rev1d</td>
<td>CGT TGG AGT CAA CTA GCA GTG AGT</td>
</tr>
<tr>
<td></td>
<td>RPL17-for1d</td>
<td>ATG GTC TGC TAC TCT CTC GAC</td>
</tr>
</tbody>
</table>
### Table 1.2. Gene-specific primers for SYBR green quantitative PCR in olive rockfish.

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Primer</th>
<th>Nucleotide Sequence (5’ to 3’)</th>
<th>Amplicon length (bp)</th>
<th>% efficiency (avg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>igf1</td>
<td>for</td>
<td>CTC TTT CCT TTC AGT GGC ATT TAT</td>
<td>90</td>
<td>102.39</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>CGC ACA GCA GGA GTA GTG AGA G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igf2</td>
<td>for</td>
<td>GCA GTT CGT CTG TGA AGA CA</td>
<td>108</td>
<td>99.19</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>CTA CGG AAA CAA CAC TCC TCT AC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp1a</td>
<td>for</td>
<td>GAC AAA CAC GCG CTC TAC AA</td>
<td>96</td>
<td>96.20</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>GGA GTT CAC GCA CCA ACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp1b</td>
<td>for</td>
<td>CTT GGA GAG AGG TTC ACA ACT T</td>
<td>131</td>
<td>96.63</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>TTC CCG TTC CAG GAA GAA AC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp2a</td>
<td>for</td>
<td>ATC CGA AAG CCC AGC AAA</td>
<td>94</td>
<td>101.41</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>TGG TCT TCA TCT TGG TCT TCA TC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp2b</td>
<td>for</td>
<td>ACA ACT CAT CCA CGG TTT AGG</td>
<td>97</td>
<td>103.02</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>GGT CCC TTG CAC CTC ATT T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp5a</td>
<td>for</td>
<td>CGA GAC GGC TTA CAC TGT TT</td>
<td>97</td>
<td>102.44</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>GCA TGA AGG ACA CTT CTA GGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp5b</td>
<td>for</td>
<td>GCA CCA ACG AGA AAG GAT ACA</td>
<td>102</td>
<td>102.64</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>GCT GCA GCT CCT CAG TAA TC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igf1rA</td>
<td>for</td>
<td>GGG CGT AGT TGT AGA AGA GAT TG</td>
<td>104</td>
<td>100.10</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>CGA CTA CCT GCT GCT GTT T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igf1rB</td>
<td>for</td>
<td>TCT GCT ACC TGG ACT CCA TAG</td>
<td>87</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>ACT CCT TGG ACT GCT TGT TC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ef-1α</td>
<td>for</td>
<td>GAG GTG AAG TCT GTG GAG ATG</td>
<td>96</td>
<td>98.84</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>CTC CTT GAC GGA CAC ATT CTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rpl17</td>
<td>for</td>
<td>CCT CCT GCA CAT GCT CAA A</td>
<td>96</td>
<td>99.97</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>GCC TTG TTG ACC TGG ATG T</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1.3. Mass and length-specific somatic growth rates (SGR) calculated across different time intervals.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mass SGR</strong></td>
<td>r = 0.4156</td>
<td>r = 0.4171</td>
<td>r = 0.4243</td>
<td>r = 0.4266</td>
<td>r = 0.2361</td>
</tr>
<tr>
<td></td>
<td>p = 0.0027</td>
<td>p = 0.0026</td>
<td>p = 0.0021</td>
<td>p = 0.0020</td>
<td>p = 0.0988</td>
</tr>
<tr>
<td><strong>Length SGR</strong></td>
<td>r = 0.3358</td>
<td>r = 0.3181</td>
<td>r = 0.3135</td>
<td>r = 0.2895</td>
<td>r = 0.3868</td>
</tr>
<tr>
<td></td>
<td>p = 0.0171</td>
<td>p = 0.0244</td>
<td>p = 0.0267</td>
<td>p = 0.0414</td>
<td>p = 0.0150</td>
</tr>
</tbody>
</table>
Table 1.4. Liver mRNA gene correlation matrix. Correlations are Pearson’s product-moment correlations. Bolded and stared values indicate significant correlations (FDR corrected P > 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Mean h</th>
<th>SD l</th>
<th>igf1 h</th>
<th>igf2 h</th>
<th>igfbp1a h</th>
<th>igfbp1b h</th>
<th>igfbp2a h</th>
<th>igfbp2b h</th>
<th>igfbp5a h</th>
<th>igfbp5b h</th>
</tr>
</thead>
<tbody>
<tr>
<td>igf1</td>
<td>h = 0.55</td>
<td>l = 0.61</td>
<td>h = 0.20</td>
<td>l = 0.23</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igf2</td>
<td>h = 0.67</td>
<td>l = 0.64</td>
<td>h = 0.32</td>
<td>h = -0.37</td>
<td>-</td>
<td>*l = 0.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp1a</td>
<td>h = 0.85</td>
<td>l = 3.26</td>
<td>h = 1.88</td>
<td>h = -0.03</td>
<td>h = 0.22</td>
<td>l = 0.33</td>
<td>*l = 0.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp1b</td>
<td>h = 0.36</td>
<td>l = 1.65</td>
<td>h = 0.59</td>
<td>h = 0.10</td>
<td>h = -0.05</td>
<td>h = 0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp2a</td>
<td>h = 0.67</td>
<td>l = 0.92</td>
<td>h = 0.40</td>
<td>h = -0.40</td>
<td>h = 0.46</td>
<td>h = 0.48</td>
<td>h = 0.33</td>
<td>*l = 0.72</td>
<td>*l = 0.46</td>
<td>1 = 0.40</td>
</tr>
<tr>
<td>igfbp2b</td>
<td>h = 0.68</td>
<td>l = 0.80</td>
<td>h = 0.31</td>
<td>h = -0.29</td>
<td>h = 0.42</td>
<td>*h = 0.56</td>
<td>h = 0.30</td>
<td>*h = 0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp5a</td>
<td>h = 0.75</td>
<td>l = 0.89</td>
<td>h = 0.40</td>
<td>h = -0.09</td>
<td>h = -0.09</td>
<td>h = 0.16</td>
<td>h = 0.12</td>
<td>h = 0.26</td>
<td>h = 0.29</td>
<td></td>
</tr>
<tr>
<td>igfbp5b</td>
<td>h = 0.92</td>
<td>l = 0.86</td>
<td>h = 0.47</td>
<td>h = -0.46</td>
<td>h = 0.42</td>
<td>h = 0.37</td>
<td>h = 0.19</td>
<td>*h = 0.94</td>
<td>*h = 0.92</td>
<td>h = 0.28</td>
</tr>
</tbody>
</table>

* Bolded values indicate significant correlations (FDR corrected P > 0.05).
Table 1.5. Muscle mRNA gene correlation matrix. Correlations are Pearson’s product-moment correlations. Bolded and stared values indicate significant correlations (FDR corrected P > 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>igf1</th>
<th>igf2</th>
<th>igfbp1a</th>
<th>igfbp1b</th>
<th>igfbp2a</th>
<th>igfbp2b</th>
<th>igfbp5a</th>
<th>igfbp5b</th>
<th>igfra</th>
<th>igfrb</th>
</tr>
</thead>
<tbody>
<tr>
<td>igf1</td>
<td>h = 0.84</td>
<td>l = 0.39</td>
<td>h = 0.37</td>
<td>l = 0.21</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igf2</td>
<td>h = 0.97</td>
<td>l = 0.82</td>
<td>h = 0.24</td>
<td>h = 0.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l = 0.82</td>
<td>l = 0.29</td>
<td>l = -0.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = 1.29</td>
<td>1 = 1.16</td>
<td>h = 0.08</td>
<td>h = 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = 1.29</td>
<td>1 = 1.16</td>
<td>l = -0.39</td>
<td>l = 0.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp1a</td>
<td>h = 0.81</td>
<td>l = 0.97</td>
<td>h = 0.99</td>
<td>h = 0.08</td>
<td>h = 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp1b</td>
<td>h = 0.81</td>
<td>l = 0.97</td>
<td>h = 0.99</td>
<td>h = 0.08</td>
<td>h = 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l = 1.13</td>
<td>l = 1.77</td>
<td>l = -0.39</td>
<td>l = 0.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>l = -0.19</td>
<td>l = -0.15</td>
<td>l = 0.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp2a</td>
<td>h = 0.95</td>
<td>l = 0.77</td>
<td>h = 0.44</td>
<td>h = 0.31</td>
<td>h = 0.40</td>
<td>*h = 0.62</td>
<td>h = -0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l = 0.77</td>
<td>l = 0.36</td>
<td>l = -0.03</td>
<td>*l = 0.67</td>
<td>l = 0.30</td>
<td>l = -0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp2b</td>
<td>h = 0.69</td>
<td>l = 1.42</td>
<td>h = 1.42</td>
<td>h = -0.12</td>
<td>h = -0.31</td>
<td>h = -0.04</td>
<td>h = 0.01</td>
<td>h = -0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l = 1.42</td>
<td>l = 2.65</td>
<td>l = -0.22</td>
<td>l = -0.03</td>
<td>l = 0.50</td>
<td>l = 0.58</td>
<td>l = -0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp5a</td>
<td>h = 0.82</td>
<td>l = 0.64</td>
<td>h = 0.27</td>
<td>h = 0.37</td>
<td>h = 0.56</td>
<td>h = 0.07</td>
<td>h = 0.02</td>
<td>h = 0.30</td>
<td>h = -0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l = 0.64</td>
<td>l = 0.24</td>
<td>*l = 0.58</td>
<td>l = 0.33</td>
<td>l = -0.08</td>
<td>l = -0.19</td>
<td>l = 0.16</td>
<td>l = -0.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfbp5b</td>
<td>h = 0.86</td>
<td>l = 0.67</td>
<td>h = 0.22</td>
<td>h = 0.50</td>
<td>h = 0.40</td>
<td>h = 0.30</td>
<td>h = 0.15</td>
<td>h = 0.53</td>
<td>h = -0.10</td>
<td>h = 0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l = 0.67</td>
<td>l = 0.19</td>
<td>l = 0.06</td>
<td>*l = 0.75</td>
<td>l = 0.34</td>
<td>l = -0.28</td>
<td>*l = 0.56</td>
<td>l = -0.11</td>
<td>l = 0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfra</td>
<td>h = 1.10</td>
<td>l = 1.21</td>
<td>h = 0.24</td>
<td>h = 0.21</td>
<td>h = 0.10</td>
<td>h = 0.43</td>
<td>h = 0.02</td>
<td>h = 0.37</td>
<td>h = 0.19</td>
<td>h = 0.25</td>
<td>*h = 0.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>l = 1.21</td>
<td>l = 0.45</td>
<td>l = -0.36</td>
<td>l = 0.44</td>
<td>l = 0.16</td>
<td>l = 0.00</td>
<td>l = 0.28</td>
<td>l = 0.06</td>
<td>l = -0.21</td>
<td>l = -0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>igfrb</td>
<td>h = 1.08</td>
<td>l = 1.07</td>
<td>h = 0.27</td>
<td>h = -0.02</td>
<td>h = 0.32</td>
<td>h = 0.42</td>
<td>h = 0.19</td>
<td>h = 0.54</td>
<td>h = -0.16</td>
<td>h = -0.02</td>
<td>h = 0.44</td>
<td>h = 0.37</td>
</tr>
<tr>
<td></td>
<td>l = 1.07</td>
<td>l = 0.44</td>
<td>l = -0.34</td>
<td>*l = 0.59</td>
<td>l = 0.48</td>
<td>l = 0.22</td>
<td>l = 0.49</td>
<td>l = 0.18</td>
<td>l = -0.03</td>
<td>l = 0.38</td>
<td>l = 0.63</td>
<td></td>
</tr>
</tbody>
</table>
1.8. Figures

Figure 1.1. Mean (±SEM) values of (A) mass, (B) standard length, and (C) condition factor for rockfish reared under high ration (4% wet wt.) or low ration (1% wet wt.) conditions. Mass and body condition factor differed between treatments beginning on day 24, while length differed beginning on day 48.
Figure 1.2. High (4%) ration fish (dark gray bars, n = 25) showed a greater (A) mass-specific and (B) length-specific somatic growth rate (SGR) than low (1%) ration fish (light gray bars, n = 26). Bars represent group means (±SEM) of percent change per day with p-values from student t-tests.
Figure 1.3. Mass and standard length (SL) relations separated by treatment before and after experimental manipulation. Initial measures prior to the experiment (day 0 - triangles) are lower and have shallower slopes than final measurements (day 98 – circles).
Figure 1.4. Comparison of mean (±SEM) plasma total Igf1 concentrations between high ration (4% wet wt.) and low ration (1% wet wt.) treatments. Rockfish in the high ration treatment ($n = 24$) had significantly higher plasma Igf1 than low ration fish ($n = 26$).
Figure 1.5. Individual (A) mass-specific specific growth rate (SGR) and (B) length-specific SGR both correlated positively to plasma Igf1 concentration. SGR values shown are calculated from the body size change across the entire experimental period (day 0 to 98). Lines represent Pearson correlation relationships for fish from both ration treatments combined (n = 50).
Figure 1.6. Relative hepatic mRNA levels from the high and low ration treatments. (A) Levels of igf1 and igf2 did not differ between treatments. (B) Transcripts encoding Igf binding proteins igfbp1a and igfbp1b were expressed at higher relative levels in rockfish reared under the low (1%) ration treatment. Data are shown as mean (±SEM) values. Lines indicate significant differences between treatments (Student’s t-test: *P < 0.05, **P < 0.01, ***P < 0.0001).
Figure 1.7. Individual variation in liver *igf1* mRNA levels correlated positively with plasma Igf1 concentrations. Pearson’s product-moment correlation line shows the relationship for all fish combined (*n = 50*). Ration treatments are shown for reference.
Figure 1.8. Individual variation in liver igf1 mRNA levels correlated positively with plasma Igf1 concentrations within both (A) high ($n = 24$) and (B) low ($n = 19$) treatments. Pearson’s product-moment correlation line shows the relationship for fish in each treatment.
Figure 1.9. Relative mRNA levels for *igf1*, *igf2*, and several genes encoding Igfbps in skeletal muscle of rockfish from the high (4%) and low (1%) ration treatments. (A) Transcript abundance for *igf1*, but not *igf2*, was higher in fish from the high ration treatment. (B) Only *igfbp5a* and *igfbp5b* mRNA levels in muscle differed between the two ration treatment groups. (C) There were no differences between treatments for both Igf receptors. Data are plotted as mean (±SEM) values, and lines indicate significant differences between treatments (Student’s t-test: *P* < 0.05, **P** < 0.01, ***P** < 0.0001).
Figure 1.10. Correlation between plasma Igf1 concentrations and relative levels of mRNAs encoding *igf1* in skeletal muscle for (A) all fish combined (*n* = 50), (B) high treatment, and (C) low treatment fish. The line represents a significant Pearson correlation relationship for Treatments shown for reference in all fish combined.
CHAPTER 2

Spatial and temporal variation in plasma insulin-like growth factor-1 (Igf1) in blue rockfish (Sebastes mystinus) in MPAs

2.1. Abstract

Marine protected areas (MPAs) were created to protect the marine environment and sustain fisheries, yet monitoring of these areas has been difficult as current methods for quantifying growth rates of wild fish require terminal sampling (otolith analysis) or time-consuming tagging (mark-recapture). The development of rapid, non-lethal methods for quantifying fish growth rates is needed to better evaluate the performance of MPAs and manage the incorporated fish stocks. Blood concentrations of the hormone insulin like growth factor-1 (Igf1) relate positively with individual growth rate in several fishes, including Pacific rockfishes. Given the relationship between plasma Igf1 and growth, we explored spatial and temporal patterns of Igf1 concentrations in Blue Rockfish (Sebastes mystinus), one of several Sebastes rockfishes important to commercial and recreational fisheries. By quantifying circulating Igf1 concentrations in blue rockfish caught within and outside MPAs on the Central California coast, we were able to test whether Igf1 concentrations varied in patterns associated with habitat protection status, which would imply differences in fish growth rates between MPA and non-MPA sites. Blue Rockfish were caught by hook-and-line within and adjacent to the Piedras Blancas and Point Buchon MPAs in August and September 2016. Circulating Igf1 concentrations in Blue Rockfish associated positively with body size regardless of habitat protection status, as has been observed in other fishes. After controlling for size variation, we detected higher
Igf1 concentrations in Blue Rockfish within the Piedras Blancas MPA compared to its non-MPA reference site. Point Buchon MPA, however, showed no difference in fish Igf1 concentrations. We also observed declining Igf1 levels from August to September at both locations. These patterns of Igf1 variation imply spatial patterns of growth in Blue Rockfish that do not link simply to protection status and suggest that this hormonal ‘bioindicator’ approach might help identify local habitats supporting faster fish growth.
2.2. Introduction

The population dynamics of marine fishes are influenced by a variety of ecological processes including bottom-up forces such as water temperature, upwelling intensity, and the quality and quantity of food resources (e.g., Frank et al., 2007; Frederiksen et al., 2006; Harley et al., 2006; Hunt and McKinnell 2006). The relevance of these bottom-up influences varies depending on ocean conditions (Gertseva et al., 2017; von Biela et al., 2015, 2016), which in turn alter food availability to affect population-level processes including reproductive output, recruitment success, and density-dependent growth (Caselle et al., 2010; VenTresca et al., 1996; White and Caselle, 2008). Food resource availability is therefore often considered a limiting factor for density-dependent processes such as adult fecundity, juvenile growth and survival, and habitat selection, which together can influence the size and age structure of marine fish populations (Le Pape and Bonhommeau, 2013). Given the proposed importance of food resource variation as a regulatory influence on marine fish populations, studies evaluating the role of nutritional availability on growth rates in wild fishes are crucial to predicting variation in marine fish populations relevant to commercial and recreational fisheries.

Obtaining data on individual growth rates of wild fish, however, is challenging. The most commonly used method for obtaining growth rate data is capture-mark-recapture, but this method requires the tagging of large numbers of fish and subsequently recapturing those same individuals (Pradel, 1996); such recaptures can be particularly challenging in large ocean areas. A common alternative to capture-mark-recapture is the use of otolith structure to back-calculate past growth (Campana, 1990). However, calculating fish growth from otoliths has its own disadvantages, perhaps the greatest of
which is that fish under investigation must be terminally sampled. Other methods that have also been proposed for assessing growth, such as quantifying RNA:DNA ratios (Chícaro and Chícaro, 2008), can have restricted utility depending on the ecological conditions and the range of growth rate variation being sampled (Kaneko et al., 2015).

Even so, data obtained from approaches such as those stated above are used to provide growth rate estimates that can then be examined in the context of abiotic and biotic oceanographic determinants such as water temperature, chlorophyll a concentration, or estimates of prey abundance (e.g., Hahlbeck et al., 2007; Jennings and Collingridge, 2015; Malick et al., 2015; Ware and Thompson, 2005). While useful, growth measures using capture-mark-recapture or otoliths may not successfully link oceanographic conditions to growth variation, given that the time frame of growth measures may not relate clearly to the temporal scale of variation in ocean conditions, and that the time durations across which growth is measured often varies among individual fish examined using these techniques. In light of those challenges, the development and application of new methods that provide accurate estimates of individual growth rate have the potential to provide valuable insights into how the growth rates of wild fish are affected by changing food availability, temperature, or other ecologically-relevant factors, especially if the growth rate estimation method reliably reflects a fish’s recent nutritional experience.

Physiological approaches can provide tractable, quantitative tools for measuring nutritional status, and there is now abundant evidence that the blood concentrations of some hormones serve as accessible indicators of growth rate and metabolic state in fishes (Beckman, 2011; Picha et al., 2008; Reinecke, 2010). Of the many hormones that have
been studied in fish, insulin-like growth factor-1 (Igf1) has been shown to be the best
direct indicator of growth rate due to the low clearance rate, delayed onset, and strongest
relationship that circulating concentrations of this hormone exhibit to body growth
(Larsen et al., 2001; Wilkinson et al., 2006). Igf1 is a protein hormone that is synthesized
and released by the liver into blood circulation in response to growth hormone (GH)
stimulation from the pituitary gland. Igf1 in circulation then regulates growth by
promoting cell proliferation, cartilage growth, and skeletal elongation (Reinecke et al.,
2005; Duan, 1997; Chen, et al. 2000; Wood et al., 2005). Plasma concentrations of Igf1
have been shown in laboratory studies to positively associate with specific growth rates
(SGR) in several fish species including coho salmon (Onchorhynchus kisutch; Beckman et
al. 2004a,b; Shimizu et al., 2009), Chinook salmon (O. tshawytscha; Beckman et al.,
1998), masu salmon (Kawaguchi et al., 2013), Atlantic cod (Davie et al., 2007), gilthead
sea bream (Pérez-Sánchez et al., 1995; Mingarro et al., 2002), tilapia (Oreochromis
mossambicus; Uchida et al., 2003), and several other fishes (e.g., Dyer et al., 2004; Picha
et al., 2006). Such studies have revealed that individual variation in plasma Igf1
concentration associates with individual variation in somatic growth rate across a variety
of teleost fish (Picha et al., 2008; Beckman, 2011).

Previous experimental studies in juvenile Sebastes rockfishes have shown that
individual plasma Igf1 concentrations correlate positively with individual SGR variation
(Chapter 1; Hack et al., unpub. results), indicating that concentrations of Igf1 can provide
an instantaneous picture of an individual’s relative growth rate in these fishes. Here, Igf1
hormone concentrations are assessed in blue rockfish (Sebastes mystinus) with relation to
differences in habitat protection status off the coast of Central California, USA to
evaluate the use of this hormone as a biomarker for growth in wild rockfishes. Specifically, rockfish were collected by hook-and-line fishing within and outside of two Marine Protected Areas (MPAs) along California’s coast: the Piedras Blancas MPA and the Point Buchon MPA. These two MPAs are part of a network of no-take reserves established in central California in 2007 to protect abundance and diversity of marine life in these coastal waters (Gleason et al., 2013). The collection of S. mystinus blood samples from these MPAs was conducted as part of the California Collaborative Fisheries Research Program (CCFRP), which surveyed fishes weekly from commercial passenger fishing vessels in order to monitor and assess the effectiveness of these MPAs for protecting populations of nearshore fishes—mainly rockfishes (Starr et al., 2015; Wendt and Starr, 2009; Yochum et al., 2011). Spatial and temporal patterns of variation in plasma Igf1 levels of blue rockfish in these MPAs and in two adjacent, non-protected (i.e., non-MPA) locations were analyzed to assess its use as an index of relative growth. These comparisons were made to evaluate whether Igf1 levels were higher in blue rockfish caught within the protected MPAs, and to assess whether the two MPA sites generate similar patterns of growth variation in these species.

2.3. Methods

2.3.1. Animals

Rockfishes of the genus Sebastes are important species for commercial and recreational hook-and-line fisheries in the North Pacific Ocean (e.g., Miller et al., 2014; Parker et al., 2000). Blue Rockfish (Sebastes mystinus) are an abundant, semi-pelagic
nearshore species that inhabits rock reefs and kelp forests in areas generally less than 100 m in depth from the Gulf of Alaska to Baja California (Allen et al., 2006; Love et al., 2002). Blue rockfish are considered a planktivore that feeds largely on pelagic gelatinous taxa including ctenophores, thaliaceans, and schyphozonans, but will also consume pelagic hydrozoans and gastropods, and young-of-the-year fishes and squid (Hallacher and Roberts 1985; Hobson and Chess, 1988; Love et al., 2002). Tagging studies indicate that blue rockfish typically have small home ranges (~0.23 km²) and high residency to particular habitat sites (Green et al., 2014), and movement of fish is generally limited to than less than 100 m from the central core of their range (Jorgensen et al., 2006). These home range sites are often associated spatially with submarine structures including rock pinnacles and the seaward edges of kelp beds (Jorgensen et al., 2006; Hallacher and Roberts 1985, Hobson et al. 1996). Notably, however, a minor proportion of blue rockfish tagged and tracked in the Monterey Bay region of Central California were observed to shift home ranges when tracked over time scales of ~1 yr (Green et al., 2014). Those home range shifts were observed following a 4-6 month period of residency, and the range shifts occurred between April and June, when upwelling activity is pronounced along the California coast (Green et al., 2014).

2.3.2. Study Sites

Immature blue rockfish under 270 mm in total length (TL) were caught by hook-and-line fishing from commercial passenger vessels in August and September 2016 at two MPA locations: the Piedras Blancas MPA (26.9 km²) (PBL) and the Point Buchon MPA (17.4 km²) (PBN) (Fig. 1). Both MPAs are closed to all commercial and
recreational fishing. These MPAs were established in 2007 along with several other MPA sites along the central coastline of California (Gleason et al., 2013).

Fishing occurred during 15 min drifts inside 500 m² cells within the two MPAs and associated ‘reference’ (REF) sites (see also Starr et al., 2015), which had no recreational or commercial fishing restrictions. Cells were positioned over rocky habitats in water less than 40 m in depth. The two REF sites were located 0.5–10 km away from their corresponding MPA, and were positioned within areas open to both recreational and commercial fishing. The Piedras Blancas site had a total of 57 cells, while the Point Buchon site had 22 cells. Which cells were selected for fish sampling on a given sampling day was determined randomly prior to departing for fishing. Sampling at each site followed a paired sampling design, so that at each location an MPA was sampled on one day and the corresponding REF site was sampled the following day—weather permitting. All paired sites were sampled within 48 hours of each other.

Fish were collected between 8 am and 3 pm by volunteer anglers using a mixture of fishing gear including barbless baited hooks, lures and metal jigs. Each captured fish was identified to species and measured to total length (measured from the tip of the snout to the posterior edge of the flattened caudal fin). The latitude and longitude location and depth of the site where each fish was collected was recorded. A ~2 mL blood sample was then collected from the caudal vein of each fish. Fish were wrapped in a wet towel and processed in under 2 min in order to ensure proper recovery from handling. Blood samples were collected using sterile needles (¼ in diameter) and syringes, and transferred to heparinized microcentrifuge tubes (1.5 ml) that were maintained on ice. Blood was
then centrifuged at 3,000 x g for 10 min at 4°C, and the resulting plasma was collected and stored at -80°C. All fish were then tagged with a T-bar anchor tag and released.

A total of 401 blood samples were collected from blue rockfish between June and September 2016. A subset of 264 of those samples were analyzed for plasma Igf1 concentrations resulting in a total of 127 fish from Piedras Blancas and 137 from Point Buchon. At Piedras Blancas, $n = 78$ and $n = 49$ fish were sampled from the MPA and REF sites respectively. At Point Buchon, blood samples were analyzed from $n = 89$ fish from the MPA and $n = 48$ fish from the REF. Fish were not sexed due to inability to externally sex rockfish and immaturity of fish collected (< 270 mm in TL). The estimate size for blue rockfish at 50% maturity is 270 mm for males and 290 mm for females, with 1st maturity for both sexes being seen at 220 mm (Echeverria, 1987).

2.3.3. Plasma Igf1 Quantification

Plasma total Igf1 (combined bound and unbound to Igfbps) concentrations were determined using a time-resolved fluoroimmunoassay (TR-FIA) using DELFIA assay reagents (Perkin-Elmer) and anti-Igf1 antiserum to barramundi (*Lates calcarifer*) (GroPep BioReagents, Ltd., Thebarton, SA, Australia). This TR-FIA and barramundi anti-IGF-I antiserum was previously validated for use in *Sebastes* rockfishes. Plasma samples (25 μl) were assayed in duplicate, and the %B/Bo values for all samples ranged from 40-80% on the standard curve. Any sample duplicates with a % CV greater than 12% were re-assayed. The resulting % CV for the assay was $4.78 \pm 3.34 \%$ (mean ± SD).
2.3.4. Statistical Analyses

Body size (TL) of rockfish were examined using a two-factor ANOVA model with site (Piedras Blancas vs. Point Buchon) and habitat protection status (MPA vs. REF) and the interaction between these factors. Student’s t-tests were subsequently used to examine pairwise comparisons between levels of factors found to have significant effects on variation in body size.

Any outlier ± 3SD from the total mean of plasma Igf1 values were excluded from analysis, which resulted in only one sample being excluded. Because plasma Igf1 concentration have been observed to be associated positively with body size in fishes (Beckman et al., 2004b; Ferris et al., 2014; Mingarro et al., 2002; Uchida et al., 2003), we first tested for a relationship between plasma Igf1 concentration and body size (TL) using a Pearson’s product moment correlation. Since a significant positive relationship between Igf1 and TL was observed (see Results below), plasma Igf1 levels were standardized using a linear regression model where Igf1 values are adjusted to incorporate the effect of body size (TL). Residual Igf1 values from this regression model were then used as ‘standardized Igf1’ (Igf1\text{STD}) hormone values in all further analyses. We then used a linear regression model to test for differences in Igf1\text{STD} levels between locations (PBL vs. PBN), protection status (MPA vs. REF), and paired sampling dates. Pairwise comparisons were subsequently calculated using Student’s t-tests. All statistics were conducted using R v3.3.2 through RStudio v1.0136. ESRI ArcGIS was also used to show kernel densities of standardized Igf1 concentrations using GPS coordinates for each fish.
Principal Components analysis was conducted on wind speed, swell height, wave height, wave direction, turbidity (secchi depth), surface temperature, and depth of collection. Wave height, wave direction, and temperature were supplied by NOAA’s Diablo Canyon Waverider Buoy (station 46215) which is 0.46 m below the water line. Wind speed and swell height were estimated by sight and depths were recorded by onboard vessel instruments averaged for each drift. Secchi depth was measured on the calmer side of the vessel not in a shadow and without the use of sunglasses using marked line attached to a secchi disk at half meter increments. Final principal components were selected using Kaiser’s criterion and significance in predicting Igf1_STD.

2.4. Results

2.4.1. Body size

The mean body length (TL) of blue rockfish evaluated for plasma Igf1 concentrations was 20.81 ± 3.39 cm (mean ± SD, N = 269). The body length of sampled rockfish varied with both site and habitat protection status (two-factor ANOVA, site * protection status interaction: F1,269 = 5.255, p = 0.023). Overall, blue rockfish collected at Piedras Blancas (MPA and REF combined) were larger in size (21.87 ± 3.73 cm, mean ± SD, n = 128) compared to conspecifics collected from Point Buchon (19.86 ± 2.72 cm) (t = -5.002, p < 0.0001; Fig. 2). Within each sample site, the mean size of blue rockfish collected with the protected MPA habitat and associated REF locations were similar in body size (Fig. 2).
However, the group of blue rockfish sampled on 1 August 2016 within the boundaries of the Piedras Blancas MPA were significantly smaller than those caught in the corresponding REF locations the following day (Fig. 3a, \( t = 2.056, p = 0.0439 \)). Blue rockfish collected at Point Buchon on 8 and 9 August 2016 showed the opposite pattern of size variation, with fish sampled within the REF locations being smaller in size compared to fish caught within the MPA (Fig. 3b, \( t = 2.951, p = 0.0046 \)); this size variation, however, was only observed at Point Buchon on the first set of paired sampling dates (\( t = 1.785, p = 0.0783 \)). While we observed differences in the length of blue rockfish collected within and outside of these MPAs on select paired sampling dates, a more extensive evaluation of the size of blue rockfish caught between 2007 and 2013 at these MPAs, as well as two other MPAs along the Central California coast, did not observe any differences in the length of blue rockfish within our outside of the MPAs (Starr et al., 2015).

2.4.2. Plasma Igf1 standardization

Unstandardized plasma Igf1 concentrations showed a significant positive relationship with body length (Fig. 4; \( F_{1,267} = 19.822, p < 0.0001 \)). That relationship did not differ between groups of fish collected at Piedras Blancas or Point Buchon, so all fish from both MPA sites were analyzed together for standardization of plasma Igf1 levels. After standardizing plasma Igf1 levels for this significant positive relationship to body length, \( \text{Igf1}_{\text{STD}} \) did not correlate to length (Fig. 5, \( F_{1,262} < 0.0001, p = 1.00 \)).
2.4.3. Plasma Igf1 variation with habitat protection status

Plasma Igf1\textsubscript{STD} differed between MPA sites, with rockfish collected in the Piedras Blancas MPA and adjacent REF locations having significantly higher Igf1\textsubscript{STD} levels than fish collected from Point Buchon (Fig. 6, t = -5.67, p < 0.0001). Plasma Igf1\textsubscript{STD} levels were also observed to be significantly higher in blue rockfish collected within the Piedras Blancas MPA compared to the adjacent REF locations for this MPA (Fig. 6; t = -2.753, p = 0.0068). This habitat protection effect, however, was limited to Piedras Blancas, as Igf1\textsubscript{STD} levels were similar for blue rockfish collected within and outside of the Point Buchon MPA boundaries (Fig. 6; t = -0.386, p = 0.7001).

More detailed examinations of Igf1\textsubscript{STD} levels by paired sampling date revealed that blue rockfish collected within the boundaries of the Piedras Blancas MPA showed elevated plasma Igf1\textsubscript{STD} levels on the 1-2 August 2016 sampling dates (Fig. 7a; t = -2.478, p = 0.0158), but not on the subsequent 15-17 August 2016 dates (Fig. 7a; t = -1.755, p = 0.0844). At Point Buchon, plasma Igf1\textsubscript{STD} levels were consistently similar between fish collected within or outside of the MPA boundaries on both paired sampling dates (Fig. 7b; Aug: t = 0.630, p = 0.0531; Sept: t = 0.121, p = 0.904).

Plasma Igf1 levels standardized to regional lengths on paired sampling dates showed particular hot spots for relatively high or low levels within Piedras Blancas (Fig. 8; relative kernel densities) and Point Buchon (Fig. 9). Hot and cold spots relate to the relative mean of paired sampling dates for each location and increase or decrease in hue with values farther from the mean as well as with more fish per a given GPS location.
2.4.4. Environmental influences on plasma Igf1

Plasma concentrations of Igf1 can be influenced by several ecological factors including water temperature and photoperiod (Gabillard et al., 2003; Hevroy et al., 2013; Hevroy et al., 2015). We observed that mean plasma Igf1STD levels in blue rockfish declined from early to mid August at Piedras Blancas (t = -1.991 p = 0.0486) and from early August to early September at Point Buchon (t = -3.339, p = 0.0011) (Fig. 10).

Principal Components Analysis (PCA) shows variable abiotic factors between locations (Fig. 11), protection status (Fig. 12), and sampling dates (Fig. 13). Principal components 1-3 explain 32.8%, 24.8%, and 16.1% of the variance respectively which totals to 73.7% explained variance. There is a significant effect of PC1 (p = 0.0248), PC2 (p = 0.0012), PC3 (p = 0.0003), as well as PC5 (p = 0.0002) on IgfSTD when run in a multivariate model (Fig. 14). PC1 is comprised mainly by wave height and wave direction in one direction and temperature in the negative direction while PC2 is primarily secchi depth (i.e. water turbidity) in the positive direction and wind speed and swell in the negative direction (Table 1.). On the other hand, PC3 is mainly explained by depth and PC5 includes wind speed and swell (Fig. 15).

2.5. Discussion

This study reports spatial and temporal patterns of variation in plasma Igf1 levels in blue rockfish in Point Buchon and Piedras Blancas MPAs and in two adjacent, non-protected (i.e., non-MPA) reference locations along the West Coast of North America. Plasma Igf1 concentrations have been validated as a physiological index of growth rate in
a variety of fishes under controlled, laboratory studies (Beckman et al., 1998; Perez-Sanchez et al., 1995; Pierce et al., 2001; for review, see Beckman, 2011). And, recent experimental work in *Sebastes* rockfishes confirmed that individual variation in plasma Igf1 likewise associates positively to growth rate. Igf1 concentrations in olive rockfish (*Sebastes serranoides*) reared in captivity with differing positive rates of growth generated by varied feeding rates showed a positive correlation with mass- and length-specific growth rate (see: Chpt 1). Similarly, individual variation in plasma Igf1 was observed to correlate positively to individual growth rate in juvenile copper rockfish (*Sebastes caurinus*) raised in captivity under differing conditions of food availability (Hack et al., unpub. data).

Given the relationship between plasma Igf1 and growth in fishes, a variety of studies have begun using plasma Igf1 as a physiological index to identify spatial or temporal (i.e., seasonal, annual) variation in growth in wild fish (Andrews et al., 2001; Beckman et al., 2000, 2004; Beaudreau et al., 2011; Ferriss et al., 2014; Wechter et al., 2017). The relationships between plasma Igf1 levels and growth rate arise from the positive influence that food consumption has on pituitary gland production of GH, which stimulates the liver to produce Igf1 and enhance somatic growth (e.g., Picha et al., 2008; Reinecke, 2010).

### 2.5.1. Plasma Igf1 variation with habitat protection status

As has been observed in other fishes (e.g, Ferris et al., 2014; Beckman et al., 2011; Uchida et al., 2003; Picha et al., 2006), plasma Igf1 levels correlated positively to body size in wild blue rockfish. We therefore standardized plasma Igf1 values to remove
the influence of body size and analyzed differences between MPAs and reference sites within each location as well as, patterns between locations.

After standardizing hormone values for body size, we found that plasma Igf1$_{STD}$ levels were higher in blue rockfish within the Piedras Blancas MPA compared to fish caught in the adjacent unprotected reference site. Fish caught in this MPA on 1 August 2016 were also smaller in size but had significantly higher Igf1$_{STD}$ than those caught in the corresponding reference site the following day. This reinforces the finding that although fish in the MPA were smaller, they experienced more rapid growth in the recent past than fish in the reference site.

At the Point Buchon location, however, mean Igf1$_{STD}$ levels were found to be similar in blue rockfish sampled within the Point Buchon MPA and adjacent fished area on both paired sampling dates. This lack of variation between protected and unprotected areas illustrates how this approach of using Igf1 as a physiological index can provide important, overlooked information about MPA efficacy. While there are typically larger fish caught within MPAs compared to unfished reference sites (Starr et al., 2015), we did not see the same trend overall. Although there were larger fish caught within the Point Buchon MPA on 09 Aug 2016, the Igf1$_{STD}$ values indicate that these fish are feeding and growing at a rate similar to smaller blue rockfish caught in the associated reference site. Without the added information from Igf1, managers could assume Point Buchon MPA was effective at supporting a more productive ecosystem for blue rockfish, as counts and size of fish are some of the main parameters used currently for monitoring fish stocks in these MPAs (Murphy and Jenkins, 2010).
2.5.2. Environmental influences on plasma Igf1

Ecological factors such as water temperature, upwelling intensity, and turbidity influence quantity of food resources available to coastal marine species thus indirectly affecting reproductive output, recruitment success, and density-dependent growth (e.g., Frank et al., 2007; Frederiksen et al., 2006; Harley et al., 2006; Hunt and McKinnell 2006; Caselle et al., 2010; VenTresca et al., 1996; White and Caselle, 2008). In this way, food limitation impacts size and age structure of marine fish populations, which in turn dictates population fecundity, juvenile growth and survival, and habitat selection (Le Pape and Bonhommeau, 2013).

Environmental conditions on the days that fish were sampled varied between sampling locations, and it is possible that some of that environmental variation contributed to patterns of Igf1 variation. Here we found environmental differences in location including wave activity and temperature with Point Buchon having higher temperatures and less wave activity. Alternately, differences in protection status were due to aspects of turbidity likely caused from upwelling. Interestingly, reference sites showed higher turbidity and less swell and wind. California’s longshore current typically runs North to South pushing surface water into protected coves and bays. This causes upwelling along exposed coasts and calm, clearer water leeward of coastal heads. The reference sites for the two locations sampled here have opposite locations—Piedras Blancas downcurrent and Point Buchon upcurrent. Although these coastlines vary in contour, they should have similar exposures indicating that these differences in wave activity are likely due to daily variation in weather patterns. That daily variation becomes evident when comparing identical locations between days. Green and colleagues (2014)
found that blue rockfish in California occurred at deeper depths when there was increased wave height and lower water temperatures. Variation in Igf1 concentrations similarly tracked these ecological changes represented by the greater significance of PCs compared to location or protection status. Because of this, future use of Igf1 in monitoring growth in wild fish will need to take into account ecological parameters and time of year in order to properly assess health of stocks.

Hormonal biomarkers are currently mostly used within the aquaculture industry to monitor growth and health of farmed stocks (Picha et al, 2008). By expanding the assessment to recreationally and commercially important species, this tool could be used to measure the regional variation in production and size of wild fish populations, as well as how population parameters relate to ecosystem processes, fishing pressures, and decadal oscillations. Consideration of biomarker data in relation to other management practices will also help determine whether MPAs are achieving their expected outcome of enhancing fish growth, and thus contribute to efforts to redefine MPAs to best maintain rockfish populations—species that serve a critical role as predatory fish in California’s kelp forest ecosystems. Data on spatial variation in fish growth is essential to quantify both the impact MPAs are having on fish populations, and the environmental health status of coastal ocean systems broadly. Having an accurate way to access growth rate of many individuals will lead to better identification of the factors that might modulate growth of fish populations, thus ensuring ecosystem functions are maintained and help accurately determine how fish growth rates link to local environmental conditions in light of current overexploitation of fish stocks and climate change.
2.6. Conclusion

In this study, blue rockfish were collected from within and outside two MPAs on the central coast of California to validate the use of Igf1 as a biomarker for growth. After adjusting for the positive relationship between plasma Igf1 and body size, we saw higher levels of Igf1 in Piedras Blancas than Point Buchon as well as higher levels within Piedras Blancas MPA compared to the reference site. These differences are likely attributed to the ecological parameters of each habitat but to a larger extent due to changes in weather patterns that shift regional ecological factors on a daily basis.
2.7. Tables

Table 2.1. Principal component loadings. Variable in order are wind speed, swell height, wave height, wave cardinal direction, secchi depth, surface temperature, and depth.

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
<th>PC6</th>
<th>PC7</th>
</tr>
</thead>
<tbody>
<tr>
<td>wind</td>
<td>0.176378</td>
<td>-0.57061</td>
<td>0.061878</td>
<td>-0.39538</td>
<td>0.588246</td>
<td>-0.32259</td>
<td>0.181788</td>
</tr>
<tr>
<td>swell</td>
<td>0.251546</td>
<td>-0.52544</td>
<td>0.047245</td>
<td>-0.37166</td>
<td>-0.63274</td>
<td>0.339599</td>
<td>-0.0677</td>
</tr>
<tr>
<td>wave ht</td>
<td>0.522429</td>
<td>0.277007</td>
<td>-0.32247</td>
<td>-0.04516</td>
<td>0.049755</td>
<td>0.266548</td>
<td>0.686137</td>
</tr>
<tr>
<td>wave dir</td>
<td>0.470697</td>
<td>0.265961</td>
<td>-0.42383</td>
<td>-0.2835</td>
<td>0.113986</td>
<td>-0.1058</td>
<td>-0.65078</td>
</tr>
<tr>
<td>secchi</td>
<td>-0.05253</td>
<td>0.481096</td>
<td>0.445795</td>
<td>-0.625</td>
<td>-0.21221</td>
<td>-0.32694</td>
<td>0.15654</td>
</tr>
<tr>
<td>temp</td>
<td>-0.51712</td>
<td>0.096047</td>
<td>-0.21823</td>
<td>-0.46607</td>
<td>0.300925</td>
<td>0.605567</td>
<td>-0.03535</td>
</tr>
<tr>
<td>depth</td>
<td>0.375442</td>
<td>0.100819</td>
<td>0.681145</td>
<td>0.123639</td>
<td>0.320235</td>
<td>0.474099</td>
<td>-0.2057</td>
</tr>
</tbody>
</table>
2.8. Figures

Figure 2.1. Map showing sampling locations in Central California with Marine Protected Areas (MPA) shown in blue. Sampled 500 m$^2$ cells were fished within (1) Piedras Blancas and (2) Point Buchon MPAs and also in associated reference sites (REF).
Figure 2.2. Mean (±SEM) values of total lengths for blue rockfish caught at Piedras Blancas (PBL) and Point Buchon (PBN) within the marine protected area (MPA) and at adjacent reference sites (REF). Fish caught at Piedras Blancas were significantly larger than those caught at Point Buchon. Number of samples are indicated inside each bar. Stars indicate significant differences between treatments (Student’s t-test: NS - not significant, *P < 0.05, **P < 0.01, ***P < 0.0001).
Figure 2.3. Mean (±SEM) values of total lengths for blue rockfish caught at (A) Piedras Blancas and (B) Point Buchon within the marine protected area (MPA) and at adjacent reference sites (REF) separated by sampling date. Number of samples are indicated inside each bar. Stars indicate significant differences between treatments (Student’s t-test: NS - not significant, *P < 0.05, **P < 0.01, ***P < 0.0001).
Figure 2.4. Linear regression ($r^2 = 0.066$) of total lengths by plasma Igf1 concentrations for all fish combined. Regression and standard error (gray) include both locations and all sampled cells. Protection status only shown for reference.
Figure 2.5. Linear regression ($r^2 = -0.004$) of total lengths by length standardized plasma Igf1 concentrations for all fish combined. Protection status only shown for reference.
Figure 2.6. Mean (±SEM) values of length standardize plasma Igf1 for blue rockfish caught at (A) Piedras Blancas and (B) Point Buchon within the marine protected area (MPA) and at adjacent reference sites (REF). Stars indicate significant differences between treatments (Student’s t-test: NS - not significant, *P < 0.05, **P < 0.01, ***P < 0.0001).
Figure 2.7. Mean (±SEM) values of length standardize plasma Igf1 for blue rockfish caught at (A) Piedras Blancas and (B) Point Buchon within the marine protected area (MPA) and at adjacent reference sites (REF) separated by sampling date. Number of samples are indicated inside each bar. Stars indicate significant differences between treatments (Student’s t-test: NS - not significant, *P < 0.05, **P < 0.01, ***P < 0.0001).
Figure 2.8. Kernel densities for length standardized plasma Igf1 concentration at Piedras Blancas. Igf1 concentrations are standardized to regional lengths on paired sampling dates. All dates are shown.
Figure 2.9. Kernel densities for length standardized plasma Igf1 concentration at Point Buchon. Igf1 concentrations are standardized to regional lengths on paired sampling dates. All dates are shown.
Figure 2.10. Individual plasma Igf1 values standardized to total length for all fish caught in Piedras Blancas (PBL) and Point Buchon (PBN) marine protected areas (MPA) and at adjacent reference sites (REF) separated by sampling date.
Figure 2.11. Principal components analysis showing relation of highest components in relation to Location. Piedras Blancas (PBL, green) exhibits higher wave action (wave height and secchi turbidity) while Point Buchon (PBN, yellow) has higher temperatures.
Figure 2.12. Principal components analysis showing relation of highest components in relation to protection status. Marine Protected Areas (MPA) had higher swell and wind speed while reference sites (REF) has higher turbidity (secchi).
Figure 2.13. Principal components analysis showing relation of two highest components in relation to sampling date. August 17, 2016 has notably high wave height and cloud cover.
**Figure 2.14.** Principal components analysis showing relation of two highest components in relation to total length standardized Igf1. Green are higher than expected Igf1 levels for their length and redder points are lower than expected Igf1 levels.
Figure 2.15. Principal components analysis showing relation of highest components in relation to protection status. Marine Protected Areas (MPA) had higher swell and wind speed while reference sites (REF) has higher turbidity (secchi).
References


Hobson ES, Chess JR, Howard DF., 1996. Zooplankters consumed by blue rockfish during brief access to a current off California’s Sonoma coast. Calif Fish Game 82:87–92


salmon: relationship with growth rate and changes during downstream and coastal migration in northeastern Hokkaido, Japan. Fish Physiol. Biochem. 41, 991–1003.


Le Pape, O., Bonhommeau, S., 2013. The food limitation hypothesis for juvenile marine fish. Fish and Fisheries 16, 373-398.


Pérez-Sánchez, J., Martí-Palanca, H., Kaushik, S.J., 1995. Ration size and protein intake affect circulating growth hormone concentrations, hepatic growth hormone
binding and plasma insulin-like growth factor-I immunoreactivity in a marine teleost, the gilthead sea bream (Sparus aurata). J. Nutrition 125, 546-552.


