Fall 2020

Gene Expression Responses of Larval Gopher (Sebastes carnatus) and Blue (S. mystinus) Rockfish to Ocean Acidification and Hypoxia

Jacoby Baker

GENE EXPRESSION RESPONSES OF LARVAL GOPHER (*SEBASTES CARNATUS*) AND BLUE (*S. MYSTINUS*) ROCKFISH TO OCEAN ACIDIFICATION AND HYPOXIA

A Thesis

Presented to the

Faculty of

Moss Landing Marine Laboratories

California State University Monterey Bay

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Marine Science

---

by

Jacoby Baker

Fall 2020

CALIFORNIA STATE UNIVERSITY MONTEREY BAY

The Undersigned Faculty Committee Approves the

Thesis of Jacoby Baker:

GENE EXPRESSION RESPONSES OF LARVAL GOPHER (*SEBASTES CARNATUS*) AND BLUE (*S. MYSTINUS*) ROCKFISH TO OCEAN ACIDIFICATION AND HYPOXIA

Scott Hamilton, Chair
Moss Landing Marine Laboratories

Cheryl Logan, Primary Research Advisor
California State Monterey Bay

Jonathan Geller
Moss Landing Marine Laboratories

Nathaniel Jue
California State Monterey Bay

Daniel Shapiro, Interim Dean
Associate VP for Academic Programs and Dean of Undergraduate and Graduate Studies

12/11/2020

Approval Date

ABSTRACT

Gene Expression Responses of Larval Gopher (*Sebastes carnatus*) and Blue (*S. mystinus*) Rockfish to Ocean Acidification and Hypoxia
by
Jacoby Baker
Master of Science in Marine Science
California State University Monterey Bay, 2020

Global climate change is driving shifts in ocean chemistry, which combined with intensification of coastal upwelling, reduces ocean pH and dissolved oxygen (DO) content in the nearshore habitats of the California Current System. Physiological plasticity, within and across generations, might be especially important for long-lived, late-to-mature species, like rockfishes (genus *Sebastes*), that may be unable to keep pace with climate change via genetic adaptation. Rockfishes exhibit matrotrophic viviparity and may be able to buffer their offspring from environmental stress through early developmental exposure or transgenerational plasticity (non-genetic inheritance of phenotypes). In this study, mature female gopher (*S. carnatus*) and blue (*S. mystinus*) rockfish were pre-exposed to one of four treatments; 1) control conditions, 2) low pH, 3) low DO, or 4) combined low pH/DO stressors during embryonic growth (i.e. fertilization and gestation), followed by a 5-day larval exposure after birth in either the same or a different treatment received by mothers. I used RNA sequencing to determine how the maternal environment affected larval rockfish gene expression (GE) at birth, after the 5-day larval exposure in either the same maternal treatment or a novel pH/DO environment, and between larvae sampled at birth and after the 5-day larval exposure within each treatment. For both species, I found that the maternal exposure drove larval GE patterns regardless of sampling time point or treatment. Furthermore, the maternal environment continued to strongly influence larval GE for at least the first five days after birth. In gopher rockfish, larvae differentially expressed fewer genes at birth between the control and hypoxic groups than larvae that gestated in and remained in the same treatment and were sampled after the 5-day larval exposure. Gene functions also shifted; at day 5, there was an increase in differentially expressed genes that were related to metabolic pathways, implying that the larvae in the hypoxic treatment are responding to the stressor. In both species, I found that larvae which experienced a pH and/or hypoxic stressor during the maternal exposure had fewer differentially expressed genes across time compared to larvae that experienced control conditions. This pattern remained consistent, even if the larvae were placed into control conditions for the 5-day larval exposure, indicating that exposure to low pH/DO stressors might cause a delay in development. These data suggest that rockfish may not be able to buffer their offspring from environmental stressors, highlighting the important role of the maternal environment during gestation. Between the two species, however, blue rockfish may in fact fare better in future conditions as their reproductive season occurs before the onset of strong spring upwelling, when more hypoxic and low pH water intrudes the nearshore. However, if future climate models are correct,

shifts in the timing and intensity of upwelling season may overlap with the reproductive season in blue rockfish. Elucidating the critical role of the maternal environment on offspring physiology can help us better understand how economically and ecologically important species will fare in the face of climate change.

TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

ACKNOWLEDGEMENTS

This thesis was made possible with the help of many collaborators across multiple institutions. Foremost, I would like to thank my primary research advisor Dr. Cheryl Logan who has guided me throughout my master's career by providing key insight into the world of molecular physiology, helping me gain confidence in the lab, and always having an open-door policy whenever I had questions. Additionally, I would like to thank my co-advisor Dr. Scott Hamilton, without whom I would not have been introduced to this project, nor had the opportunities to hone my fieldwork skillset. Furthermore, they each provided key insight into this project from physiological processes and ecological relevance of results to writing and communication of science. I would like to thank my thesis committee member Dr. Nathaniel Jue for his insight on the statistical design of the project as well as preparing me for working through the bioinformatic pipelines involved in these datasets. I would also like to thank my fourth thesis committee member Dr. Jonathan Geller for his insight on the molecular approaches on this project. I would like to thank the other two PIs on this project, Dr. Susan Sogard and Dr. Giacomo Bernardi, without whom this project would not have been possible.

Special shout-out to my fellow graduate student collaborator, Kristin Saksa, for partnering with me on this project, running the physiological trials, and taking lead on fish husbandry at NOAA NMFS. Other key team members include Neosha Kashef, Dave Stafford, and Melissa Palmisciano, all of whom had integral roles in the project but also helped make the team at NMFS feel like a second home. Other students and interns of the aquarium team that helped this project happen include Helaina Lindsey, Mari Figueroa, Melissa Gutterman, Maggie Gusman, Mark Schumacher, and Steven Wertheimer.

Thank you to all those who have mentored me over the years. My undergraduate advisor who gave me confidence that I could succeed in the sciences, Dr. Shannon Bros-Seemann. Fellow ichthyology lab member, Evan Mattiasen, mentored me while I was in undergrad and helped me break out of my shell and really gain an appreciation for research. I'd also like to thank Kristin Hunter-Thomson and Dr. Laura Good showing me how important and rewarding science education and outreach is.

A big thank you to my fellow Logan Lab members for helping support me over the years and listening to multiple iterations of my talks to make sure they were polished. Special shout-out to Jake Cline who not only showed me the ropes around the lab, but always checked in on me and made sure I had food and coffee during the long 10-hour protocol days; he was always willing to get a beer afterward to unwind. Big thanks to Holly Doer who was always around to bounce ideas off of, help debug code, and get together to just relax. Thank you to Melissa Meredith and Jacob Green who were big helps in the bioinformatic world whenever I had any questions. I would also like to thank Lauren Kashiwabara, David Deering, and Wenda Ly who helped in various areas with sample processing and analysis.

Another big thank you to my fellow ichthyology lab members who helped in this project as well as those who have been great friends and colleagues over the years during my time at MLML: Laurel Lam, Lauren Parker, June Shrestha, Bonnie Basnett, Vivian Ton, Rachel Brooks, Katie Neylan, Stephen Pang, and Devona Yates.

Finally, I would like to thank my family and friends who helped support me during my time at MLML. My uncle, Cam Hardwig, grandma, Inez Hardwig, and the rest of my Minnesotan brethren. All the amazing friends I have made at MLML: Jason Adelaars, Angela Zepp, Heather Barrett, Sharon Hsu, Mason Cole, and Sloane Lofy. And, of course, Jenni Johnson, who has been a great friend, classmate, and pain in the…neck for the last six years.

Lastly, I would like to thank the California Sea Grant (R/HCME-25A~C), National Oceanographic and Atmospheric Administration (NA16NMF4270255), and NSF (EF-1416895 to C.A.L.) which provided the funding which made this project possible. I would also like to thank additional funding sources including the Simpkins Family Marine Science Scholarship, the Myers Trust, CSUPERB, and NCGAS which helped support me, my research, and travel during my graduate career.

INTRODUCTION

Anthropogenic induced climate change is altering ocean chemistry, creating potentially physiologically taxing environments for marine organisms. Changes in ocean conditions include increasing temperatures (warming), decreasing pH (acidification), and decreasing dissolved oxygen levels (hypoxia) (Henson et al., 2017). The California Current System (CCS) is a dynamic and highly productive environment with relatively high biodiversity. Upwelling, a natural phenomenon along eastern boundary currents, like the CCS, occurs when strong winds drive coastal surface waters offshore (Bakun and Nelson, 1991). Surface waters are replaced by deep water characterized by cold temperatures and high nutrients. These upwelled waters, however, are also characterized by low levels of pH and dissolved oxygen (DO). Snyder et al. (2003) suggested that upwelling conditions may become more intense with increases in CO_2 emissions and rising global temperatures that increase the land-ocean temperature gradient and resulting alongshore winds. Climate models project an increase in wind intensity along many eastern boundary current systems, including the CCS, which can alter both the frequency and duration of these events (Patti et al., 2010; Sydeman et al., 2014). Although this may bring more nutrient rich waters to the surface, it can also create physiologically stressful conditions for fishes that reside off the western coast of the United States (Gruber et al., 2012).

Rockfishes are a diverse family (Sebastidae), represented by over 70 species in the CCS along the North American Pacific coast (Love et al., 2002). As rockfishes are economically and ecologically important species, it is important to understand how changing levels of pH and DO may affect their populations. Rockfish differ from most teleosts in their mode of reproduction in that they are viviparous with internal fertilization (Boehlert and Yoklavich, 1984; Love et al., 2002; MacFarlane and Bowers, 1995). After fertilization, embryogenesis occurs within the ovaries of the female fish until developed larvae are released during a parturition event, or birth, as free-swimming larvae (Love et al., 2002). This mode of reproduction may allow for additional maternal influences on larval physiology and survival. For example, a previous study in black rockfish (*Sebastes melanops*) found that larvae from older mothers exhibited higher growth rates and larger

oil globules (Berkeley et al., 2004). Maternal effects on oil globule size have also been observed in blue rockfish (*Sebastes mystinus*), gopher rockfish (*Sebastes carnatus*), and yellowtail rockfish (*Sebastes flavidus*), suggesting that larger females can produce larvae that may have increased survivability in more stressful conditions (Sogard et al., 2008). Additionally, rockfishes are a highly fecund genus, where each parturition can result in the range of ~18,000 (dwarf calico rockfish *Sebastes dalli*) to 2,700,000 larvae (yelloweye rockfish *Sebastes ruberrimus*) (Love et al., 2002).

Maternal effects represent a potential mechanism by which fishes could rapidly respond to environmental change. This mechanism may be particularly important in long-lived, late to mature species, like rockfishes, where genetic adaptation may be relatively slow. Maternal effects are a form of transgenerational plasticity, which could aid future generations by providing offspring with a higher acclimation potential through non-genetic parental influences (Bossdorf et al., 2008; Salinas and Munch, 2012; Wong et al., 2018). During gestation, the external environment that mothers are exposed to could influence the phenotypes of offspring, potentially making larvae either more or less fit when exposed to that stressor at birth.

Understanding the effects of low pH and low DO on larval rockfishes is important in determining how their populations will respond to climate change. Transcriptomics, or the study of genome wide changes in gene expression, is a powerful tool that can be used to investigate the underlying mechanisms that drive physiological responses (Connon et al., 2018). This method can be an incredibly powerful means of inferring physiological status, especially for tiny larvae for which more traditional physiological measurements might be difficult. RNA sequencing (RNAseq), is a sequencing-based technique for measuring changes in gene expression that enables researchers to examine molecular responses to climate change, even in non-model species that have no prior genomic sequencing information (Connon et al., 2018). For example, Hamilton et al., (2017) found that when two juvenile rockfish congeners were exposed to high pCO_2, copper rockfish (*Sebastes caurinus*) experienced changes in behavior and physiology while blue rockfish experienced no significant change at the behavioral and organismal physiology level. However, when gene expression was analyzed in white muscle tissue, they found that blue rockfish differentially expressed a higher number of genes, many of which were

related to genes encoding muscular restructuring proteins. Juvenile copper rockfish, on the other hand, differentially expressed fewer genes, but more of these genes were related to the cellular stress response, a sign that macromolecular damage had occurred in response to the same high pCO_2 environment. The integration of RNAseq revealed physiological changes at the cellular level that otherwise may have been missed and possible mechanisms underpinning physiological and behavioral changes. RNAseq can also uncover molecular changes associated with acclimatization processes and acute or chronic stress responses. For ecologically important species, transcriptomics is helping to uncover the interaction between the genotype and phenotypic plasticity in a changing environment (Oomen and Hutchings, 2017).

RNAseq has now been utilized in a multitude of studies to examine the effects of various climate change stressors on marine organisms. This technique has been used to demonstrate molecular changes that aid in acclimation processes of different species. rapid evolution and acclimation of species in the face of climate change. Pespeni et al. (2013) found larval purple sea urchins (*Strongylocentrotus purpuratus*) have the capacity to rapidly evolve in response to ocean acidification with large genetic variation within populations. Hamilton et al. (2017) found that juvenile rockfish (*Sebastes spp.*) had differing molecular response mechanisms to high pCO_2 levels with one species having more acclimatization potential. A study on the thermal stress in corals (*Acropora hyacinthus*) revealed fundamental cellular processes leading to increased stress tolerances (Barshis et al., 2013). Finally, Long et al. (2013) showed that zebrafish (*Danio rerio*) have the ability to build cold-tolerance when pre-exposed to a cold stress during early life. These studies reveal the ability of RNAseq to reveal adaptive mechanisms in organisms that may be missed by more traditional physiological studies.

In this study, I investigated if and how the maternal environment affects rockfish larval physiology. To examine this, I measured gene expression responses of gopher rockfish (*Sebastes carnatus*) and blue rockfish (*Sebastes mystinus*) larvae at birth when mothers were exposed to normoxic (normal oxygen), hypoxic (low oxygen), low pH, or combined hypoxic/low pH conditions during gestation. If the maternal environment affects rockfish larval physiology (negatively or positively), I expected larvae to show differences in gene expression patterns at birth that varied by maternal treatment. If the

maternal environment did not have effect, I expected little to no differential gene expression at birth. Additionally, I examined the possible influence of the maternal environment on the early larval stages after parturition. I placed a subset of each larval brood ($_{L}$) into environments that were either the same or different from the maternal treatment ($_{M}$), and measured gene expression again after five days. If the maternal environment does not have an effect or has only a transient effect immediately after birth, I expected gene expression profiles to be driven by larval treatment post-birth. Alternatively, if maternal environment does have a longer lasting effect on larval physiology, I expected to see gene expression patterns that were driven by maternal treatment even after five days. In addition to testing the role of the maternal environment on rockfish larval physiology, I also used RNAseq as an exploratory tool to examine molecular level responses to environmental stress in rockfish larvae. In these next two chapters I will subdivide the research in the following way: Chapter 1 will cover the effects of hypoxia on the gene expression of larval gopher rockfish, while Chapter 2 will investigate the effects of low pH and combined low pH/hypoxia on gene expression responses of larval blue rockfish.

CHAPTER 1

GOPHER ROCKFISH

Introduction

Changes in dissolved oxygen (DO) concentrations in the oceans can be attributed to both anthropogenic and natural influences, including warming waters, eutrophication, increased stratification, and biological respiration (Altieri and Gedan, 2015; Breitburg et al., 2018; Diaz and Rosenberg, 2008; Keeling and Garcia, 2002; Keeling et al., 2010; Long et al., 2016). Eutrophic waters, from both natural and anthropogenic influences, supply an abundance of nutrients that can fuel algal and phytoplankton blooms within coastal waters. When left un-grazed, these primary producers sink below the pycnocline where microbes feed upon the organisms, depleting oxygen through microbial respiration and decomposition (Rabalais et al., 2010). DO availability also shifts with changing water temperatures; warmer waters have a lower capacity to hold DO therefore reducing the overall concentration of DO. Furthermore, rising ocean temperatures can also increase stratification of the water column. This can stimulate the shoaling of the oxygen minimum zone (OMZ) drawing suboxic and anoxic water (low oxygen and oxygen deficient, respectively) closer to the continental shelf (Grantham et al., 2004). To date, DO concentrations have decreased by 2% globally since 1960 (Schmidtko et al., 2017), and it is predicted that the oceans will continue to deoxygenate, reducing total DO availability by an additional 1-7% by the year 2100 (Keeling et al., 2010; Long et al., 2016). Some locations, including along the West Coast of the U.S., are predicted to see even greater declines in DO in the future.

The California Current System (CCS) is a highly productive environment with relatively high biodiversity. The CCS is a dynamic environment that is seasonally subjected to bouts of hypoxic waters during the upwelling season (April – September) (Connolly et al., 2010; Walter et al., 2014) when DO concentrations can drop low as 3 mg L^{-1} (Bakun and Nelson, 1991; Mattiasen et al., 2020). Intense hypoxic events in nearshore habitats off the coast of Oregon have persisted for weeks or months and have led to mass die offs of fishes and invertebrates (Chan et al., 2008a; Grantham et al.,

2004). Global climate change is predicted to expand the upwelling season and cause upwelling events to occur more frequently, resulting in more hypoxic water intrusions. Marine fishes residing in the nearshore ecosystems that are exposed to hypoxic conditions may be faced with increased physiological challenges (Grantham et al., 2004).

Hypoxic intrusions into the nearshore habitat can be problematic to fish populations as most fishes rely on aerobic metabolism. Fishes can respond to hypoxic conditions through mechanisms to increase oxygen supply (e.g., increased ventilation or oxygen carrying capacity in the blood) or decrease oxygen demand (e.g., decreased activity, growth rates, and reproduction). Previous work demonstrates a variety of these strategies are employed when fishes encounter low oxygen conditions, including decreased growth (Pichavant et al., 2001), reduced swimming ability and predator avoidance (Domenici et al., 2007a), lower reproductive output (Wu et al., 2003), and decreased aerobic scope (Richards, 2009). At the molecular level, reductions in metabolic activity are achieved by reducing expression of genes and proteins related to energy intensive processes like Na+/K+-ATPase activity, protein synthesis, and ATP production (Richards, 2009). Over evolutionary timescales, some fishes have developed adaptations for survival under long-term hypoxia, e.g., such as aerial respiration, adaptive remodeling of gill tissue, or the conversion of lactate to ethanol (Kramer and McClure, 1982; Richards, 2011; Shoubridge and Hochachka, 1980; Sollid and Nilsson, 2006). Recent evidence also indicates the potential for transgenerational plasticity in fishes, such that a long-term exposure to hypoxia (> 2 weeks) in the parental generation of zebrafish can increase offspring tolerance to hypoxia (Ho and Burggren, 2012). In this sense, phenotypic responses employed by parental generations may influence progeny phenotypes, potentially preparing them to deal with current stressors during embryonic development (Petitjean et al., 2019).

Knowing how marine species respond to decreased oxygen availability is central to understanding how it will fare under climate change. To address this question, it is important to understand how hypoxia exposure affects different life history stages. It is thought that the early life history stages of fishes are likely to be more vulnerable to hypoxia because of under-developed regulatory systems (Ishimatsu et al., 2008; Melzner et al., 2009; Rombough, 2007). However, studying larval physiology can be a difficult

task, considering their small size. To circumvent the challenges of doing traditional physiological studies on small larvae, examination of molecular-level phenotypes can provide insight into how stress levels affect larval physiology. Transcriptomics, or the study of genome wide changes in gene expression, is a powerful tool that can be used to investigate the underlying mechanisms that drive physiological responses (Connon et al., 2018). With this technique, researchers can examine how environmental hypoxia may alter gene expression patterns in larval fishes.

Gene expression can change relatively rapidly when organisms are exposed to a new environment. Gracey et al. (2001) saw gene expression changes in adult goby fish (*Gillichthys mirabilis*) after only eight hours of hypoxic exposure, where changes in muscle tissue occurred to likely reduce energy expenditure. Cline et al. (2020) also observed relatively rapid gene expression changes in juvenile rockfishes (*Sebastes spp.*) after 12 hours of a hypoxic exposure. The juvenile rockfish continued to experience changes in gene expression across time, with changes occurring after a two-week exposure to hypoxia. These two studies show that changes in gene expression can occur over acute and longer-term timescales. At birth, rockfish larvae are exposed to a novel environment outside of their mother and will, therefore, likely respond to the new environment by altering gene expression. When this change is also accompanied by a direct exposure to a hypoxia, larvae may elicit even more changes in gene expression to combat the stressful conditions.

Rockfish are an economically important group of fishes that are targeted by both recreational and commercial fisheries along the California coast. In addition, rockfish are ecologically important as predators and by serving as prey resource to multiple organisms throughout their life. Gopher rockfish, (*Sebastes carnatus*), inhabit nearshore rocky reef areas as adults and tend to inhabit rocky crevices during the daytime. Gopher rockfish are matrotrophic viviparous fishes and carry their embryos during embryogenesis; their gestational period is between 30-50 days (Boehlert and Yoklavich, 1984; Love et al., 2002; MacFarlane and Bowers, 1995). As rockfish mothers can provide nutrients to their young during development, they may also be able to provide higher levels of oxygen during gestation and additional maternal influences to increase larval resilience to environmental stressors. For example, two previous studies found that maternal

influences can provide several rockfish species, including blue rockfish (*Sebastes mystinus*) and gopher rockfish (*Sebastes carnatus*), with increased oil globule sizes (Berkeley et al., 2004; Sogard et al., 2008), which would provide larvae with increased energy stores. Additionally, adult rockfish may be able to regulate their internal chemistry through various physiological and behavioral means. Therefore, mother rockfish may be able to provision higher oxygen levels to their larvae during gestation, despite being in oxygen poor conditions. Alternatively, as oxygen demands are high during gestation (Boehlert et al., 1991), mother rockfishes may not be able to extract enough oxygen to compensate fully for their metabolic needs, resulting in increased hypoxic stress for their larvae.

Parturition occurs from January to July in gopher rockfish, with the later range coinciding with the start of the upwelling season. After parturition, many developmental changes occur in the early larval stages of rockfishes, such as fin, spine, and pigmentation development and notochord flexion (Laroche and Richardson, 1981; Nagasawa and Kobayashi, 1995; Richardson and Laroche, 1979) to prepare for life in the open ocean. Gopher rockfish have a pelagic larval duration of 1-2 months and recruit to the nearshore kelp canopy in June and July (Lenarz et al., 1991a; Love et al., 2002). These habitats experience intrusions of seasonal hypoxic waters during upwelling, exposing gopher rockfish to low levels of DO for extended periods of time (Booth et al., 2012; Mattiasen et al., 2020). Exposure to hypoxia in juvenile rockfish has been found to cause reductions in aerobic scope, increases in ventilation rates, and shifts in the expression of genes to cope with increased hypoxic stress (Cline et al., 2020; Mattiasen et al., 2020).

As rockfishes are internal fertilizers, mothers may possess the ability to protect gestating larvae from environmental hypoxia by altering their behavior and physiology. Nevertheless, rockfishes have been observed to increase oxygen uptake during gestation to offset increased metabolic needs for developing larvae (Boehlert et al., 1991) and may or may not be able to employ compensatory mechanisms to regulate oxygen levels in their internal environment for developing larvae. If mother rockfish are able to adjust their metabolic needs through behavior and uptake more oxygen from their environment, then they may be able to provide a more stable environment for their larvae during gestation. Alternatively, larval development may be affected if mothers are unable to

maintain a well-oxygenated environment for developing offspring or if forced reallocation of energy from growth and development to compensatory mechanisms affects resource allocation to larvae.

During the first few days of life post-birth, rockfish larvae rely upon oil globules for energy (Berkeley et al., 2004). The oil globule provides energy stores for a myriad of changes that occur in the early larval period, when larvae need to adjust to free-swimming life outside of their mother, including feeding and growth along with fin, spine, and skeletal development (Moser, 1967). Additionally, major gene expression changes drive early development. In zebrafish, massive gene expression changes (>20,000 genes of 32,312 total genes) occur between time of hatch and one-week post-hatch (Yang et al. 2013). In response to environmental stress, larval fish may need to alter energy allocation to genes involved in compensatory, stress, or response mechanisms, potentially slowing growth and development.

The goal of this experiment was to determine if and how hypoxic exposure during gestation and early larval stages affects gopher rockfish physiology. Specifically, I was interested to test whether rockfish are able to protect or "buffer" developing larvae from environmental hypoxia. If rockfish are able to buffer their offspring from the external environment, I expected to see few differences in gene expression between larvae at birth that gestated in different conditions. After birth, I expected to see gene expression differences driven by larval environment rather than gestational environmental. Alternatively, if mothers are not able to fully buffer larvae from environmental hypoxia during gestation, I expected larval gene expression would be driven by maternal treatment and that this effect might be amplified after continued larval exposure to hypoxia. Overall, I expected hypoxia to induce changes in genes involved the cellular stress response (CSR), metabolic processes (to reduce oxygen demand or increase oxygen supply), and in developmental processes (if hypoxia delays development). To assess these responses, I evaluated transcriptome-wide gene expression changes in larvae sampled in the normoxic and hypoxic treatments sampled immediately after parturition and after a 5-day exposure to reciprocal treatments.

Methods

Experimental Setup

Gopher rockfish were caught on hook-and-line from rocky reefs (15-30 m depth) near Monterey, CA during the middle of the reproductive season from February-March 2017. Each fish was sexed and, if female, was checked for pregnancy stage by extracting eggs from the ovary using a soft catheter. I collected adult females at stage II pregnancy (i.e., those that have mated and stored sperm, holding fully developed, but non-fertilized eggs). The fish were transported to the National Marine Fisheries Service (NMFS) lab in Santa Cruz, CA for subsequent experiments. Gopher rockfish were initially held in a large holding tank and allowed to acclimate to laboratory conditions for one week. After this period, fish were individually tagged using a Passive Integrated Transponder (P.I.T) tag. Newly tagged fish were randomly assigned to a control (8.0 mg O_2/L) or low DO treatment (4.0 mg O_2/L) and transferred to 110-gallon flow-through sea water tanks containing control water with two fish per tank and two replicates per treatment, fed with different source water (n=4 females per treatment). Control seawater in the tanks was slowly changed to achieve the desired treatment levels over a 3-4-hour period. The low DO treatment used in this experiment was based on current DO levels observed in Monterey Bay, CA (Booth et al., 2012), including near future predicted sublethal low DO levels representing hypoxic water intrusions. Currently, during an upwelling event, DO levels can drop as low as 4.0 mg/L (Booth et al., 2012; Mattiasen et al., 2020) and these events are expected to occur more frequently and can produce even more hypoxic waters (Patti et al., 2010; Sydeman et al., 2014).

Adult females remained in their respective treatment waters through all stages of embryonic development, including egg fertilization, gestation, and larval release. Periodically (every 3-7 d), a soft catheter was used to extract eggs from the ovaries of each fish to assess the timing of fertilization. Following fertilization, eggs were extracted with a soft catheter (Berkeley et al., 2004) from the ovaries of each female on a weekly basis and examined to assess the developmental progress of the embryos and to predict the date of parturition. Upon parturition, a subsample of 13,000 larvae were collected to perform physiological experiments (Saksa, in prep) and RNA sequencing. Another subsample of 1,200 larvae was collected and subsequently placed into a new flow-through seawater tank with either the control or hypoxic exposure to assess physiological

and gene expression responses for five days after birth (**Fig. 1**). This was done to differentiate maternal treatment effects (M) at parturition from larval treatment effects (L) (after larvae were directly exposed to the treatments). Samples collected at birth will be referred to as either $_{M}$Control or $_{M}$Hypoxia; samples collected after the larval exposure will be referred to using both the maternal and larval treatment: $_{M}$Control_ $_{L}$Control, $_{M}$Control_ $_{L}$Hypoxia, $_{M}$Hypoxia $_{L}$Hypoxia or $_{M}$Hypoxia $_{L}$Control).

Larval Collection

Whole larvae were pooled (~66 larvae) for gene expression sample analysis to achieve a mass of ~15 mg. Pooled larvae also allowed for a more representative sample of each brood for sequencing. To account for natural die offs of deformed larvae, only visually healthy larvae were collected for transcriptomic work. Pooled larval samples were placed into a 1.5 mL cryotube, removing as much seawater as possible with a disposable plastic transfer pipette. A line was then drawn on the tube to indicate the volume of larvae needed to achieve ~15 mg of tissue. This tube was used as a guide to standardize the volume of larvae collected for each sample. After each parturition event, larvae were counted and collected into a single water source and concentrated by pouring the larvae over a sieve. Larvae were then carefully pipetted into each cryotube, taking care to not damage larvae. Pooled samples were flash frozen in liquid nitrogen and stored at -80°C.

Total RNA Extraction

Total RNA was extracted from 12 pooled larval samples. At birth (time point 1), there were a total of 4 pooled samples (2 gestational treatments x 2 mothers=4). After five days (time point 2), there were an additional eight pooled samples (2 gestational treatments x 2 mothers x 2 larval treatments=8). RNA extractions were performed using a Qiagen RNeasy Mini Plus Kit (Cat. No. 47134). RNA quality was assessed using 1% agarose gel electrophoresis, a Nanodrop 2000 Spectrophotometer, and an Advanced Analytical® Fragment Analyzer™. RNA yields were quantified using the Qubit® RNA Broad Range Assay kit (catalog number Q10210). The 1% agarose gel was used to assess RNA degradation and the Nanodrop Spectrophotometer was used to assess RNA purity.

Only samples with RNA Quality Numbers (RQN) > 8 were used for cDNA library preparation. One microgram (1ug) of total RNA from each pooled larval sample was used for mRNA isolation and subsequent complementary DNA (cDNA) library preparation.

mRNA Isolation and cDNA Synthesis

Messenger RNA (mRNA) from each sample was isolated and reverse transcribed to create complementary DNA (cDNA) libraries following the protocol provided by NEBNext in the Ultra II Directional RNA Library Prep Kit for Illumina® (#E7760L, Lot: 0021703). Adapter indices from the NEBNext® Multiplex Oligos for Illumina® kit were used to ligate unique indices to each sample for multiplexing. After the double stranded cDNA was synthesized and indexed, I used Aline PCR Clean™ DX (Cat. No. C-1003, Lot No. 161229) beads to purify cDNA. The cDNA libraries were amplified using PCR to increase the yield of cDNA for sequencing using 13 cycles. I quantified cDNA libraries using a Qubit and validated library size using an Advanced Analytical® Fragment Analyzer™ with the High Sensitivity Large Fragment Analysis Kit (Advanced Analytical®, catalog number DNF-493-0500). After the samples were assessed for quality, they were sent to the Vincent J. Coates Lab at UC Berkeley for qPCR quantification and randomly assigned and loaded in equal amounts on to either a 150 bp paired end (PE) or a 100 bp single end (SE) lane HiSeq 4000 sequencing lane.

Read processing, de novo Assembly, and Annotation

Raw fastq reads were processed with Trimmomatic (version 0.36) using parameter recommendations from MacManes (2014) (phred33, MINLEN:25, SLIDINGWINDOW:4:2, LEADING:2, TRAILING:2) to remove short reads, poor-quality reads, and the adapter indices (Bolger et al., 2014; MacManes, 2014). Trinity (version 2.4.0, default parameters, including in silico normalization, with the argument –SS_lib_type RF) (Grabherr et al., 2011) was used to assemble a *de novo* reference transcriptome using the 150 bp PE reads from two of the pooled larval gopher rockfish samples. One sample from this experiment (a day-5 larval sample that gestated in control exposure then had a subsequent hypoxic larval exposure) and one sample from a concurrent experiment (larvae sampled at the day of parturition in a low pH treatment)

were used to create the *de novo* transcriptome. These samples were chosen to maximize the expression of transcripts across treatments and time points in this experiment and a concurrent experiment on gopher rockfish larvae. I assessed the quality of assembly using the built-in trinity stats program (TrinityStats.pl version 2.4.0) and BUSCO (Benchmarking Universal Single-Copy Orthologs) (version 2.0.1) cross-referenced against the Actinopterygii (ray-finned fishes) database (Simao et al., 2015). I annotated the transcriptome assembly using DIAMOND (version 0.9.24.125) (Buchfink et al., 2014) against the UniProtKB/Swiss-Prot database (Suzek et al., 2015).

Differential Gene Expression Quantification

Samples sequenced at 150 bp PE reads for the *de novo* transcriptome were trimmed to 100 bp to match the read length of the other samples used in the differential gene expression analysis. I used the R1 single end reads from those samples for downstream analysis. QC reads were aligned to the larval gopher rockfish *de novo* transcriptome using the program Bowtie (version 1.2.2) (Langmead, 2010). I used RSEM (version 1.3.0) (Li and Dewey, 2011) to estimate relative gene abundance, including transcripts per million transcripts (TPM) values. For each pairwise comparison in the analyses I used edgeR (version 3.32.0) (Robinson et al., 2010) to determine the relative differential gene expression. EdgeR is designed to analyze replicated count-based expression data and uses a negative binomial distribution to model gene counts (Robinson and Smyth, 2007; Robinson and Smyth, 2008). It is designed to detect differences in expression between two or more groups (Robinson et al., 2010) and is robust to small sample sizes (Cole et al., 2016).

I used the edgeR program to identify differentially expressed genes for each pairwise comparison within the following four analyses (described below). EdgeR compares the relative expression of each gene to the mean expression value of that gene expressed across all samples within the comparison. Differentially expressed genes were selected using a p-value for false-discovery rate of 0.05; no fold change cut-off was used.

Analysis 1&2: Maternal and larval treatment effects on gene expression

To test whether the maternal or larval environment had a stronger effect on larval gene expression patterns in gopher rockfish larvae, I examined DGE patterns when all samples were included in the same comparison. For this first analysis, I included larval samples taken at birth in each treatment (representing the maternal exposure), and after the 5-day larval exposure in either the same or reciprocal treatment as the maternal exposure (representing the larval exposure).

To test how the maternal environment influenced the response to the larval environment, my second analysis compared larvae only after the 5-day larval exposure for larvae that either gestated in the same treatment but were then exposed to different treatments (e.g., $_{M}$Control$__{L}$Control vs $_{M}$Control$__{L}$Hypoxia) (**Table 3**) or larvae that gestated in different treatments and were exposed to the same treatment for the 5-day larval exposure (e.g., $_{M}$Control$__{L}$Control vs $_{M}$Hypoxia$__{L}$Control).

Analysis 3: Treatment effects at each time point

To test how gene expression responses differed between time of birth and after a 5-day larval exposure, my third analysis consisted of treatment comparisons at each time point: a) larvae sampled at birth that were exposed to different maternal treatments (maternal control exposure vs maternal hypoxia exposure) and b) larvae after the 5-day larval exposure that remained in the same treatment that they were in for the maternal exposure (maternal and larval control exposure vs maternal and larval hypoxia exposure).

Analysis 4: Treatment effects over time in the same or reciprocal treatment

To test how early larval development is affected by a hypoxic exposure during the maternal and/or larval exposure, my fourth analysis had two comparisons: a) larvae within the same treatment but across time (e.g., comparing larvae that experienced control conditions during the maternal exposure with larvae that experienced control conditions during both maternal and the larval exposure), and b) larvae that gestated in one treatment but were then transferred to the other treatment for the 5-day larval exposure (e.g., comparing larvae that experienced control conditions during the maternal exposure with larvae that experienced control conditions during the maternal exposure but were placed into hypoxia for the larval exposure).

For all analyses, heatmaps were used to visualize the edgeR (version 3.32.0) differential expression data and were produced with the ggplot2 package in R where columns (samples) and rows (genes) were hierarchically clustered by gene expression similarity in R using the package *stats* version 4.0.2 (R Team, 2018). Heatmaps were used to examine gene expression trends for each analysis. Box figures (e.g., **Fig. 3**) were created with information derived from lists of differentially expressed genes created from the edgeR program and used to represent the number of differentially expressed genes between each pairwise comparison in each analysis. These general steps were used to identify and visualize differentially expressed gene lists.

To identify gene ontology categories within gene lists of interest, I used the UniProt website Retrieve/ID mapping tool to translate the SwissprotID names associated with each annotated gene into gene names. I then used PANTHER (version 15.0) (Mi et al., 2017) to identify the biological processes of the genes. The list of differentially expressed genes was input into PANTHER with *Danio rerio* (zebrafish) as the selected organism, and the functional classifications of the list of genes was outputted for zebrafish. I then used PANTHER to identify the percentage of genes within each child category of biological processes. I used ShinyGO (version 0.61, Ensmbl release 96, Ensmbl Metazoa release 43) (Ge et al., 2019) on the larger gene lists to run a gene enrichment analysis, using zebrafish (*Danio rerio*) as the reference species with a P-value cutoff (FDR) of 0.05 to identify the biological processes of genes that were overrepresented. Gene lists that yielded fewer than 100 annotated genes were not used in comparative measures within the analyses as I was unable to have high confidence in gene ontology function/patterns with the short lists. Therefore, pairwise comparisons that produced small gene lists were manually analyzed for expression patterns with some accompanying gene ontology information, and not compared to other treatments.

Results

Fish Husbandry and Larval Collections

No adult fish mortality was observed within the experiment. However, of the four adult fish originally placed into each treatment, only two fish per treatment fertilized

eggs, gestated, and released larvae. Thus, I only used larvae from two adult females per treatment. The fish that gave birth ranged in size from 250 mm FL to 293 mm FL at time of birth; post-birth weights ranging from 249 g to 700.5 g (**Table 2**). Adult fish were exposed to the treatment conditions for 41-61 days, depending on time of fertilization and parturition.

RNA Sequencing and De Novo Transcriptome Assembly

Single end 100 bp samples ranged from 13-18 million reads. Paired end 150 bp samples ranged from 27-46 million reads before trimming to 100 bp. The larval gopher rockfish Trinity *de novo* transcriptome assembly contains 158,008 total contigs ("genes") and 275,950 transcripts ("isoforms"). The N_{50} value for the transcripts was 2,009 bp, a median contig length of 435 bp, and an average contig length of 981.91 (**Table 1**). The assembly contained 89.3% of the Actinopterygii BUSCOs (43.6% single-copy orthologs; 45.7% duplicated orthologs). Samples mapped to the transcriptome assembly using RSEM (version 1.3.0) ranged from 79.58-83.30% reads. The transcriptome as annotated against the UniProtKB/Swiss-Prot database and returned an annotation rate of 35%.

Effects of Maternal Environment on Larval Gene Expression Patterns

Gene expression in larvae was strongly influenced by the maternal treatment. For my first analysis, I compared larvae sampled in both treatments at time of birth ($_{M}$Control and $_{M}$Hypoxia) and after the subsequent 5-day larval exposure to the same ($_{M}$Control_$_{L}$Control and $_{M}$Hypoxia_$_{L}$Hypoxia) or different ($_{M}$Control_$_{L}$Hypoxia and $_{M}$Hypoxia_$_{L}$Control) treatment. I found that larval gene expression patterns clustered based on their maternal treatment, regardless of sampling time point or subsequent larval exposure (**Fig. 2**). In this comparison, I identified 109 differentially expressed genes (DEGs) between larvae from the control and low DO treatments and across both time points where 24 genes were upregulated and 85 genes downregulated (false discovery rate, FDR = 0.05). I also observed variation in expression patterns between maternal replicates within the same treatment. For example, larvae that gestated in the control treatment had slightly different expression patterns between replicate mothers where a suite of 13 genes were upregulated in one replicate and downregulated in the other.

To determine the influence of the maternal environment on larval gene expression five days after birth in either the same environment as gestation or after experiencing a new environment, my second analysis compared larvae sampled only after the larval exposure. For this analysis, I ran multiple pairwise comparisons at day 5 (**Fig. 3**). This second analysis a) compared larvae that had the same maternal exposure, but experienced different larval environments ($_{M}$Control_$_{L}$Control vs $_{M}$Control_$_{L}$Hypoxia or $_{M}$Hypoxia_$_{L}$Control vs $_{M}$Hypoxia_$_{L}$Hypoxia) and b) compared larvae that had differing maternal environments, but the same larval environment ($_{M}$Control_$_{L}$Hypoxia vs $_{M}$Hypoxia_$_{L}$Hypoxia or $_{M}$Control_$_{L}$Control vs $_{M}$Hypoxia_$_{L}$Control).

I found that exposure to hypoxia either during gestation or during the first 5 days of the larval stage resulted in few DEGs between larval samples. Larvae that gestated in control but experienced different larval treatments ($_{M}$Control_$_{L}$Control vs $_{M}$Control_$_{L}$Hypoxia) had 29 DEGs between the two sample groups with 1 gene upregulated and 28 genes downregulated in the hypoxic treatment (**Fig. 3A**). Larvae gestated in hypoxia and sampled after direct larval exposure to hypoxia or control conditions for 5 days ($_{M}$Hypoxia_$_{L}$Control vs $_{M}$Hypoxia_$_{L}$Hypoxia), had only 1 DEG between the two larval treatment groups, which was upregulated in the hypoxic treatment (**Fig. 3B**).

An exposure to hypoxia during the larval environment also reduced gene expression variance in gopher rockfish larvae. Larvae that gestated in either treatment but were subsequently placed into the control treatment for the 5-day larval exposure ($_{M}$Control_$_{L}$Control vs $_{M}$Hypoxia_$_{L}$Control) had 1446 DEGs between the two maternal groups with 587 genes upregulated and 859 gene downregulated from the hypoxia treatment larvae (**Fig. 3C**). Of the 1446 DEGs, there were many genes enriched that were related to developmental and metabolic processes as well as response to stress (**Table S1**). There were 23 genes related to development, including functions of anatomical structure development, brain development, nervous system development, and axon guidance (**Table S2**). Larvae that gestated in either treatment but were placed into the hypoxic treatment for a 5-day larval exposure ($_{M}$Control_$_{L}$Hypoxia vs $_{M}$Hypoxia_$_{L}$Hypoxia) differentially expressed fewer genes with only 32 DEGs between the gestational treatments, 15 of which were upregulated in larvae that gestated in

hypoxia and 17 that were upregulated in larvae that gestated in control conditions (**Fig. 3D**).

Maternal Influence Amplified Over Time

To determine the effects of hypoxia at each time point, my third analysis first compared larvae between control and hypoxia at parturition (maternal environment), then compared larvae between control and hypoxia after the 5-day larval exposure (larval environment). To investigate how the maternal environment influenced larval gene expression over time, I examined the relative number of genes that were differentially expressed between the treatments at each time point. I found that, at the time of parturition, larvae differentially fewer genes than larvae sampled after the 5-day larval exposure. Larvae from the two maternal treatments sampled directly after parturition differed in the expression of 31 genes (14 upregulated;17 downregulated in the hypoxic treatment) (**Fig. 4A**). Larvae sampled at 5-days post-parturition, after exposure to the same maternal treatment, differed in the expression of 430 genes, with 247 upregulated and 183 downregulated in the hypoxic treatment (**Fig. 4B**). There were 10 genes shared between the day 1 pairwise comparison and the day 5 pairwise comparison, one of which were annotated; an Interferon-induced very large GTPase 1 responsible for GTP binding. Of the 430 DEGs between control and hypoxia at day 5 ($_{M}$Control$_{}_{L}$Control vs $_{M}$Hypoxia$_{}_{L}$Hypoxia), there were 132 annotated DEGs that had functional classification hits in biological processes, including 39 genes related to metabolic processes (**Fig. 5**). Furthermore, there were also 7 genes related to muscle fiber development, muscle contraction, and muscle differentiation (**Table S3**).

To determine how the maternal exposure influenced larval responses, the first part of my fourth analysis compared samples within the same treatment, but across time points. I found that larvae differentially expressed fewer genes across time within the hypoxic treatment ($_{M}$Hypoxia vs $_{M}$Hypoxia$_{}_{L}$Hypoxia) compared to the control treatment ($_{M}$Control vs $_{M}$Control$_{}_{L}$Control). In the control treatment, I observed 8262 DEGs between parturition and the 5-day larval exposure (4416 upregulated ;3846 downregulated). In the hypoxic treatment, I observed 3987 DEGs between parturition and the 5-day direct exposure (1911 genes upregulated; 2076 genes downregulated). There

are 3209 (1887 annotated) genes shared between the two treatments with 5053 (2357 annotated) genes unique to the control treatment and 778 (343 annotated) genes uniqueto the hypoxic treatment. Figure 6 shows the percentage of shared genes that were involved in functional classifications of biological processes. There were 11 developmental genes (5 upregulated, 7 downregulated) at day 5 unique to the hypoxic treatment across time (**Table S4**). Of genes unique to the hypoxic treatment, a gene enrichment analysis showed an overrepresentation (p-value = 0.037) of mitochondrial transporter family (SLC25) genes involved in the TCA cycle (tricarboxylic acid cycle), particularly genes involved in Oxaloacetate and Malate transport within the mitochondrial matrix (**Table S5**). Enriched genes unique to larvae in the control treatment differed in function and included genes related to multiple metabolic processes (cellular nitrogen, organic cyclic compound, heterocycle, cellular aromatic compound, small molecule, oxoacid carboxylic acid, organic acid, and Nucleobase-containing compound), developmental processes (animal organ, anatomical structure, multicellular organism, and system), biosynthetic processes (organic substance and cellular biosynthetic), and oxidation-reduction process. (**Table S6**).

The second part of my fourth analysis examined how the maternal treatment influenced larvae across time when they experienced a reciprocal treatment to their maternal exposure. When larvae gestated in normoxic conditions were subsequently transferred into a hypoxic environment (i.e., $_{M}$Control vs $_{M}$Control_$_{L}$Hypoxia), I identified 7180 DEGs between parturition and the 5-day direct exposure (3306 upregulated; 3874 downregulated). Larvae that gestated in hypoxic conditions and were then placed in normoxic conditions differentially expressed 4680 genes between parturition and the 5-day direct exposure (2166 upregulated; 2514 downregulated relative to the day 5 normoxic exposure). I compared the gene lists between the four across-time pairwise comparisons and found that there were 2561 DEGs in common. These shared genes were differentially expressed across time in all four comparisons and are likely needed for larval developmental processes, regardless of treatment exposure.

Discussion

In this study I assessed gene expression responses in gopher rockfish when exposed to hypoxia during early life stages. Adult mothers were exposed to either hypoxia ($_{M}$Hypoxia) or control ($_{M}$Control) conditions followed by reciprocal exposure of larvae to the same or different treatments for the first five days after birth. I expected gene expression would be similar at birth if the mothers are able to buffer their larvae from environmental hypoxia. Furthermore, I predicted that larval DGE patterns after birth would be driven by larval treatment ($_{L}$) rather than the maternal treatment ($_{M}$) as changes in gene expression would happen during the larval exposure if mothers were able to buffer their larvae. Interestingly, these results suggest that larval DGE patterns are heavily influenced by the maternal environment, and this effect remains regardless of larval exposure post-birth. After continued exposure to hypoxia after birth ($_{M}$Hypoxia_$_{L}$Hypoxia), these larvae differentially expressed genes related to metabolic processes (**Fig. 5**) and developmental processes (including anatomical structure development, morphology, and formation). I also found high differential expression between day 1 and day 5, which is likely a result of early developmental changes. Finally, exposure to hypoxia during gestation is associated with less differential gene expression across time, indicating a potential delay in early larval development.

Maternal Influence on Larval Gene Expression Patterns

If maternal environment affects larval physiology, I expected any gene expression differences at birth to be driven by maternal treatment. I compared larvae sampled at parturition (maternal treatment) and after a 5-day post-parturition direct exposure to hypoxia or control conditions (larval treatment) (**Fig 2**). I found that even after direct larval exposure to a different treatment for five days, larval DGE profiles grouped by maternal environment rather than larval environment. This suggests that the maternal environment influences larval physiology at birth and for at least five days after birth. On one hand, this maternal effect could be beneficial, e.g., if it results in a stress hardened larval phenotype more tolerant of a hypoxic environment (Ho and Burggren, 2012). On the other hand, this could be harmful, e.g., if it results in developmental delays or deformities upon birth (Shang and Wu, 2004).

I also found that larvae which gestated in hypoxic conditions produced almost three times the number of deformities, compared to those that gestated in control conditions, with the prevailing type consisting of spinal deformities (Saksa, in prep). As I only sampled live and non-deformed larvae, I was unable to capture any of the genes that caused the deformities in the hypoxic treatment. However, in larvae that were sampled at the 5-day time point between the hypoxia and normoxia ($_{M}$Control$_{_L}$Control vs $_{M}$Hypoxia$_{_L}$Hypoxia), I did observe differentially expressed genes related to muscle fiber development, muscle contraction, and muscle differentiation (**Table S3**), which could lead to deformities to arise in larvae as they continue aging in hypoxic conditions (Hassell et al., 2008). In zebrafish eggs, a short-term (24hr) direct hypoxic exposure from 24-48 hours post-fertilization was found to significantly alter gene expression, but when returned to normoxic conditions for 5 hours, gene expression reverted to normal expression levels (Ton et al., 2003). Here I found that the environment which the mother is exposed to during fertilization and gestation (~1 month of hypoxic exposure), heavily influences larval gene expression, regardless of subsequent larval exposure to normoxic or hypoxic conditions, at least for a period of 5 days. This pattern holds true regardless of sampling time point, suggesting that gopher rockfish gestational environment is critical in influencing larvae after parturition.

The sustained differences in gene expression after five days suggests that maternal exposure might induce developmental plasticity in gopher rockfish (Ho and Burggren, 2012). This has been demonstrated in European seabass (*Dicentrarchus labrax*) where a hypoxic exposure during larval stages yielded prolonged physiological effects, though it did not increase hypoxia tolerance (Vanderplancke et al., 2015). Future work could evaluate whether hypoxia induced gene expression increases hypoxia tolerance over longer timescales or, alternatively, if gene expression patterns return to normal levels over a longer normoxic period. Although my data point to a clear effect of the maternal environment, an experiment that tracks longer-term effects on gene expression and survival would be needed to determine whether these effects are fixed for life and if they adaptive. Potential signs of developmental delay suggest that the effect may be detrimental, as its delayed development might lead to higher larval mortality.

Inter-individual differences among mothers is known to affect larval quality and condition among rockfish, including gopher rockfish (Sogard et al., 2008). I found variability in gene expression patterns between larval broods from mothers within the same treatment. Within the control maternal treatment, an entire suite of genes (13 of 109 genes) was upregulated in larvae from one replicate mother that were down regulated in larvae from the other mother (**Fig. 2**), though I was unable to annotate this suite of genes. Research has also shown that differences between mothers can have a large impact on larval survivability in rockfish, where some mothers provision more energy to larvae, supplying them with larger oil globules and increasing larval growth rates (Berkeley et al., 2004). In this experiment, only two of the mothers were age validated so, unfortunately, I was unable to confidently attribute differences in gene expression between mothers to age differences. However, it is interesting to note that a difference in maternal traits could account for differences in the response to hypoxia. A higher variation in responses to hypoxia could increase adaptive potential in fishes by providing more phenotypes for selection to act upon. Thus, inter-individual variability between mothers may also affect the ability of larvae to respond to hypoxic conditions.

Maternal Influences Amplified Over Time

Exposure to hypoxia may have altered developmentally related gene expression. Larvae sampled directly after birth ($_{M}$Control vs $_{M}$Hypoxia) differentially expressed fewer genes (31 genes) than larvae sampled after a 5-day larval exposure (430 genes) to the same treatment ($_{M}$Control$_{_L}$Control vs $_{M}$Hypoxia$_{_L}$Hypoxia) (**Fig. 4 A&B**). The increase in DEGs seen in the 5-day comparison would then be attributed to hypoxia affecting genes related to early development. This could also explain the large number of DEGs observed between the two sampling time points within each treatment, where thousands of genes changed expression profiles between parturition and 5-days post birth. Furthermore, larvae that gestated in the hypoxic treatment differentially expressed fewer genes between sampling time points than larvae that gestated in the control treatment, regardless of subsequent larval exposure. Fewer DEGs between two samples means the samples are more similar to each other, suggesting that there are fewer developmental processes occurring in the hypoxic treatment (*expanded further in this section*).

Hypoxia also induced a change in the types of genes that larvae expressed. For example, I found an increase in DEGs related to metabolic pathways (**Fig. 5**) in larvae that gestated in hypoxia and remained in the same treatment for the 5-day direct exposure ($_{M}$Hypoxia_$_{L}$Hypoxia). This increase in genes related to metabolic functions is likely due to the need to respond to the hypoxic stress in the environment outside of the mother. During gestation, the larvae may have had a lessened hypoxic stress as the mother may have been able to provision enough oxygen for her larvae during development. Fishes can employ tactics to increase oxygen uptake or reduce metabolic demands, including increasing ventilation rates, altering gill morphology to increase surface area for oxygen uptake, or even decrease overall activity to conserve energy (Mattiasen et al., 2020; Randall, 1982; Richards, 2009; Richards, 2011; Sollid and Nilsson, 2006; Sollid et al., 2003). However, the change in DEGs related to metabolic pathways in larvae that experienced the hypoxic environment shows that larvae are making changes to their metabolism to respond to the hypoxic stressor. These effects are likely carrying over from the hypoxic exposure during the maternal treatment, indicating that rockfish are not able to buffer their larvae from environmental hypoxia.

The effect of the maternal environment on larval gene expression was amplified over time. For example, at day 5, 1446 genes were differentially expressed between larvae exposed only to control conditions after birth but from different maternal treatments ($_{M}$Control_$_{L}$Control vs $_{M}$Hypoxia_$_{L}$Control). Among the 1446 DEGs, 23 were related to development including functions of anatomical structure development, brain development, nervous system development, and axon guidance (**Table S6**). Additionally, a gene enrichment analysis showed an increased proportion of differentially expressed genes related to multiple metabolic processes, developmental processes, and response to stress, which is unexpected as the larvae were in the 5-day normoxic exposure and not experiencing a hypoxic stress (**Table S5**). These DEGs show that there is a lasting effect of gestating in a hypoxic environment for at least the first five days after birth, affecting both development and metabolic processes. In model systems, gene expression data has been used to examine developmental delay in response to stress. Fan et al. (2010) used a subset of genes as developmental markers to examine how zebrafish changed the timing of expression in response to a neurotoxicant. One of the genes, gap43 (neuromodulin),

was also differentially expressed in the 1446 DEG list ($_{M}$Control_$_{L}$Control vs $_{M}$Hypoxia_$_{L}$Control) and was upregulated in larvae that gestated in hypoxia. Neuromodulin is expressed at high levels during zebrafish development and axonal regeneration. Both marine fish and invertebrate species have been shown to change development due to hypoxic exposure during early life history stages (Cancino et al., 2003; Chan et al., 2008b; Hassell et al., 2008; Shang and Wu, 2004; Ton et al., 2003). For example, if hypoxic exposure during gestation and early larval stages delays development, developmental DGE patterns might be expected later in the hypoxic group. Developmental delay due to a hypoxic exposure during gestation has been observed in black bream eggs resulting in delayed time to hatch, decreased survivorship, and increased deformities (Hassell et al., 2008). We observed similar responses in gopher rockfish larvae, with a trend of increased deformities and decreased survival in the hypoxic treatment (Saksa et al., in prep). If gopher rockfish are regularly exposed to hypoxia during gestation and early larval stages, these detrimental effects could result in reduced species abundance.

Our results also suggest that hypoxic exposure during gestation affects larval development more than the larval exposure. Larvae that gestated in hypoxic conditions differentially expressed fewer genes between birth and day five, regardless of larval treatment, than larvae that gestated in normoxic conditions. Larvae that gestated and remained in the hypoxic environment decreased regulation of genes related to varying morphological growth processes (BRINP1, Slitrk2, ihhb, ism1, tbxta, Col7a1, CHAD) (**Table S4**). Additionally, larvae that gestated in hypoxia and then experienced the 5-day larval exposure in control conditions exhibited fewer DGEs between parturition and day 5 than larvae that gestated in normoxia and experienced a treatment change (**Fig. 3**).

Gestational exposure to hypoxia reduces gene expression variability in larvae

The maternal environment continued to drive gene expression responses, even when larvae were exposed to a reciprocal treatment. Larvae that gestated in control conditions then either remained in control or were transferred to hypoxia ($_{M}$Control_$_{L}$Control vs $_{M}$Control_$_{L}$Hypoxia) had 29 DEGs between the larval groups. In contrast, larvae that gestated in hypoxia then either remained in hypoxia or were

transferred to control ($_{M}$Hypoxia_$_{L}$Hypoxia vs $_{M}$Hypoxia_$_{L}$Control) only had 1 DEG between the larval groups (**Fig. 3**). This low number of DEGs in larvae that gestated in hypoxia could be due to reduced phenotypic variation under stress, whereby interindividual variation is reduced when individuals display a consistent physiological response (Oleksiak and Crawford, 2012). An alternate explanation could be that larvae that gestated in hypoxia could have experienced a selection event, whereby larvae with a more adaptive hypoxia response had higher survival. *Fundulus heteroclitus* (mummichongs) embryos and larvae, when exposed to environmental-contaminant exposures, were shown to have "unnatural" selection events, selecting for embryos and larvae that are resistant to short-term toxic effects (Nacci et al., 1999). This could be done by examining changes in SNP variants between larval samples across time. Exposure to another low pH stress during gestation has shown to change allele frequencies for certain genes in larval stages of purple sea urchins (*Strongylocentrotus purpuratus*), selecting for individuals that had improved performance under the stressor (Pespeni et al., 2013).

Gopher rockfish larvae that gestate in hypoxia experience latent responses to hypoxia after birth. Larvae that gestated in separate conditions then were exposed to normoxic conditions after birth differentially expressed far more genes between each other (1446 genes) than larvae exposed to hypoxic conditions after birth (32 genes) (**Fig. 3C&D**). This large number of DEGs could be resultant of latent maternal influences in larvae that gestated in the hypoxic treatment, such as delayed development and phenotypic preparedness for hypoxia. The marine gastropod *Crepipatella dilatate* experienced a similar latent effect from a hypoxic exposure during embryonic development that compromised juvenile growth and development for 30 days in normoxic water (Segura et al., 2014). Of the 1446 DEGs, there were many genes enriched that were related to developmental and metabolic processes, which is unexpected as the larvae were in the 5-day normoxic exposure and not experiencing a hypoxic stress (**Table S1**). This lagged response could be attributed to a latent response to hypoxia, affecting development and growth rates in after a prolonged embryonic exposure, similar to what has been observed in marine gastropods (Li and Chiu, 2013; Segura et al., 2014). This could be due to the need to respond to the hypoxic environment

in a specific manner, causing a reduction in the number of genes that are affected between the sets of larvae that gestated in different treatments.

Limitations in the Project

Despite the robust signal of maternal environment in our gene expression dataset, some aspects of our experimental design and statistical analysis may have limited our power to detect differences among treatments. First, as only two mothers per treatment gave birth, I only have two replicates and therefore may be missing information on how these species as a whole may be responding to hypoxia. As I observed inter-individual differences in some of the gene expression patterns in the gopher rockfish, I may have picked up more variability with increased sample size. However, edgeR is known to be more robust and reliable with error rate control with low replicates in the analysis (Robinson et al., 2010). Second, I used the classic edgeR method to identify differentially expressed genes within multiple pairwise comparisons. This approach, however, has some drawbacks as it is not able to tease apart the potential interacting effects of the maternal treatment on the larval treatment that a generalized linear model approach could identify. Because of this, I may be missing some key information on how the larvae are responding after the five-day larval exposure. Additionally, due to the statistical design, running pairwise comparisons may have a higher false-positive rate due to lower sample numbers within the analysis itself.

The construction of our de novo assembly could have also limited or biased our analysis. Ideally, the transcriptome would have contained samples all treatments to capture the highest diversity of genes expressed in the larvae. However, as each sample contained pooled larvae, the number of individuals in the transcriptome was high and created a transcriptome was too large to conduct downstream analyses computationally on our server (high number of contigs with low size). Creating at transcriptome with two samples provided normal levels of contigs with an N50 value within the range we would expect to see for a successful transcriptome assembly.

How gopher rockfish will fare under future hypoxic conditions

To date, most studies on the effects of a hypoxic exposure to larval fishes have focused on oviparous fishes, however, little research has been conducted on matrotrophic viviparous fishes, like rockfish. In this experiment, larvae potentially experienced a delay in development which could be a result of a reallocation of energy from development to metabolism (Wendelaar Bonga, 1997). Furthermore, larvae that gestated in hypoxic conditions showed similar expression profiles, independent of their secondary exposure to either hypoxia or normoxia. This continued maternal effect could be fixing certain phenotypes, even when larvae are no longer experiencing hypoxic conditions, and alter molecular pathways during the larval phase. In situ, however, oxygen levels are fluctuating and mothers with developing larvae will experience a hypoxic intrusion between a few hours to a week at most in central California, however, further north along the Oregon coast upwelling events can last over a month (Booth et al., 2012; Grantham et al., 2004). Acute hypoxic exposures during gestations are less likely to be problematic for developing larvae as development may be arrested in the short term like what was observed in zebrafish (Ton et al., 2003). An extended exposure, as seen in this study, could have lasting effects into the early larval period. Further research could focus on replicating realistic upwelling exposures to gestating mothers along with extending the secondary larval exposure to further tease out how long these maternal effects last in the larval phase.

CHAPTER 2

BLUE ROCKFISH

Introduction

Rising carbon emissions are increasing atmospheric carbon dioxide (CO_2) concentrations. This atmospheric CO_2 is partially absorbed by the oceans and dissociates into bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and hydrogen protons (H^+). Increased concentrations of free-floating hydrogen protons decrease ocean pH, a phenomenon known as ocean acidification, which also disrupts other aspects of carbonate chemistry. Already, the average pH of the ocean has decreased by 0.1 pH units (Caldeira and Wickett, 2003), and if emissions are left unchecked, models suggest that pH levels could decrease by another 0.4 units by the end of this century (Orr et al., 2005). Within the California Current System (CCS), pH levels are likely to decrease by 0.2 pH by the year 2050 (Gruber et al., 2012), creating more physiologically stressful environments for marine organisms within the next thirty years.

Changes in ocean chemistry can negatively affect marine organisms. Fishes were initially thought to be more tolerant to changes in pH than invertebrates due to their high capacity for acid-base regulation (Heuer and Grosell, 2014; Kroeker et al., 2010). However, exposure to acidic waters has been shown to cause hypercapnia in some fishes, causing downstream physiological consequences (Heuer and Grosell, 2014). At the molecular level, changes in internal pH can affect enzyme conformation including enzyme substrate binding sites, and hemoglobin-oxygen binding (Benesch et al., 1969; Grasso et al., 2015). Exposure to more acidic water has been also shown to negatively affect fishes at the behavioral, physiological, and molecular level (Altieri and Gedan, 2015; Hamilton et al., 2017; Lucon-Xiccato et al., 2014). Prolonged exposure to low pH water in teleost fishes can lead to changes in brain function (behavioral lateralization) (Domenici et al., 2012; Hamilton et al., 2017), limit aerobic activity (Hamilton et al., 2017; Munday et al., 2009a), impair olfactory abilities (Munday et al., 2009b), and cause a downregulation of carbonic anhydrase, an important enzyme related to acid-base regulation (Esbaugh et al., 2012).

As discussed in Chapter 1, the marine environment is also undergoing deoxygenation and creating more hypoxic intrusions within the CCS. Dissolved Oxygen (DO) concentrations in the ocean have already decreased by 2% since 1960 (Schmidtko et al., 2017). Due to climate change, the oceans are likely to continue to experience deoxygenation, reducing DO availability by 1-7% globally by the year 2100 (Keeling et al., 2010; Long et al., 2016). Reduced oxygen availability also has negative effects on marine fishes. Fishes, like all aerobic animals, need oxygen for aerobic cellular respiration, which is the most common oxidizing agent in the Krebs cycle. With less oxygen available, fishes may employ compensatory mechanisms to reduce oxygen demand, like reducing physical and metabolic activity, or through an increasing oxygen supply, e.g., by increasing water flow over the gills to increase oxygen uptake (Wu, 2002). Studies have shown that exposure to low levels of DO can affect fishes in a multitude of ways, including inhibiting growth (Pichavant et al., 2001), swimming ability and predator avoidance (Domenici et al., 2007b), reproductive output (Wu et al., 2003), and the capacity for aerobic activity (Richards, 2009).

The CCS is an excellent study system to test how these climate change stressors will affect local species. This highly dynamic environment is seasonally subjected to bouts of low pH and hypoxic waters during the upwelling season (April-September) (Connolly et al., 2010; Feely et al., 2008; Walter et al., 2014). During upwelling events, nearshore waters experience periods of coinciding low pH and hypoxic water intrusions (Booth et al., 2012; Feely et al., 2008) lasting for hours, days, or even weeks (Booth et al., 2012; Leary et al., 2017; Walter et al., 2014). Current pH levels in Central California range from an average of 8.0 pH in surface waters to 7.8 pH at 100 m depth (Hauri et al., 2013), however, in nearshore waters pH levels can drop as low as 7.5 pH (Hamilton et al., 2017). With the influence of anthropogenic ocean acidification, projected levels of mean ocean pH are expected to reach 7.4 by the year 2100 (Orr et al., 2005). Currently, during an upwelling event, pH levels can reach as low as 7.44 in nearshore waters and DO levels can drop to 4.0 mg/L (Booth et al., 2012). Dissolved oxygen levels have even dropped below 3.0 mg/L for short durations in Carmel Bay, CA (Mattiasen et al., 2020). Intense hypoxic events in nearshore habitats of the CCS off the coast of Oregon have persisted for weeks or months and have led to mass die offs of rockfishes (Chan et al.,

2008a; Grantham et al., 2004). These upwelling events are predicted to become more intense and increase in frequency and duration with global climate change, creating longer upwelling seasons, with more frequent and stronger events (Patti et al., 2010; Snyder et al., 2003; Sydeman et al., 2014). As multiple environmental stressors like ocean acidification and hypoxia intensify in this ecosystem, it is important to test how combined stressors affect marine organisms.

Combined stressor effects can be classified as either additive, synergistic, or antagonistic. Responses are considered additive when the combined response is equal to the sum of both independent stressors. Synergistic responses occur when the combined response is amplified and is greater than the sum of the independent stressors. Antagonistic responses occur when the combined response is diminished and less than the sum of the individual stressors. Two recent studies found that the combined effects of low pH and low DO had an additive effect in marine fish and squid. Gobler and Baumann (2016) found that these stressors negatively impacted larval development of inland silversides, Atlantic silversides, and sheepshead minnows, while Navarro et al. (2016) measured a decrease in embryonic growth and development of market squid (*Doryteuthis opalescens*) when exposed to combined low pH/low DO. It has also been found that the co-occurring stressors of ocean acidification and hypoxia can have a synergistic effect. DePasquale et al. (2015) found that these co-occurring stressors had an additive effect, leading to decreased larval survival of *Menidia beryllina* but an increased negative response on *M. menidia* larval survival, indicating a synergistic effect. These studies suggest that, when co-occurring, ocean acidification and hypoxia have the potential to reduce development, growth, and metabolic performance of the early life history stages of multiple organisms, including those found along the CCS.

Rockfishes are a diverse family (Sebastidae) with high diversity (represented by over 70 species) in the CCS along the North American Pacific coast (Love et al., 2002). Adaptation to specific habitats may affect the resilience of these fishes to the stressors of low pH and low DO. For example, rockfishes living in deeper waters are already experiencing low levels of pH and DO, so those species may be more tolerant to further reductions in pH and DO (Bjorkstedt et al., 2002; Lenarz et al., 1991b; Love et al., 2002). Rockfishes living more nearshore, like in kelp forest ecosystems, may be less

physiologically plastic to further decreases in pH and DO as they have not been experiencing these levels as much in their recent evolutionary history (Davis et al., 2018; Hamilton et al., 2017; Mattiasen et al., 2020; Cline et al. 2020).

Recent work has examined the effects of low pH and low DO, both as independent and combined stressors, on rockfishes. Hamilton et al. (2017) found that juvenile blue rockfish and copper rockfish responded differently to low pH exposure, with blue rockfish exhibiting higher resilience to the stressor. Mattiasen et al. (2020) found that juvenile blue rockfish were also more resilient to hypoxia than juvenile copper rockfish. Davis et al. (2018) found that juvenile rockfishes in the KGB-C (kelp, gopher, black and yellow, and copper) complex had a synergistic response to the combined stressors of low pH and low DO, however, after three weeks of exposure, physiological and behavioral changes were moderately compensated for. Finally, Cline et al. (2020) suggested that juvenile blue rockfish are likely resilient to combined low pH and low DO stressors. These studies have all focused on the juvenile stages of rockfish and indicate that blue rockfish are more resilient to these stressors than rockfishes in the KGB-C complex. In an effort to gain a more comprehensive understanding on how the early life history of blue rockfish respond to these stressors, I examined how these stressors affect reproduction and early larval stages of rockfish.

Adult blue rockfish (*S. mystinus*) inhabit the mid-water column in kelp forests and seek shelter among the rocky reef at night. Their parturition season ranges from October to March, and peaks in December. Larval blue rockfish spend 3-5 months in a pelagic larval/juvenile stage, and late-stage larvae and pelagic juveniles occupy deeper depths in the water column than then gopher rockfish described in Chapter 1. Recruitment of blue rockfish to nearshore rocky reefs and kelp forests occurs during the upwelling season between April and June (Lenarz et al., 1991a; Love et al., 2002). As blue rockfish reside deeper in the water column during their pelagic larval stage, they may already possess some resilience to more acidic and hypoxic conditions if blue rockfish larvae have adapted or acclimatized to those environmental conditions. Furthermore, rockfish larvae are oftentimes concentrated around upwelling fronts, where they would repeatedly experience varying levels of pH and DO (Bjorkstedt et al., 2002).

Rockfishes are viviparous reproducers with internal fertilization, embryogenesis, and egg hatching, leading to free swimming larvae at parturition (birth) (Boehlert and Yoklavich, 1984; Love et al., 2002; MacFarlane and Bowers, 1995). As rockfish mothers can provide nutrients to their young during development, they may also be able to provide additional maternal influences to increase larval resilience to environmental stressors. For example, two previous studies found that maternal influences can provide black rockfish (*Sebastes melanops*), blue rockfish (*Sebastes mystinus*), gopher rockfish (*Sebastes carnatus*), and yellowtail rockfish (*Sebastes flavidus*), with increased oil globule sizes (Berkeley et al., 2004; Sogard et al., 2008), which would provide larvae with increased energy stores. Larval resilience to environmental stressors could also occur through buffering capacity of the mother (physiological plasticity of the mother), through larval response to the stressors (developmental plasticity), or through the maternal environment interacting with the larval environment to determine larval phenotype (transgenerational plasticity) (Donelson et al., 2011; 2018). These mechanisms may provide rockfish with options on how to respond to climate change induced environmental stress by offering acclimatory responses for larvae.

Alternatively, the need to respond to environmental stressors could affect larval development by a forced reallocation of energy from growth and development to compensatory mechanisms. During the first five days of life, rockfish larvae are mainly relying upon their oil globules for energy (Berkeley et al., 2004). The oil globule provides energy stores for a myriad of changes that occur in the early larval period, when larvae need to adjust to free-swimming life outside of their mother, including feeding and growth along with fin, spine, and skeletal development (Moser, 1967). Additionally, there are many changes in gene expression that occur during the early larval phase. Yang et al. (2013) identified genes that were preferentially expressed at different developmental stages in zebrafish, with the highest proportion between time of hatch and after a week in the early larval stage (2905/4288 genes expressed between 64 cell stage and early larval stage). In the same study, zebrafish at the one-week post-hatch stage also had the highest number of expressed genes between all the stages (>20,000 of 32,312 total genes). To respond to environmental stress, larval fish may need to alter energy allocation to genes

involved in compensatory, stress, or response mechanisms, rather than to growth and development, potentially slowing development.

Changes in larval physiology in response to climate change stressors can be hard to detect, given the small size and delicate nature of larvae. Molecular techniques can be used to observe changes on a scale that may otherwise be missed by traditional physiological studies. Transcriptomics, the study of genome wide changes in gene expression, allows researchers to identify shifts in gene expression when organisms are exposed to different environments (Connon et al., 2018). To assess the molecular physiological changes in blue rockfish in response to low pH and hypoxia, this study used next generation sequencing (RNAseq) to examine changes in gene expression in the early life stages of blue rockfish while exploring how gene expression differs between the time of birth and during the early larval period. This was done by assessing if there are any maternal influences on larval gene expression patterns by sampling larvae: a) immediately after parturition ($_{M}$) when mother rockfish were exposed to low pH and/or low DO during larval gestation, and b) after a five-day direct exposure ($_{L}$) to low pH and/or low DO following parturition. Furthermore, to determine the effects of a similar or different larval environment, I sampled larvae that remained in the same treatment during maternal gestation and early larval development (e.g., $_{M}$Control_$_{L}$Control) (**Table 5**) and larvae that had differing maternal gestation and early larval exposures (e.g., $_{M}$Control_$_{L}$Low pH).

In this Chapter, I aimed to determine how low pH alone and low pH and low DO as combined stressors affect the early life history stage of blue rockfish. I hypothesized that (1) environmental stress might delay development. If the adult female rockfish are not able to buffer their larvae then the larvae will likely need to respond to the stressor by reallocating energy to compensatory mechanisms. This change of energy allocation could shift energy away from growth and development. This hypothesis would be supported if larval rockfish differentially express fewer genes between day 1 and day 5 when exposed to stress versus control. I also expect (2) larval gene expression patterns would be influenced by the maternal ($_{M}$) exposure. As seen in Chapter 1, gopher rockfish larval gene expression patterns were driven by the maternal exposure at birth and after five days. Even though the blue rockfish are experiencing different stressors, I expect this

pattern would remain the same. To assess these responses, I evaluated transcriptome-wide gene expression changes in larvae sampled in the normoxic, low pH and low pH/hypoxic combined treatments, sampled both directly after parturition and after a 5-day larval exposure.

Methods

Experimental Setup

Blue rockfish were caught using hook-and-line fishing techniques near Monterey, CA in December of 2017 in the middle of their reproductive season. I collected adult females at stage II pregnancy (i.e., fish have mated, stored sperm, have fully developed eggs, but have not yet fertilized their eggs). After collections, the fish were transported to the National Marine Fisheries Services (NMFS) lab in Santa Cruz, CA, and placed into holding tanks for one week to act as an acclimation period and reduce stress of capture and handling. After the acclimation period, the fish were tagged with Passive Integrated Transponder (P.I.T.) tags to identify individuals within the tank, then randomly assigned to a treatment. The fish were then transferred to 110-gallon flow-through sea water tanks in control water with two fish in each tank. A replicate tank fed from a different water source was placed adjacent to each treatment with two additional fish. Tank water was brought to the respective treatment level over the course of four hours.

The treatments used in the experiment are based on current pH and DO levels in Monterey Bay, CA (Booth et al., 2012; Hamilton et al., 2017; Mattiasen et al., 2020), including near future predicted levels of pH (Gruber et al., 2012; Orr et al., 2005), sublethal low DO levels representing hypoxic water intrusions (Keeling and Garcia, 2002; Keeling et al., 2010; Long et al., 2016), and a treatment with both stressors co-occurring (expected conditions during future upwelling events). Based on this information, the treatments used in this experiment were: 1) control (~8.0 pH and ~8.0 mg O_2/L), 2) low pH (7.5 pH, ~8.0 mg O_2/L), and 3) a co-occurring stressor treatment (7.5 pH, 4.0 mg O_2/L). Originally, I had a single stressor low DO treatment, however, only one mother gave birth in that treatment and I was unable to use the data due to lack of statistical power.

The fish remained in the tanks for the duration of the experiment, allowing fertilization, gestation, and larval release to occur within each respective treatment. The fish were monitored for their gestation time by using a soft catheter (Berkeley et al., 2004) to extract eggs from the ovaries of the fishes every 3-7 days. The eggs were photographed and examined to determine their developmental stage, which was used to predict parturition dates for each of the broods. After parturition, subsamples of larvae were collected for physiological experiments, morphometrics (Saksa, in prep), enzyme activity assays, and RNA sequencing (see methods below). Another subsample of live, healthy larvae was collected and subsequently placed into larval holding tanks containing each treatment for a 5-day direct larval exposure (**Fig. 1**). These larvae were then used in additional physiological and gene expression experiments.

Larval Collections

Each sample of larvae was whole-pooled (~80 larvae) to achieve a mass of ~15 mg, the mass required for RNA extractions. Using pooled larvae also allows for a more representative sample of each brood for RNA sequencing, though it prevents us from examining individual larval transcriptomes. To account for natural die offs of deformed larvae, only visually healthy larvae were collected for transcriptomic work. The whole-pooled larvae were placed into a 1.5 mL cryotube, siphoning off as much water as possible using a disposable plastic transfer pipette. A line was then drawn on the tube to indicate the approximate volume of larvae needed to achieve ~15 mg of tissue. This tube was used as a guide to standardize the volume of larvae collected for each sample. After each parturition, larvae were counted (for fecundity analysis), collected into a single water source, then concentrated by pouring the larvae over a sieve. The larvae were then carefully pipetted into each cryotube, taking care to not damage any of the larvae. The samples were immediately flash frozen in liquid nitrogen to prevent the degradation of RNA in each sample. Samples collected at birth will be referred to by their maternal treatment: $_{M}$Control, $_{M}$Low pH or $_{M}$Combined. Samples collected after the larval exposure will be referred to using the maternal and larval treatment for each combination (e.g., $_{M}$Control_ $_{M}$Control, $_{M}$Control_$_{L}$Low pH, $_{M}$Control_$_{L}$Combined Stressor, etc.)

Total RNA Extraction

Total RNA was extracted from a total of 20 whole pooled larval samples using a Qiagen RNeasy Mini Plus Kit (Cat. No. 47134). RNA quality was assessed using agarose gel electrophoresis, a Nanodrop 2000 Spectrophotometer, and an Advanced Analytical® Fragment Analyzer™. RNA yields were quantified using the Qubit® RNA Broad Range Assay kit (catalog number Q10210). A 1% agarose gel was used to assess RNA degradation and to ensure that the 18S and 28S ribosomal subunit bands were clear. A Nanodrop Spectrophotometer was used to assess RNA purity (no DNA or protein contamination) by examining sample fluorescence at the 230 nm, 260 nm, and 280 nm wavelengths. The Nanodrop Spectrophotometer was also used to assess a rough estimation of the concentration of RNA in the sample. The Advanced Analytical® Fragment Analyzer™ used capillary electrophoresis to assess degradation of RNA, determine RNA fragment sizes, and provide an RNA Quality Number that is a metric of overall quality of the sample. A Qubit Flourometer was used to accurately determine the concentration of RNA in the sample using dyes that bind to RNA and fluoresce after they are bound to their target. One microgram of total RNA from each pooled larval sample was used for mRNA isolation and subsequent complementary DNA (cDNA) library preparation.

mRNA Isolation and cDNA Synthesis

Messenger RNA (mRNA) from each sample was isolated and reverse transcribed to create complementary DNA (cDNA) libraries following the protocol provided by NEBNext in the Ultra II Directional RNA Library Prep Kit for Illumina® (#E7760L, Lot: 0021703). Adapter indices from the NEBNext® Multiplex Oligos for Illumina® kit were used to bind unique indices to each sample to bioinformatically distinguish samples after sequencing. After the double stranded cDNA was synthesized and indexed, I used Aline PCR Clean™ DX (Cat. No. C-1003, Lot No. 161229) beads with a magnetic stand to purify the cDNA of any contaminants, primer dimers, or adapter dimers which can reduce the overall yield of informative sequencing reads. The samples were then amplified using PCR to increase the yield of cDNA for sequencing. The samples underwent 13 PCR cycles to decrease the overamplification of replicated cDNA. I

validated cDNA libraries by using Qubit, Nanodrop, and an Advanced Analytical® Fragment Analyzer™ with the High Sensitivity Large Fragment Analysis Kit (Advanced Analytical®, catalog number DNF-493-0500). After the samples were checked for quality, they were sent to Novogene in Sacramento, CA. The twenty experimental samples were pooled with four samples from a sister experiment, then sequenced in a single lane on an Illumina HiSeq X Platform for sequencing at 150bp paired end (PE) reads.

Read processing, de novo Assembly and Annotation

I used Trimmomatic (version 0.36) to remove short reads, poor-quality reads, and the adapter indices from the fastq read files using parameters (phred33, MINLEN:25, SLIDINGWINDOW:4:2, LEADING:2, TRAILING:2) from MacManes (2014) and Bolger et al. (2014). Trinity (version 2.4.0, default parameters, including in silico normalization, with the argument –SS_lib_type RF) was used to assemble a *de novo* reference transcriptome, with the default parameters including normalization, using the 150bp PE reads from two of the larval blue rockfish samples (Grabherr et al., 2011; Haas et al., 2013). The samples included larvae exposed to a range of stressors over time to capture stress-responsive transcripts within the reference assembly. The first was sampled at the day of parturition after maternal exposure to low pH treatment. The second was from larvae that gestated in control conditions and exposed to hypoxia for five days after birth. I assessed the quality of assembly using the built-in Trinity stats program (TrinityStats.pl version 2.4.0) and BUSCO (Benchmarking Universal Single-Copy Orthologs) (version 2.0.1) cross-referenced against the Actinopterygii (ray-finned fishes) database (Simao et al., 2015)I annotated the transcriptome assembly using DIAMOND (version0.9.24.125) (Buchfink et al., 2014) against the UniProtKB/Swiss-Prot database (Suzek et al., 2015). I used the human curated UniProtKB/Swiss-Prot database to provide higher confidence in the annotations.

Differential Gene Expression Quantification

QC reads were aligned to the larval blue rockfish *de novo* transcriptome using Bowtie (version 1.2.2) (Langmead, 2010) and RSEM (version 1.3.1) was used to estimate

relative gene abundance, and normalized to transcripts per million (TPM) values to account for differences in sequencing depth among samples (Li and Dewey, 2011). EdgeR (version 3.32.0) was used to determine the relative differential gene expression in each of the analyses below (Robinson et al., 2010). EdgeR is designed to analyze replicated count-based expression data and uses a negative binomial distribution to model gene counts (Robinson and Smyth, 2007; 2008). The program compares relative expression of each gene to the mean expression value of that gene expressed across all samples within the comparison. I chose edgeR because it has been shown to detect differences in expression between two or more groups (Robinson et al., 2010) and is robust to small sample sizes (Cole et al., 2016).

Analysis 1: Treatment effects over time in the same or reciprocal treatment

I performed three analyses to determine how larval blue rockfish gene expression varied by maternal treatment, larval treatment, and sampling time point. To test my first hypothesis, I wanted to determine how early larval development of blue rockfish was affected by an exposure to a stressor over time. Here, I compared larvae sampled at birth with larvae sampled after the 5-day larval exposure for each treatment: a) larvae within the same treatment but across time (e.g., $_{M}$Control vs $_{M}$Control$_{_L}$Control). Then, to determine the influence of the maternal treatment on larval development across time, I b) sampled larvae that gestated in one treatment but were exposed to a different treatment for the 5-day larval exposure (e.g., $_{M}$Control vs $_{M}$Control$_{_L}$Combined Stressor).

Analysis 2&3: Maternal and larval treatment effects on gene expression

To test my second hypothesis, I performed two analyses to examine how the maternal environment influenced the response to the same or different larval environment. For analysis two, I compared larvae only after the 5-day larval exposure. I compared larvae that gestated in the same treatment then were exposed to different treatments for the 5-day larval exposure (e.g., $_{M}$Control$_{_L}$Control vs $_{M}$Control$_{_L}$Low pH). To test whether the maternal or larval environment had a stronger effect on larval gene expression patterns in gopher rockfish larvae I examined DGE patterns when all samples were included in the same comparison. For my third analysis, I included larval samples

taken at birth in each treatment (representing the maternal exposure), and after the 5-day larval exposure in either the same or reciprocal treatment as the maternal exposure (representing the larval exposure). In other words, I ran an analysis with all datapoints to determine if there was a defining factor that influenced larval gene expression.

Visualization of Gene Expression Data

For each of the three analyses described above, differentially expressed genes were selected using a p-value for false-discovery rate of 0.05 with no fold change cut-off. Heatmaps were used to visualize the edgeR (version 3.32.0) expression data and were produced with the ggplot2 package in R where columns (samples) and rows (genes) were hierarchically clustered by gene expression similarity in R using the R package *stats* version 4.0.2 (R Team, 2018). Heatmaps were used to examine gene expression trends between pairwise comparisons within and between analyses. Lists of differentially expressed genes generated from edgeR (version 3.32.0) were used to compare expression patterns and trends within each analysis. These general steps were used to identify and visualize the differentially expressed genes in each of the three analyses.

After identifying lists of differentially expressed genes for each analysis, I performed a gene ontology analysis to determine the functions of the differentially expressed genes. First, I used the UniProt Retrieve/ID mapping tool to translate the SwissprotID names associated with each annotated gene into gene names. I then input gene names into PANTHER (version 15.0) (Mi et al., 2017) to identify the biological processes of the genes using *Danio rerio* (zebrafish) as the selected organism. I used *D. rerio* as the selected organism as rockfish do not have a published genome to reference against and *D. rerio* is a well-studied teleost fish. This allowed for more confidence in gene functions rather than using other organisms (e.g., mice, humans, bacterium) where gene functions may be different. Functional classifications of the list of genes were extracted as well as the percentage of genes within each child category for Biological Process. I used ShinyGO (version 0.61, Ensmbl release 96, Ensmbl Metazoa release 43) (Ge et al., 2019)to run a gene enrichment analysis with a P-value cutoff (FDR) of 0.05 to identify the biological processes of genes that were overrepresented. I also used zebrafish (*Danio rerio*) as the reference species within Shiny GO. In-depth gene ontology analysis

was performed on pairwise comparisons that yielded over 100 annotated genes. Gene lists that yielded fewer than 100 annotated genes were not used in comparative measures within the analyses as I was unable to have high confidence in gene ontology function/patterns with the short lists.

Results

Fish Husbandry and Larval Collections

No mortality of adult female rockfish was observed within the experiment. Two mothers in the control, low pH, and combined stressor treatments gave birth to larvae, whereas only one mother in the low DO treatment gave birth. Due to lack of statistical replicates in the low DO treatment I was unable to use the data in the analysis. When placed into treatment, adult female blue rockfish ranged in size between 279 mm FL and 327 mm FL; weights were between 452.7 g and 756.7 g. They were in treatment waters anywhere between 28 – 82 days, depending on time of fertilization and gestation length (**Table 4**).

RNA Sequencing and De Novo Transcriptome Assembly

Paired end 150 bp samples ranged from 15.5-30.8 million reads. The larval blue rockfish transcriptome assembly contained 131,017 total contigs ("genes") and 231,646 transcripts ("isoforms"). The N_{50} value for the transcripts was 2,287 bp, a median contig length of 468 bp, and an average contig length of 821.72 bp. The assembly contained 91.3% of Actinopterygii BUSCOs (46.6% single-copy orthologs; 44.7% duplicated orthologs) (**Table 1**). Samples were mapped to the blue rockfish transcriptome assembly using RSEM (version 1.3.1) and ranged from 43-58%. The transcriptome annotation rate to the UniProtKB/Swiss-Prot database was 36%.

Analysis 1: Environmental effects on development

Fewer genes were differentially expressed between birth and after the 5-day larval exposure in larvae that gestated and remained in a stressor treatment compared to the control. To determine the effect of the environmental stressors on development, my first analysis examined differential gene expression over time within each treatment ($_{M}$Control

vs. $_{L}$Control, $_{M}$Low pH vs. $_{L}$Low pH, or $_{M}$Combined vs. $_{L}$Combined). In the control only treatment, I identified 2215 genes that changed in expression over the five-day period. Fewer genes changed in expression between day 1 and day 5 in the pH only treatment (1287 genes) and combined stressor only treatments (545 genes) (**Fig. 8**). Among all gene lists, 212 genes were conserved in their expression over time regardless of treatment. I also found that 834 genes were shared between the control and low pH treatments, 251 between the control and combined stressor treatment, and 303 genes shared between the low pH and combined stressor treatment (**Fig. 9**).

To determine the unique responses across time (e.g., $_{M}$Control vs $_{M}$Control$_{_L}$Control) in each treatment, I compared the gene lists with each other to determine which genes were shared between the treatments and which genes were unique to each treatment across time (**Fig. 9**). Larvae in the control treatment across time exhibited higher levels of differential gene expression than the other two stressor treatments. Between the larval and maternal exposure there were 1342 DEGs unique to the control ($_{M}$Control vs $_{M}$Control$_{_L}$Control), 362 DEGs unique to the low pH treatment ($_{M}$Low pH vs $_{M}$Low pH$_{_L}$Low pH), and 203 DEGs unique to the combined stressor treatment ($_{M}$Combined vs $_{M}$Combined$_{_L}$Combined). A gene ontology analysis revealed the biological processes of the DEGs in each unique list, and after normalizing the data to a percentage of genes expressed in each list, I was able to identify differences between treatments (**Table 6**). Across time, larvae in the control treatment differentially expressed a higher proportion of genes related to cellular processes, metabolic processes, and biological regulation compared to the stressor treatments (**Fig. 10**). A gene enrichment analysis on this list of genes revealed functional categories that were over-represented in larvae in the control treatment including multiple metabolic processes, RNA processing, gene expression, autophagy, and catabolic processes (**Table S7**).

Larvae that gestated and remained in that same environment during the early larval phase (e.g., $_{M}$Control vs $_{M}$Control$_{_L}$Control) showed unique differential gene expression patterns as a function of their maternal treatment. Between day 1 and day 5, larvae in the low pH treatment differentially expressed a higher proportion of genes related to cellular component organization, reproduction, growth, and locomotion compared to control and the combined stressor (**Fig 10**). DEGs related to development

and response to stress unique to the low pH treatment can be found in Table S8. Response to stress genes were involved in DNA repair and response to DNA damage, all of which were down regulated. Up regulated developmental process genes were involved in central nervous system and skeletal development whereas down regulated genes were involved in tissue, skeletal muscle, muscle structure, axon extension, regulation of the MAPK cascade (cell differentiation). Larvae in the combined stressor treatment between day 1 and day 5 differentially expressed a higher proportion of genes related to localization (transportation within a cell, including ion transport), developmental processes, and multicellular organismal processes (including but not limited to response to stress, behavior, development, and growth) compared to control and low pH (**Fig 10**). DEGs related to development and stress unique to the combined stressor across time can be found in Table S9. Only two response to stress genes were differentially expressed unique the combined stressor, one down regulated and the other upregulated. One developmental process gene (hoxc5a) was down regulated and was involved in regulation of transcription of DNA/RNA. Five developmental process genes were upregulated (PCK1, NR5A2, CRYBB1, crygnb, and Elf3) and were involved in tissue development, eye development, and cell differentiation. As mentioned earlier, larvae in the two stressor treatments differentially expressed fewer genes overall across time, with larvae in the low pH treatment differentially expressing about half of the number of genes as the control larvae. The larvae in the combined stressor treatment differentially expressed even fewer genes, about a quarter of the number as control.

To examine the effect of exposure to a different treatment after birth, I compared gene expression differences between birth and day 5 in larvae that gestated in one treatment then were placed into a different treatment for the 5-day larval exposure (**Fig. 8**). I found that the maternal environment influenced the magnitude of gene expression across time, where larvae that gestated in a stressor differentially expressed fewer genes across time than larvae that gestated in control conditions, even when they were returned to control conditions for the 5-day larval exposure. Larvae that gestated in control conditions and were then exposed to low pH conditions for the 5-day larval exposure ($_{M}$Control vs $_{M}$Control_$_{L}$Low pH) differentially expressed 3489 genes. Larvae that were placed into the combined stressor for the 5-day larval exposure ($_{M}$Control vs

$_{M}$Control_$_{L}$Combined) differentially expressed 3797 genes. Larvae that gestated in the low pH treatment and were then placed into the control treatment ($_{M}$Low pH vs $_{M}$Low pH_$_{L}$Control) differentially expressed 887 genes. Larvae that gestated in the combined stressor treatment then were placed into the control treatment ($_{M}$Combined vs $_{M}$Combined_$_{L}$Control) differentially expressed 408 genes.

Analysis 2 & 3: Effects of Maternal Environment on Larval Gene Expression Patterns

To determine the influence of the maternal environment on larval gene expression five days after birth in either the same environment as gestation or after experiencing a new environment my second analysis compared larvae sampled only after the larval exposure. The influence of the maternal treatment is shown by relatively few differences between larvae that gestated in one treatment but experienced different treatments for the 5-day larval exposure. Larvae that gestated in control water then experienced the control or low pH treatment for 5 days ($_{M}$Control_$_{L}$Control vs $_{M}$Control_$_{L}$Low pH) had 205 DEGs when compared to each other after the 5-day larval exposure. Larvae that gestated in the control treatment and were put into the control or combined stressor ($_{M}$Control_$_{L}$Control vs $_{M}$Control_$_{L}$Combined) treatment shared 58 DEGs between the two larval groups. Larvae that gestated in low pH then experienced either low pH or the control treatment ($_{M}$Low pH_$_{L}$Control vs $_{M}$Low pH_$_{L}$Low ph) shared 3 DEGs after the 5-day larval exposure. Larvae that gestated in the combined stressor, then experienced the control or the combined stressor treatment ($_{M}$Combined_$_{L}$Control vs $_{M}$Combined_$_{L}$Combined) for 5-days shared 0 DEGs.

Gene expression in larvae was strongly influenced by the maternal treatment. For my third analysis, I compared larvae sampled in both treatments at time of birth ($_{M}$Control, $_{M}$Low pH, and $_{M}$Combined) and after the subsequent 5-day larval exposure to the same ($_{M}$Control_$_{L}$Control, $_{M}$Low pH_$_{L}$Low pH, and $_{M}$Combined_$_{L}$Combined) or different ($_{M}$Control_$_{L}$Low pH, $_{M}$Control_$_{L}$Combined, $_{M}$Low pH_$_{L}$Control, and $_{M}$Combined_$_{L}$Control) treatment. To examine this, I compared the larval blue rockfish samples at the time of birth ($_{M}$Control, $_{M}$Low pH, and $_{M}$Combined) and after the subsequent 5-day larval exposure to the same ($_{M}$Control_$_{L}$Control, $_{M}$Low pH_$_{L}$Low pH, and $_{M}$Combined_$_{L}$Combined) or different ($_{M}$Control_$_{L}$Low pH, $_{M}$Control_$_{L}$Combined,

$_{M}$Low pH$_{_L}$Control, and $_{M}$Combined$_{_L}$Control) treatment. I found that between all treatment and time point comparisons there were 415 DEGs and that the larval gene expression patterns, when hierarchically clustered by gene expression similarity in R using the R package *stats* version 4.0.2, clustered based on their maternal treatment (**Fig 11A**), with one exception of a larval sample collected at birth in the low pH group clustering with the combined stressor samples taken at birth (**Fig. 11A**). Independent pairwise comparisons between the control treatment and each stressor over the two time points (**Fig. 11B & 11C**) showed that the samples clustered completely by maternal treatment. There were 51 DEGs in the control vs low pH comparison (**Fig. 11B**) and 231 DEGs in the control vs combined stressor comparison (**Fig. 11C**). This trend was observed in both the low pH stressor and the combined low pH/DO stressor treatments. In comparing the control to the low pH treatment, larval samples sub-clustered by replicate mothers, whereas comparing the control to the combined stressor treatment revealed that larval samples first sub-clustered by time point, then by replicate mother.

Discussion

In this study, I assessed gene expression responses of larval blue rockfish to an exposure of low pH or a combined stressor (low pH and low oxygen) treatment during early development (e.g., fertilization, embryogenesis) and the early larval period. Adult female rockfish were exposed to control conditions ($_{M}$Control), low pH ($_{M}$Low pH), or a combined stressor ($_{M}$Combined) treatment, followed by a reciprocal exposure of larvae to the same or different environment for the first five days after birth. Based on data from Chapter 1, I expected that larval gene expression patterns would be driven by the maternal environment rather than the larval environment. Furthermore, I expected to see fewer DEGs across time in the stressor treatments than in the control treatment, a possible sign of developmental delay. This might be due to reallocation of energy from growth and development to coping with environmental stress. I found that larval gene expression patterns were largely driven by the maternal environment rather than the larval environment, similar to my results from Chapter 1. In addition, I found evidence that the maternal environment influenced larval gene expression for at least the first five days after birth. Larvae that gestated in one of the two stressor treatments and remained

in the same larval environment after birth expressed fewer genes across time than those developing wholly in the control treatment, such that larvae in the combined stressor were more affected than larvae in the single pH stressor treatment. Overall, my results suggest that the maternal environment may strongly influence blue rockfish larval physiology and development.

Maternal influence on gene expression patterns

I expected that the maternal environment would have a stronger influence on DGE than the larval environment based on Chapter 1 where I found a strong maternal influence on larvae in response to hypoxia stress (**Fig. 2**). I found that larval blue rockfish gene expression profiles clustered by their maternal exposure, to either OA or a combined hypoxia and OA treatment (**Fig. 11**). Similarly, I also found that the maternal influence lingered after the five-day larval exposure. A previous study on zebrafish eggs found that developing embryos altered their gene expression when exposed to an acute hypoxic event (24 hours) during embryonic development, but were able to revert their gene expression to normal levels after being returned to normoxic water (5 hours) (Ton et al., 2003). This study, however, suggests that when blue rockfish gestate their larvae in either low pH or the combined stressor, their larvae do not revert back to "control" gene expression profiles, at least for the first 5 days after birth. I saw the same pattern in gopher rockfish, when exposed to a different stressor (hypoxia). This similar response suggests that the maternal environment is important for the early larval stages in rockfishes with differing life histories.

Our results also suggest that there are lasting maternal influences even when larvae are exposed to a different environment after parturition (e.g., $_{M}$Low pH$_{L}$Control). Prolonged exposure to a stressor during gestation could result in developmental plasticity that modifies baseline gene expression, altering energy allocation, developmental pathways, and metabolic function. Alternatively, lasting maternal effects could be a result of transgenerational plasticity, if a non-genetic inheritance occurred (e.g., an epigenetic mark). Developmental plasticity occurs when the same genotype produces multiple phenotypes that depend on the environmental conditions in which development takes place (Lafuente and Beldade, 2019). Both types of plasticity can buffer individuals from

the negative impacts of their immediate environment and can be adaptive. Although this experimental design cannot tease apart which type of plasticity may have occurred, it is likely that one or both of these mechanisms could explain the lasting effects of the maternal environment on larval gene expression.

Exposure to environmental stressors during the larval period can potentially reduce fitness and abundance. Several studies have shown that exposure to low pH, hypoxia, or both stressors during the gestational and early larval period in fish can cause downstream negative effects. Exposure to low pH conditions for 36 days post hatch in Atlantic cod (*Gadus morhua*) resulted in higher variation in gene expression within the low pH treatment than those Atlantic cod that remained in low pH conditions for 46 days, suggesting that Atlantic cod that were unable to compensate for the stress did not survive the full 46 days (Frommel et al., 2020). A hypoxic exposure during the early larval stages in European sea bass (*Dicentrarchus labrax*) was shown to negatively affect long term growth rates into the juvenile stage (Vanderplancke et al., 2015), potentially affecting future performance. Meanwhile, exposure to the combined effects of low pH and low oxygen during the egg and larval stages of *Menidia beryllina* and *M. menidia* resulted in negative effects on the survival of these two fishes (DePasquale et al., 2015). However, rockfish differ from these species because they are internal fertilizers and may be able to physically buffer or partially buffer their larvae during gestation, thereby reducing potential negative effects of exposure to environmental stressors. During gestation, mother rockfish may be able to stabilize their internal environment through their own physiological plasticity. Alternatively, the larvae may be experiencing effects of transgenerational plasticity or developmental plasticity in response to the stressors. However, the lasting influence of the maternal environment on larval gene expression patterns challenges the hypothesis that mother rockfish can buffer their larvae from environmental stressors.

Effects of the maternal environment persisted even after larvae were exposed to a new environment after birth (**Fig. 8**). For example, larvae placed in a new environment after birth differed little from larvae that were placed in the same environment as their mother. However, larvae that had a maternal control exposure, but had different larval treatments (**Fig. 8 A&C)** differentially expressed more genes between each other after

the larval exposure than larvae that had a maternal stressor exposure (**Fig. 8 B&D**). This shows that the maternal environment is continually affecting larvae for at least the first five days of life outside of their mother, indicating the importance of favorable oceanic conditions during gestation. Few DEGs in larvae that gestated in a stressor could be resultant of a prolonged effect of the maternal treatment on larvae where the larvae are experiencing reduced phenotypic variation. Reduced phenotypic variation occurs when differences between individuals are reduced due to a shared consistent physiological response (Oleksiak and Crawford, 2012). In other words, larvae that gestated in one of the stressor treatments may be altering phenotypes to respond to the stressor(s) in a similar way. By doing so, the amount of variant phenotypic expression is reduced, resulting in larvae that have more similar gene expressions.

Environmental stressors alter development

I was interested in how environmental stress would influence development during early life stages in blue rockfish. I observed a dramatic decrease in overall gene expression during early development in the stressor treatments as compared with the control treatment (**Fig. 8**), which could be a sign of developmental delay. The early larval period is comprised of a myriad of changes where larvae need to adjust to free-swimming life outside of their mother, including feeding and growth along with fin, spine, and skeletal development (Moser, 1967). During the first five days of life, rockfish larvae are mainly relying upon their oil globules for energy (Berkeley et al., 2004). However, if they need to rely on stored energy reserves to respond to external environmental stressors, larvae would then need to spend more time and energy on foraging to maintain energy levels for survival. There are many changes in gene expression that occur during the early larval phase. Yang et al. (2013) identified genes that were preferentially expressed at different developmental stages in zebrafish, with the highest proportion between time of hatch and after a week in the early larval stage (2905/4288 genes expressed between 64 cell stage and early larval stage). In this study, zebrafish at the one-week post-hatch stage also had the highest number of expressed genes between all the stages (>20,000 of 32,312 total genes). To respond to environmental stress, larval fish may need to alter energy

allocation to genes involved in compensatory, stress, or response mechanisms, rather than to growth and development, potentially slowing development.

In model systems, gene expression data has been used to look at developmental delay in response to stress. Fan et al. (2010) used a subset of genes that are used as developmental markers and examined how they changed in expression in response to a neurotoxicant. Low pH conditions have been shown to delay development in mahi mahi larvae (*Rachycentron canadum*) by up to three days during the first 14 days post hatch (Bignami et al., 2013). A developmental delay due to low pH exposure was also observed in the Japanese rice fish (*Oryzias latipes*), and was shown to have connection with a strong down-regulation of genes from major metabolic pathways (Tseng et al., 2013). A hypoxic exposure during the gestational period of black bream eggs resulted in a delayed time to hatch, decreased survivorship, and increased deformities (Hassell et al., 2008). In zebrafish, an acute hypoxic exposure during egg development caused developmental arrest at both the morphological and gene expression level (Ton et al., 2003). In this study, zebrafish embryos conserved energy during the hypoxic exposure through the down regulation of genes related to ion channel proteins, muscle contraction genes, and metabolism genes. These studies further show that an exposure to environmental stressors during the embryonic or early larval stages can have lasting effects on fish larvae by slowing development, reducing survivorship, altering metabolism, and increasing deformities. Thus, exposure to low pH and/or low oxygen conditions during gestation and the early larval period could negatively affect blue rockfish.

Some DEGs were shared between treatments, but blue rockfish also exhibited unique responses to each environment across time. Figure 9 shows the number of differentially expressed genes unique to each treatment and the number of genes shared between the treatments. As mentioned earlier, the control treatment had the most DEGs across time, and likewise, had the most DEGs unique to that exposure. These genes are likely involved in both developmental processes and response to life outside of the gestational environment when no stressors are present. In the pH and combined pH/DO stressor treatments, I observed fewer DEGs, however, there were some suites of genes that were differentially expressed in all three treatments and may be considered genes that are necessary for larval development, regardless of environmental exposure. Larvae

in the combined stressor treatment had the fewest DEGs expressed across time. When the gene lists were normalized by percentage of genes annotated to a GO term, I saw that there were a higher proportion of DEGs involved in localization (transportation within a cell, including ion transport), developmental processes, and multicellular organismal processes (including but not limited to response to stress, behavior, development, and growth) (**Fig 10**). These processes could be altered in attempt to conserve energy while these larvae are exposed to the combined stressor. Furthermore, as fewer DEGs between samples indicates the samples are more similar to each other, this suggests that the larvae that gestated and remained in a stressor treatment may have had fewer developmental changes.

Interestingly, when no stressors were present, larvae differentially expressed the fewest proportion of developmental genes, indicating an array of other genes that are normally expressed during early larval development. With more DEGs expressed overall across time in the control treatment, it seemed as though there were more developmental changes occurring within the control treatment as the samples were more different from each other. However, this was not reflected in the gene ontology analysis. Instead, there was a higher proportion of DEGs related to development in the combined stressor treatment, even though there was only ¼ of the overall genes differentially expressed. These genes were involved in eye development, cell differentiation, cartilage and tissue development, and anterior/posterior pattern specification (**Table S9**). This shows that even though there were fewer differences between the larvae, a higher proportion of the differences were developmentally related. In the combined stressor treatment, differences were observed in the combined stressor treatment due to changing the activation of the genes in response to the stressors. Alternatively, larvae in the combined stressor treatment are affected by the stressors from the maternal environment onward and are experiencing a delay in development as has been observed in other teleosts exposed to low pH or low O_2 (Bignami et al., 2013; Hassell et al., 2008; Ton et al., 2003; Tseng et al., 2013). A lower proportion of genes related to development in the control treatment could be solely due to the higher number of DEGs overall where a higher of genes were differentially expressed across time due to more developmental checkpoints reached.

Project Limitations

Despite the robust signal of maternal environment in our gene expression dataset, some aspects of our experimental design and statistical analysis may have limited our power to detect differences among treatments. First, as not enough mother rockfish gave birth in the low oxygen treatment, I was unable to tease apart the stressor specific responses in the combined stressor and classify whether or not it had an additive, antagonistic, or synergistic effect on blue rockfish larvae. Furthermore, as only two mothers per treatment gave birth, I only have two replicates and therefore may be missing information on how these species as a whole may be responding to low pH and the combined stressor of low pH/hypoxia. However, edgeR is known to be more robust and reliable with error rate control with low replicates in the analysis (Robinson et al., 2010). Second, I used the classic edgeR method to identify differentially expressed genes within multiple pairwise comparisons. This approach, however, has some drawbacks as it is not able to tease apart the potential interacting effects of the maternal treatment on the larval treatment that a generalized linear model may pick up. Because of this, some key information on how the larvae are responding after the five-day larval exposure might be missing. Additionally, due to the statistical design, pairwise comparisons might have a higher false-positive rate due to lower sample numbers within the analysis itself.

The construction of our de novo assembly could have also limited or biased our analysis.Whole-pooled larval samples made it difficult to construct a comprehensive transcriptome. The larval blue rockfish transcriptome was created with only two larval samples (one from birth in a low pH treatment, one that gestated in control then went to the hypoxic treatment for the 5-day larval exposure). Ideally, the transcriptome would have contained more samples from other treatments to capture the highest diversity of genes expressed in the larvae. However, as each sample contained pooled larvae, the number of individuals in the transcriptome was high and created a transcriptome that had a high number of contigs with low size. Creating at transcriptome with two samples provided normal levels of contigs at an N50 value we would expect to see for a successful transcriptome assembly. This may also be the cause of the wide range of mapping rates in the blue rockfish samples.

Conclusions

This study shows that the maternal environment during larval fertilization and embryogenesis has a large influence on the gene expression of blue rockfish larvae. As rockfishes are viviparous, I initially thought that blue rockfish may be able to somewhat buffer their larvae from environmental stressors, however, I found little evidence of this. Instead, I found that larval rockfish gene expression patterns are driven by the maternal exposure and subsequent larval exposure to any treatment has little effect on gene expression. I also saw that larvae which gestated in a stressor treatment had fewer differentially expressed genes across time, which could be evident of a delay in development.

As in Chapter 1, this study highlights the importance of the maternal environment during larval gestation. As blue rockfish reproductive seasons (October – March) are before the typical upwelling season (April – September) the species currently doesn't need to worry about exposure to these stressors during larval gestation. However, climate change is predicted to change upwelling events creating more intense events, increase in frequency and duration, and creating longer upwelling seasons (Patti et al., 2010; Snyder et al., 2003; Sydeman et al., 2014), which may then impede on blue rockfish reproductive seasons. These results increase our knowledge of rockfish reproduction and the importance of stable conditions during gestation.

CONCLUSIONS

Rockfish larvae undergo a myriad of changes in their early larval period after birth, adjusting to needs of living out in the ocean environment (Moser, 1967). These changes include development of fins and head spines, flexion, and pigmentation. Additionally, there are many changes in gene expression that occur during the early larval phase of fish. Yang et al. (2013) identified over 20,000 of 32,312 total genes expressed in zebrafish between early egg phase and after a week into the early larval stage with almost 3,000 genes preferentially expressed between birth and one-week post-birth. Exposure to environmental stressors during fertilization, embryogenesis, and/or the early larval period after birth could affect these normal developmental activities. This research aimed to determine if rockfish held the capacity to protect their larvae from environmental stressors. Larval resilience to these environmental stressors could occur through buffering capacity of the mother (physiological plasticity of the mother), through larval response to the stressors (developmental plasticity), or through the maternal environment interacting with the larval environment to determine larval phenotype (transgenerational plasticity) (Donelson et al., 2011; Donelson et al., 2018). This thesis found that rockfish there is a persisting effect of the maternal treatment on larvae for at least the first five days after birth.

These two studies show how influential the maternal treatment is on larval rockfish gene expression, regardless of species or stressor. Initially, it seemed as though gopher rockfish may be able to somewhat buffer their larvae from hypoxia based on the low number of differentially expressed genes (DEGs) at birth. However, lasting effects of the hypoxic maternal treatment were observed in larvae, even in those that were placed into normoxic conditions for the 5-day larval exposure. Larvae that gestated in mothers who were exposed to hypoxia had much fewer DEGs across time than those that gestated in mothers who were in normoxic waters. Blue rockfish followed the same trend, with larvae differentially expressing fewer genes across time in the stressor treatments with a stronger response seen in the combined stressor than in the single stressor alone. Fewer DEGs indicate that the samples (larval pools) are more similar to each other, suggesting that the larvae may be experiencing a delay in development when exposed to a stressor

during gestation. A developmental lag could be due to a shifting of energy from developmental processes to other maintenance processes like metabolic processes (Wendelaar Bonga, 1997) in response to the stressors.

These results show the important role of the maternal environment during gestation. Rockfishes likely are not able to buffer their larvae from environmental stressors through phenotypic plasticity of the mothers. The larvae, however, may be responding to the stressors by means of developmental plasticity or through maternal influences and transgenerational plasticity. This experimental design is unable to tease apart which mode of response may be at play as only the F_0 and F_1 generations were sampled (Donelson et al., 2018), however, with the long generational time of rockfishes, sampling a F_2 generation is not feasible. Nonetheless, I do see evidence of lasting effects on larvae when they are exposed to a stressor during fertilization and embryogenesis, which could be indicative of either response mechanism.

Our findings add to a body of research showing that rockfishes can have species specific responses to stress (Hamilton et al., 2017; Mattiasen et al., 2020) with the more sedentary species, copper rockfish (*S. caurinus*) being more affected than the more mobile species, blue rockfish. As gopher rockfish exhibit similar life history traits to copper rockfish (Love et al., 2002) it is likely that they may exhibit similar responses. In this study, blue rockfish larvae may be less affected by climate change stressors than gopher rockfish larvae due to their longer lifespans. Blue rockfish had fewer DEGs across time in the control treatment than gopher rockfish by 73% and may be reaching fewer developmental benchmarks for the stressors to enact upon. Furthermore, blue rockfish reproductive season occurs earlier in the year (October – March) than gophers (January – July) and are less likely to be exposed to reduced levels of pH and dissolved oxygen during the upwelling season. However, climate change is expected to alter the timing of upwelling season (Bakun et al., 2015; Wang et al., 2015) which could then expose blue rockfish to future upwelling intensification. As blue rockfish have not dealt with upwelling stress during gestation, they will likely be more susceptible to the stress if the upwelling season impedes on their reproductive season.

Further research should focus on additional experimental parameters. To elucidate the interactive effects of low pH and hypoxia, experiments with multiple stressor

interactions can better tease apart the individual and combined effects of the stressors. To better understand the potential for acclimatory responses within and between generations, future research can extend the larval exposures and include multiple generations into the study. Furthermore, with the addition of more species of rockfishes, researchers would better understand how these congeners will respond to future climate change scenarios. As rockfishes are long-lived and late to mature, they may not be able to readily adapt to rapidly changing ocean chemistry, especially when stressors are exacerbated by upwelling events. This study provides further evidence that the maternal environment is important in larval rockfish development. This knowledge can be used to help policy makers better understand how these economically and ecologically important species will fare in the face of climate change, providing more knowledge resources to be used to help protect at risk populations to future climate change stressors.

REFERENCES

Altieri, A. H. and Gedan, K. B. (2015). Climate change and dead zones. *Glob. Chang. Biol.* **21**, 1395–1406.

Bakun, A. and Nelson, C. S. (1991). The seasonal cycle of wind-stress curl in subtropical eastern boundary current regions. *J. Phys. Oceanogr.* **21**, 1815–1834.

Bakun, A., Black, B. A., Bograd, S. J., García-Reyes, M., Miller, A. J., Rykaczewski, R. R. and Sydeman, W. J. (2015). Anticipated Effects of Climate Change on Coastal Upwelling Ecosystems. *Curr. Clim. Chang. Reports* **1**, 85–93.

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N. and Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. *Proc. Natl. Acad. Sci.* **110**, 1387–1392.

Benesch, R. E., Benesch, R. and Yu, C. I. (1969). The Oxygenation of Hemoglobin in the Presence of 2,3-Diphosphoglycerate. Effect of Temperature, pH, Ionic Strength, and Hemoglobin Concentration. *Biochemistry* **8**, 2567–2571.

Berkeley, S. A., Chapman, C. and Sogard, S. M. (2004). Maternal age as a determinate of larval growth and survival in a marine fish, Sebastes melanops. *Ecology* **85**, 1258–1264.

Bignami, S., Sponaugle, S. and Cowen, R. K. (2013). Response to ocean acidification in larvae of a large tropical marine fish, Rachycentron canadum. *Glob. Chang. Biol.* **19**, 996–1006.

Bjorkstedt, E. P., Rosenfeld, L. K., Grantham, B. A., Shkedy, Y. and Roughgarden, J. (2002). Distributions of larval rockfishes Sebastes spp. across nearshore fronts in a coastal upwelling region. *Mar. Ecol. Prog. Ser.* **242**, 215–228.

Boehlert, G. W. and Yoklavich, M. M. (1984). Reproduction, Embryonic Energetics, and the Maternal-Fetal Relationship in the Viviparous Genus Sebastes (Pisces: Scorpaenidae). *Biol. Bull.* **167**, 354–370.

Boehlert, G. W., Kusakari, M. and Yamada, J. (1991). Oxygen consumption of gestating female Sebastes schlegeli: Estimating the reproductive costs of livebearing. *Environ. Biol. Fishes* **30**, 81–90.

Bolger, A. M., Lohse, M. and Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120.

Booth, J. A. T., McPhee-Shaw, E. E., Chua, P., Kingsley, E., Denny, M., Phillips, R., Bograd, S. J., Zeidberg, L. D. and Gilly, W. F. (2012). Natural intrusions of hypoxic, low pH water into nearshore marine environments on the California coast. *Cont. Shelf Res.* **45**, 108–115.

Bossdorf, O., Richards, C. L. and Pigliucci, M. (2008). Epigenetics for ecologists. *Ecol. Lett.* **11**, 106–115.

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., et al. (2018). Declining oxygen in the global ocean and coastal waters. *Science (80-.).* **359**,.

Buchfink, B., Xie, C. and Huson, D. H. (2014). Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **12**, 59–60.

Caldeira, K. and Wickett, M. E. (2003). Oceanography: Anthropogenic carbon and ocean pH. *Nature* **425**, 365–365.

Cancino, J. M., Gallardo, J. A. and Torres, F. A. (2003). Combined effects of

dissolved oxygen concentration and water temperature on embryonic development and larval shell secretion in the marine snail Chorus giganteus (gastropoda: Muricidae). *Mar. Biol.* **142**, 133–139.

Chan, F., Barth, J. A., Lubchenco, J., Kirincich, A., Weeks, H., Peterson, W. T. and Menge, B. A. (2008a). Emergence of Anoxia in the California Current Large Marine Ecosystem. *Science (80-.).* **319**, 920–920.

Chan, H. Y., Xu, W. Z., Shin, P. K. S. and Cheung, S. G. (2008b). Prolonged exposure to low dissolved oxygen affects early development and swimming behaviour in the gastropod Nassarius festivus (Nassariidae). *Mar. Biol.* **153**, 735–743.

Cline, A. J., Hamilton, S. L. and Logan, C. A. (2020). Effects of multiple climate change stressors on gene expression in blue rockfish (Sebastes mystinus). *Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol.* **239**, 110580.

Cole, C., Schurch, N. J., Schofield, P., Gierlin, M., Sherstnev, A., Singh, V., Wrobel, N., Gharbi, K., Simpson, G. G., Owen-hughes, T. O. M., et al. (2016). How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use ? *RNA Soc.* **22**, 1–13.

Connolly, T. P., Hickey, B. M., Geier, S. L. and Cochlan, W. P. (2010). Processes influencing seasonal hypoxia in the northern California Current System. **70**, 646–656.

Connon, R. E., Jeffries, K. M., Komoroske, L. M., Todgham, A. E. and Fangue, N. A. (2018). The utility of transcriptomics in fish conservation. *J. Exp. Biol.* **221**, jeb148833.

Davis, B. E., Komoroske, L. M., Hansen, M. J., Poletto, J. B., Perry, E. N., Miller, N. A., Ehlman, S. M., Wheeler, S. G., Sih, A., Todgham, A. E., et al. (2018). Juvenile rock fish show resilience to CO 2 - acidification and hypoxia across multiple biological scales. **6**, 1–19.

DePasquale, E., Baumann, H. and Gobler, C. J. (2015). Vulnerability of early life stage Northwest Atlantic forage fish to ocean acidification and low oxygen. *Mar. Ecol. Prog. Ser.* **523**, 145–156.

Diaz, R. J. and Rosenberg, R. (2008). Spreading Consequences Dead Zones and Consequences for Marine Ecosystems. *Science (80-.).* **321**, 926–929.

Domenici, P., Claireaux, G. and McKenzie, D. . (2007a). Environmental constraints upon locomotion and predator-prey interactions in aquatic organisms: an introduction. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 1929–1936.

Domenici, P., Lefrançois, C. and Shingles, A. (2007b). Hypoxia and the antipredator behaviours of fishes. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 2105–2121.

Domenici, P., Allan, B., McCormick, M. I. and Munday, P. L. (2012). Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. *Biol. Lett.* **8**, 78–81.

Donelson, J. M., Munday, P. L., Mccormick, M. I. and Nilsson, G. E. (2011). Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. *Glob. Chang. Biol.* **17**, 1712–1719.

Donelson, J. M., Salinas, S., Munday, P. L. and Shama, L. N. S. (2018). Transgenerational plasticity and climate change experiments: Where do we go from here? *Glob. Chang. Biol.* **24**, 13–34.

Esbaugh, A. J., Heuer, R. and Grosell, M. (2012). Impacts of ocean acidification on

respiratory gas exchange and acid-base balance in a marine teleost, Opsanus beta. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **182**, 921–934.

Fan, C. Y., Cowden, J., Simmons, S. O., Padilla, S. and Ramabhadran, R. (2010). Gene expression changes in developing zebrafish as potential markers for rapid developmental neurotoxicity screening. *Neurotoxicol. Teratol.* **32**, 91–98.

Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D. and Hales, B. (2008). Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science (80-.).* **320**, 1490–1492.

Frommel, A. Y., Hermann, B. T., Michael, K., Lucassen, M., Clemmesen, C., Hanel, R. and Reusch, T. B. H. (2020). Differential gene expression patterns related to lipid metabolism in response to ocean acidification in larvae and juveniles of Atlantic cod. *Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol.* **247**, 110740.

Ge, S. X., Jung, D. and Yao, R. (2019). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics* 1–2.

Gobler, C. J. and Baumann, H. (2016). Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life. *Biol. Lett.* **12**, 20150976.

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–652.

Gracey, A. Y., Troll, J. V. and Somero, G. N. (2001). Hypoxia-induced gene expression profiling in the euryoxic fish Gillichthys mirabilis. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 1993–1998.

Grantham, B. A., Chan, F., Nielsen, K. J., Fox, D. S., Barth, J. A., Huyer, A., Lubchenco, J. and Menge, B. A. (2004). Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. *Nature* **429**, 749–754.

Grasso, G., Satriano, C. and Milardi, D. (2015). A neglected modulator of insulin-degrading enzyme activity and conformation: The pH. *Biophys. Chem.* **203**–**204**, 33–40.

Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frolicher, T. L. and Plattner, G.-K. (2012). Rapid progression of ocean acidification in the California Current System. *Science (80-.).* **337**, 220–223.

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., Couger, M. B., Eccles, D., Li, B., Lieber, M., et al. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* **8**, 1494–1512.

Hamilton, S. L., Logan, C. A., Fennie, H. W., Sogard, S. M., Barry, J. P., Makukhov, A. D., Tobosa, L. R., Boyer, K., Lovera, C. F. and Bernardi, G. (2017). Species-specific responses of juvenile rockfish to elevated pCO2: From behavior to genomics. *PLoS One* **12**, 1–23.

Hassell, K. L., Coutin, P. C. and Nugegoda, D. (2008). Hypoxia impairs embryo development and survival in black bream (Acanthopagrus butcheri). *Mar. Pollut. Bull.* **57**, 302–306.

Hauri, C., Gruber, N., Vogt, M., Doney, S. C., Feely, R. A., Lachkar, Z., Leinweber, A., McDonnell, A. M. P. and Munnich, M. (2013). Spatiotemporal variability and

long-term trends of ocean acidification in the California Current System. *Biogeosciences* **10**, 193–216.

Henson, S. A., Beaulieu, C., Ilyina, T., John, J. G., Long, M., Séférian, R., Tjiputra, J. and Sarmiento, J. L. (2017). Rapid emergence of climate change in environmental drivers of marine ecosystems. *Nat. Commun.* **8**, 1–9.

Heuer, R. M. and Grosell, M. (2014). Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *AJP Regul. Integr. Comp. Physiol.* **307**, R1061–R1084.

Ho, D. H. and Burggren, W. W. (2012). Parental hypoxic exposure confers offspring hypoxia resistance in zebrafish (Danio rerio). *J. Exp. Biol.* **215**, 4208–4216.

Ishimatsu, A., Hayashi, M. and Kikkawa, T. (2008). Fishes in high-CO2, acidified oceans. *Mar. Ecol. Prog. Ser.* **373**, 295–302.

Keeling, R. F. and Garcia, H. E. (2002). The change in oceanic O2 inventory associated with recent global warming. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 7848–7853.

Keeling, R. F., Körtzinger, A. and Gruber, N. (2010). Ocean Deoxygenation in a Warming World. *Ann. Rev. Mar. Sci.* **2**, 199–229.

Kramer, D. L. and McClure, M. (1982). Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. *Environ. Biol. Fishes* **7**, 47–55.

Kroeker, K. J., Kordas, R. L., Crim, R. N. and Singh, G. G. (2010). Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.* **13**, 1419–1434.

Lafuente, E. and Beldade, P. (2019). Genomics of developmental plasticity in animals. *Front. Genet.* **10**, 1–18.

Langmead, B. (2010). Alignment with Bowtie. *Curr Protoc Bioinforma.* 1–24.

Laroche, W. A. and Richardson, S. L. (1981). Development of Larvae and Juveniles of the Rockfishes Sebastes Entomelas and S . Zacentrus (Family Scorpaenidae) and Occurrence Off Oregon , With Notes on. *Fish. Bull.* **79**, 231–257.

Leary, P. R., Woodson, C. B., Squibb, M. E., Denny, M. W., Monismith, S. G. and Micheli, F. (2017). "Internal tide pools" prolong kelp forest hypoxic events. *Limnol. Oceanogr.* **62**, 2864–2878.

Lenarz, W. H., Larson, R. J. and Ralston, S. (1991a). Depth Distributions O F Late Larvae and Pelagic Juveniles of Some Fishes of. *CALCOFI Rep* **32**, 41–46.

Lenarz, W. H., Larson, R. J. and Ralston, S. (1991b). Depth distributions of late larvae and pelagic juveniles of some fishes of the California current. *Calif. Coop. Ocean. Fish. Investig. Rep.* **32**, 41–46.

Li, A. and Chiu, J. M. Y. (2013). Latent effects of hypoxia on the gastropod Crepidula onyx. *Mar. Ecol. Prog. Ser.* **480**, 145–154.

Li, B. and Dewey, C. N. (2011). RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12**,.

Long, Y., Song, G., Yan, J., He, X., Li, Q. and Cui, Z. (2013). Transcriptomic characterization of cold acclimation in larval zebrafish. *BMC Genomics* **14**,.

Long, M. C., Deutsch, C. and Ito, T. (2016). Finding forced trends in oceanic oxygen. *Global Biogeochem. Cycles* 381–397.

Love, M. S., Yoklavich, M. and Thorsteinson, L. (2002). *The Rockfishes of the Northeast Pacific*. Berkeley and Los Angeles, California: University of California Press.

Lucon-Xiccato, T., Nati, J. J. H., Blasco, F. R., Johansen, J. L., Steffensen, J. F. and Domenici, P. (2014). Severe hypoxia impairs lateralization in a marine teleost fish. *J. Exp. Biol.* **217**, 4115–4118.

MacFarlane, R. B. and Bowers, M. J. (1995). Matrotrophic viviparity in the yellowtail rockfish Sebastes flavidus. *J. Exp. Biol.* **198**, 1197–206.

MacManes, M. D. (2014). On the optimal trimming of high-throughput mRNA sequence data. *Front. Genet.* **5**, 1–7.

Mattiasen, E. G., Kashef, N. S., Stafford, D. M., Logan, C. A., Sogard, S. M., Bjorkstedt, E. P. and Hamilton, S. L. (2020). Effects of hypoxia on the behavior and physiology of kelp forest fishes. *Glob. Chang. Biol.* 3498–3511.

Melzner, F., Gutowska, M. a., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M. and Pörtner, H.-O. (2009). Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences Discuss.* **6**, 4693–4738.

Mi, H., Huang, X., Muruganujan, A., Tang, H., Mills, C., Kang, D. and Thomas, P. D. (2017). PANTHER version 11: Expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. *Nucleic Acids Res.* **45**, D183–D189.

Moser, H. G. (1967). Reproduction and Development of Sebastodes paucispinis and Comparison with Other Rockfishes off Southern California. *Am. Soc. Ichthologists Herpetol.* **1967**, 773–797.

Munday, P. L., Crawley, N. E. and Nilsson, G. E. (2009a). Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Mar. Ecol. Prog. Ser.* **388**, 235–242.

Munday, P. L., Dixson, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V. and Døving, K. B. (2009b). Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl. Acad. Sci.* **106**, 1848–1852.

Nacci, D., Coiro, L., Champlin, D., Jayaraman, S., McKinney, R., Gleason, T. R., Munns, W. R., Specker, J. L. and Cooper, K. R. (1999). Adaptations of wild populations of the estuarine fish Fundulus heteroclitus to persistent environmental contaminants. *Mar. Biol.* **134**, 9–17.

Nagasawa, T. and Kobayashi, T. (1995). The Early Life History of the Rockfish , Sebastes thompsoni in the Sea of Japan Toru Nagasawa1 and Tokimasa Kobayash i 2 Japan Sea National Fisheries Niigata 951 , Ja p a n Institute of Fisheries Science Kanazawa- k u , Yokohama , Kanagawa a n (Received. *Japan. J. Ichthyol.* **41**, 385–396.

Navarro, M. O., Kwan, G. T., Batalov, O., Choi, C. Y., Pierce, N. T. and Levin, L. A. (2016). Development of embryonic market squid, Doryteuthis opalescens, under chronic exposure to low environmental pH and [O2]. *PLoS One* **11**, 1–17.

Oleksiak, M. F. and Crawford, D. L. (2012). The relationship between phenotypic and environmental variation: Do physiological responses reduce interindividual differences? *Physiol. Biochem. Zool.* **85**, 571–584.

Oomen, R. A. and Hutchings, J. A. (2017). Transcriptomic responses to environmental change in fishes: Insights from RNA sequencing. *Facets* **2**, 610–641.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A.,

Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., et al. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681–686.

Patti, B., Guisande, C., Riveiro, I., Thejll, P., Cuttitta, A., Bonanno, A., Basilone, G., Buscaino, G. and Mazzola, S. (2010). Effect of atmospheric CO2and solar activity on wind regime and water column stability in the major global upwelling areas. *Estuar. Coast. Shelf Sci.* **88**, 45–52.

Pespeni, M. H., Sanford, E., Gaylord, B., Hill, T. M., Hosfelt, J. D., Jaris, H. K., LaVigne, M., Lenz, E. A., Russell, A. D., Young, M. K., et al. (2013). Evolutionary change during experimental ocean acidification. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 6937–6942.

Petitjean, Q., Jean, S., Gandar, A., Côte, J., Laffaille, P. and Jacquin, L. (2019). Stress responses in fish: From molecular to evolutionary processes. *Sci. Total Environ.* **684**, 371–380.

Pichavant, K., Person-Le-Ruyet, J., Le Bayon, N., Severe, A., Le Roux, A. and Boeuf, G. (2001). Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. *J. Fish Biol.* **59**, 875–883.

R Team, C. (2018). An introduction to dplR. *Ind. Commer. Train.* **0**, 1–16.

Rabalais, N. N., Diaz, R. J., Levin, L. A., Turner, R. E., Gilbert, D. and Zhang, J. (2010). Dynamics and distribution of natural and human-caused hympoxia. *Biogeosciences* **7**, 585–619.

Randall, B. Y. D. (1982). The Control of Respiration and Circulation in Fish During Exercise and Hypoxia. *J. Exp. Biol.* **100**, 275–288.

Richards, J. G. (2009). Metabolic and molecular responses of fish to hypoxia. **5098**,.

Richards, J. G. (2011). Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *J. Exp. Biol.* **214**, 191–199.

Richardson, S. L. and Laroche, W. A. (1979). Development and occurrence of larvae and juveniles of the rockfishes Sebastes crameri, Sebastes pinniger, and Sebastes helvomaculatus (Family Scorpaenidae) off Oregon. *Fish. Bull.* **77**, 1–46.

Robinson, M. D. and Smyth, G. K. (2007). Gene expression Moderated statistical tests for assessing differences in tag abundance. **23**, 2881–2887.

Robinson, M. D. and Smyth, G. K. (2008). Small-sample estimation of negative binomial dispersion , with applications to SAGE data. 321–332.

Robinson, M. D., McCarthy, D. J. and Smyth, G. K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140.

Rombough, P. (2007). The functional ontogeny of the teleost gill: Which comes first, gas or ion exchange? *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **148**, 732–742.

Saksa, K. Effects of low pH and low dissolved oxygen on larval rockfish (Sebastes spp.) physiology, respirometry and hypoxia tolerance.

Salinas, S. and Munch, S. B. (2012). Thermal legacies: Transgenerational effects of temperature on growth in a vertebrate. *Ecol. Lett.* **15**, 159–163.

Schmidtko, S., Stramma, L. and Visbeck, M. (2017). Decline in global oceanic oxygen content during the past five decades. *Nature* **542**, 335–339.

Segura, C. J., Chaparro, O. R., Pechenik, J. A., Paschke, K. A., Osores, S. J. A., Navarro, J. M. and Cubillos, V. M. (2014). Delayed effects of severe hypoxia experienced by marine gastropod embryos. *Mar. Ecol. Prog. Ser.* **510**, 59–71.

Shang, E. H. H. and Wu, R. S. S. (2004). Aquatic hypoxia is a teratogen and affects fish embryonic development. *Environ. Sci. Technol.* **38**, 4763–4767.

Shoubridge, E. A. and Hochachka, P. W. (1980). Ethanol: Novel end product of vertebrate anaerobic metabolism. *Science (80-.).* **209**, 308–309.

Simao, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V and Zdobnov, E. M. (2015). Genome analysis BUSCO : assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**, 3210–3212.

Snyder, M. A., Sloan, L. C., Diffenbaugh, N. S. and Bell, J. L. (2003). Future climate change and upwelling in the California Current. *Geophys. Res. Lett.* **30**, 1–4.

Sogard, S. M., Berkeley, S. A. and Fisher, R. (2008). Maternal effects in rockfishes Sebastes spp .: a comparison among species. *Mar. Ecol. Prog. Ser.* **360**, 227–236.

Sollid, J. and Nilsson, G. E. (2006). Plasticity of respiratory structures - Adaptive remodeling of fish gills induced by ambient oxygen and temperature. *Respir. Physiol. Neurobiol.* **154**, 241–251.

Sollid, J., De Angelis, P., Gundersen, K. and Nilsson, G. E. (2003). Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *J. Exp. Biol.* **206**, 3667–3673.

Suzek, B. E., Wang, Y., Huang, H., McGarvey, P. B. and Wu, C. H. (2015). UniRef clusters: A comprehensive and scalable alternative for improving sequence similarity searches. *Bioinformatics* **31**, 926–932.

Sydeman, W. J., Garcia-Reyes, M., Schoeman, D. S., Rykaczewski, R. R., Thompson, S. A., Black, B. A. and Bograd, S. J. (2014). Climate change and wind intensification in coastal upwelling ecosystems. *Science (80-.).* **345**, 77–80.

Ton, C., Stamatiou, D. and Liew, C. C. (2003). Gene expression profile of zebrafish exposed to hypoxia during development. *Physiol. Genomics* **13**, 97–106.

Tseng, Y., Hu, M. Y., Stumpp, M., Lin, L., Melzner, F. and Hwang, P. (2013). CO 2 - driven seawater acidification differentially affects development and molecular plasticity along life history of fish (Oryzias latipes). *Comp. Biochem. Physiol. Part A* **165**, 119–130.

Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferraresso, S., Sévère, A., Zambonino-Infante, J. L. and Mazurais, D. (2015). Hypoxic episode during the larval period has long-term effects on European sea bass juveniles (Dicentrarchus labrax). *Mar. Biol.* **162**, 367–376.

Walter, R. K., Woodson, C. B., Leary, P. R. and Monismith, S. G. (2014). Connecting wind-driven upwelling and offshore stratification to nearshore internal bores and oxygen variability. *J. Geophys. Res. Ocean.* **119**, 3517–3534.

Wang, D., Gouhier, T. C., Menge, B. A. and Ganguly, A. R. (2015). Intensification and spatial homogenization of coastal upwelling under climate change. *Nature* **518**, 390–394.

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

Wong, J. M., Johnson, K. M., Kelly, M. W. and Hofmann, G. E. (2018). Transcriptomics reveal transgenerational effects in purple sea urchin embryos: Adult acclimation to upwelling conditions alters the response of their progeny to

differential pCO2levels. *Mol. Ecol.* **27**, 1120–1137.

Wu, R. S. S. (2002). Hypoxia : from molecular responses to ecosystem responses. **45**, 35–45.

Wu, R. S. S., Zhou, B. S., Randall, D. J., Woo, N. Y. S. and Lam, P. K. S. (2003). Aquatic Hypoxia Is an Endocrine Disruptor and Impairs Fish Reproduction. *Environ. Sci. Technol.* **37**, 1137–1141.

Yang, H., Zhou, Y., Gu, J., Xie, S., Xu, Y., Zhu, G., Wang, L., Huang, J., Ma, H. and Yao, J. (2013). Deep mRNA Sequencing Analysis to Capture the Transcriptome Landscape of Zebrafish Embryos and Larvae. *PLoS One* **8**,.

Table 1. Transcriptome assembly statistics

Species	Total trinity 'genes'	Total trinity transcripts	Percent GC	ALL transcript - N50	ALL transcript - Median contig length	Longest isoform - N50	Longest isoform - median contig length	Average contig length
Gopher whole-pooled larvae	158008	275950	47.1	2009	435	1490	352	981.91
Blues whole-pooled larvae	131017	231646	47.4	2287	468	1772	365	821.72

4097 (89.3%)	2000 (43.6%)	20.97 (45.7%)	284 (6.2%)	203 (4.5%)	79.85 - 83.30%
4185 (91.3%)	2138 (46.6%)	2047 (44.7%)	231 (5.0%)	168 (3.7%)	43.04 - 57.76%

Table 2. Gopher rockfish mother data: Length, weight, and time in treatment for adult female gopher rockfish.

ID	Species	Treatment	Days in Treatment	Length (mm)	Weight (g)
G72	Gopher	Control	54	293	700.5
G73	Gopher	Control	60	250	366.5
G79	Gopher	4DO	61	293	566.1
G91	Gopher	4DO	41	256	249.5

Table 3. Gopher rockfish larvae treatment denotation

Time Point	Maternal Environment	Larval Environment	Denotation
At birth	Control	N/A	$_{M}$Control
At birth	Hypoxia	N/A	$_{M}$Hypoxia
After larval exposure	Control	Control	$_{M}$Control_$_{L}$Control
After larval exposure	Control	Hypoxia	$_{M}$Control_$_{L}$Hypoxia
After larval exposure	Hypoxia	Hypoxia	$_{M}$Hypoxia_$_{L}$Hypoxia
After larval exposure	Hypoxia	Control	$_{M}$Hypoxia_$_{L}$Control

Table 4. Blue rockfish mother data: Length, weight, and time in treatment for adult female blue rockfish.

ID	Species	Treatment	Days in Treatment	Length (mm)	Weight (g)
TB225	Blue	Control	50	327	756.7
TB206	Blue	Control	82	279	452.7
TB220	Blue	Cross	51	308	579
TB231	Blue	Cross	44	319	694.6
TB222	Blue	7.5 pH	41	310	629.6
TB237	Blue	7.5 pH	28	294	484.6

Table 5. Blue rockfish larvae treatment denotations

Time Point	Maternal Environment	Larval Environment	Denotation
At birth	Control	N/A	$_{M}$Control
At birth	Hypoxia	N/A	$_{M}$Hypoxia
After larval exposure	Control	Control	$_{M}$Control$_{_L}$Control
After larval exposure	Control	Low pH	$_{M}$Control$_{_L}$Low pH
After larval exposure	Control	Combined Stressor	$_{M}$Control$_{_L}$Combined
After larval exposure	Low pH	Low pH	$_{M}$Low pH$_{_L}$Low pH
After larval exposure	Low pH	Control	$_{M}$Low pH$_{_M}$Control
After larval exposure	Combined Stressor	Combined Stressor	$_{M}$Combined$_{_L}$Combined
After larval exposure	Combined Stressor	Control	$_{M}$Combined$_{_L}$Control

Table 6. Biological Process of DEGs across time unique to each treatment in blue rockfish

Category	Con D1>D5 raw numbers	Low pH D1>D5 raw numbers	Combined D1>D5 raw numbers	Con D1>D5 normalized by % annotated genes	Low pH D1>D5 normalized by % annotated genes	Combined D1>D5 normalized by % annotated genes
cellular process (GO:0009987)	230	55	25	0.276442308	0.251141553	0.225225225
metabolic process (GO:0008152)	175	34	18	0.210336538	0.155251142	0.162162162
biological regulation (GO:0065007)	113	16	12	0.135817308	0.073059361	0.108108108
cellular component organization or biogenesis (GO:0071840)	83	25	9	0.099759615	0.114155251	0.081081081
localization (GO:0051179)	60	16	11	0.072115385	0.073059361	0.099099099
response to stimulus (GO:0050896)	60	14	5	0.072115385	0.063926941	0.045045045
signaling (GO:0023052)	36	6	2	0.043269231	0.02739726	0.018018018
developmental process (GO:0032502)	29	8	6	0.034855769	0.03652968	0.054054054
multicellular organismal process (GO:0032501)	24	8	6	0.028846154	0.03652968	0.054054054
locomotion (GO:0040011)	8	3	0	0.009615385	0.01369863	0
cell population proliferation (GO:0008283)	5	0	0	0.006009615	0	0
biological adhesion (GO:0022610)	5	2	0	0.006009615	0.00913242	0
immune system process (GO:0002376)	4	2	0	0.004807692	0.00913242	0
multi-organism process (GO:0051704)	3	1	0	0.003605769	0.00456621	0
reproduction (GO:0000003)	2	5	0	0.002403846	0.02283105	0
reproductive process (GO:0022414)	2	5	0	0.002403846	0.02283105	0
growth (GO:0040007)	1	3	0	0.001201923	0.01369863	0
biological phase (GO:0044848)	1	1	0	0.001201923	0.00456621	0
rhythmic process (GO:0048511)	1	0	0	0.001201923	0	0

Figure 1.

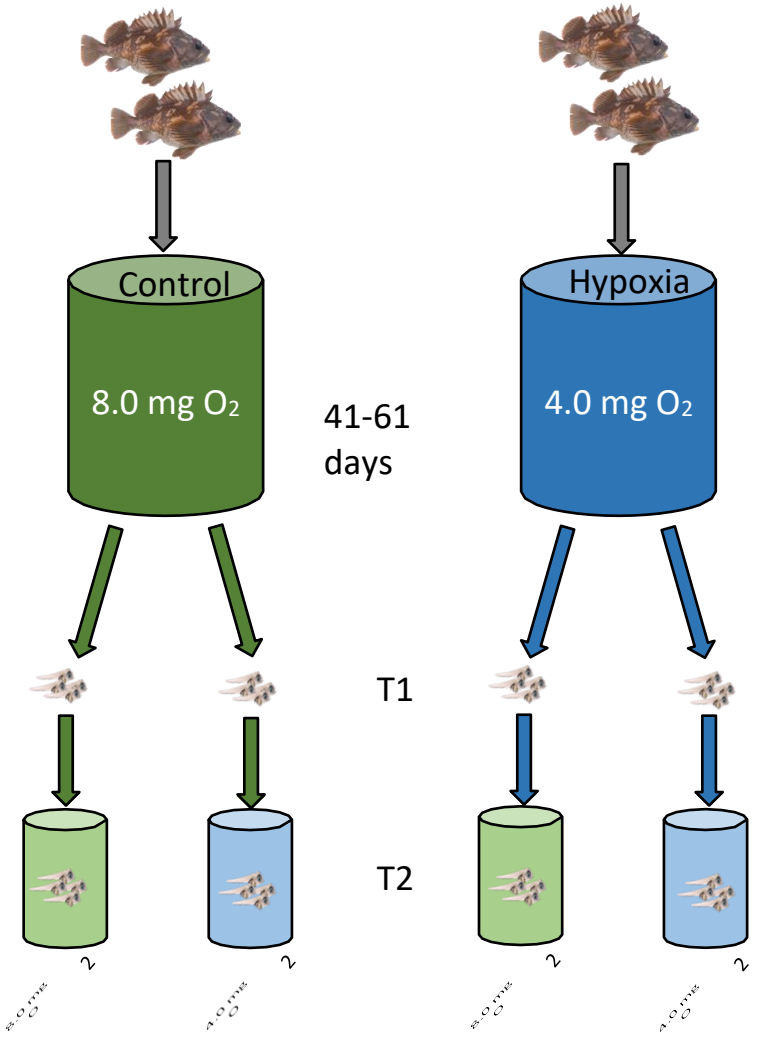


Figure 2.

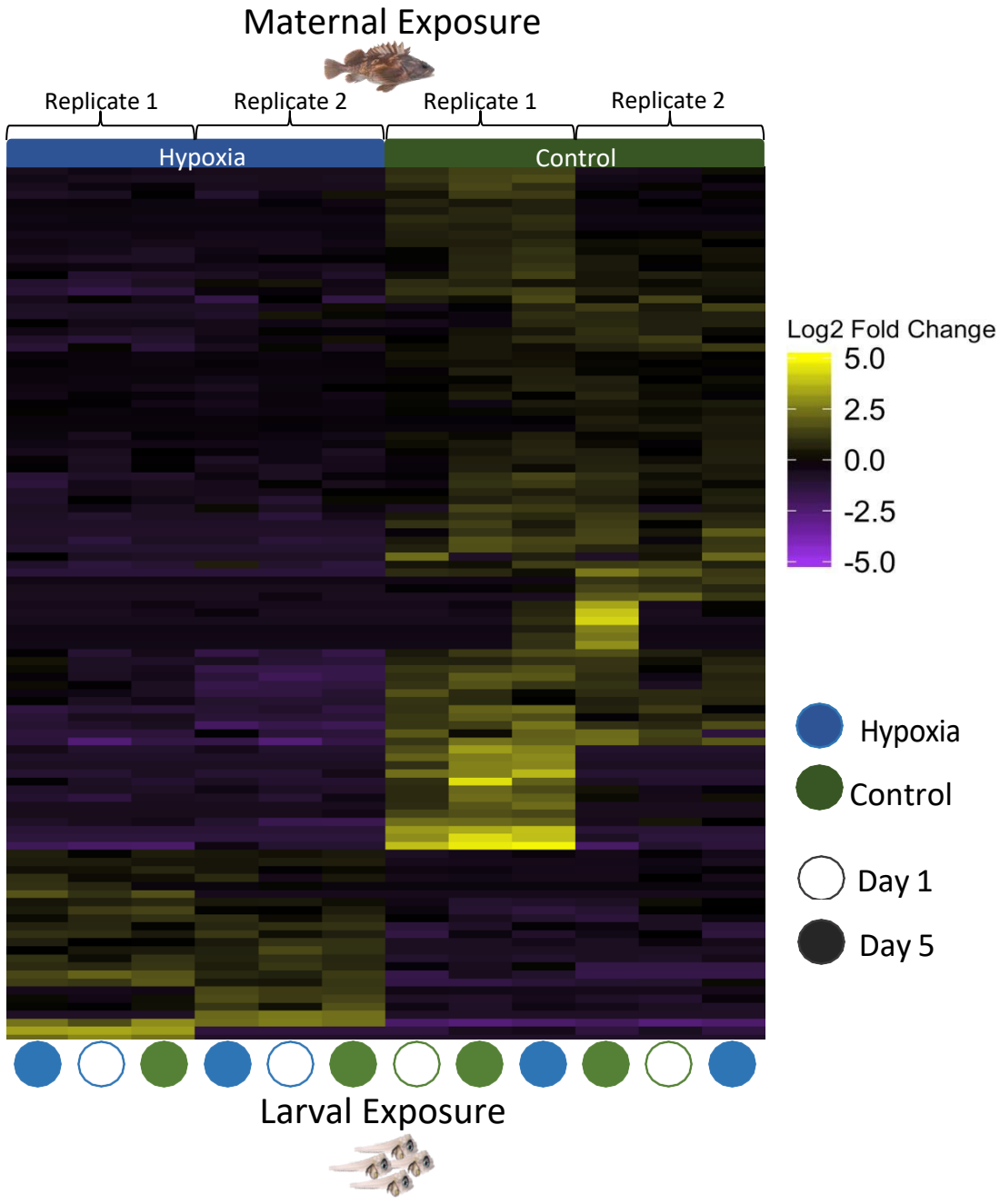

Maternal Exposure
Replicate 1
Replicate 2
Replicate 1
Replicate 2
Hypoxia
Control
Log2 Fold Change
5.0
2.5
0.0
-2.5
-5.0
Hypoxia
Control
Day 1
Day 5
Larval Exposure

Figure 3.

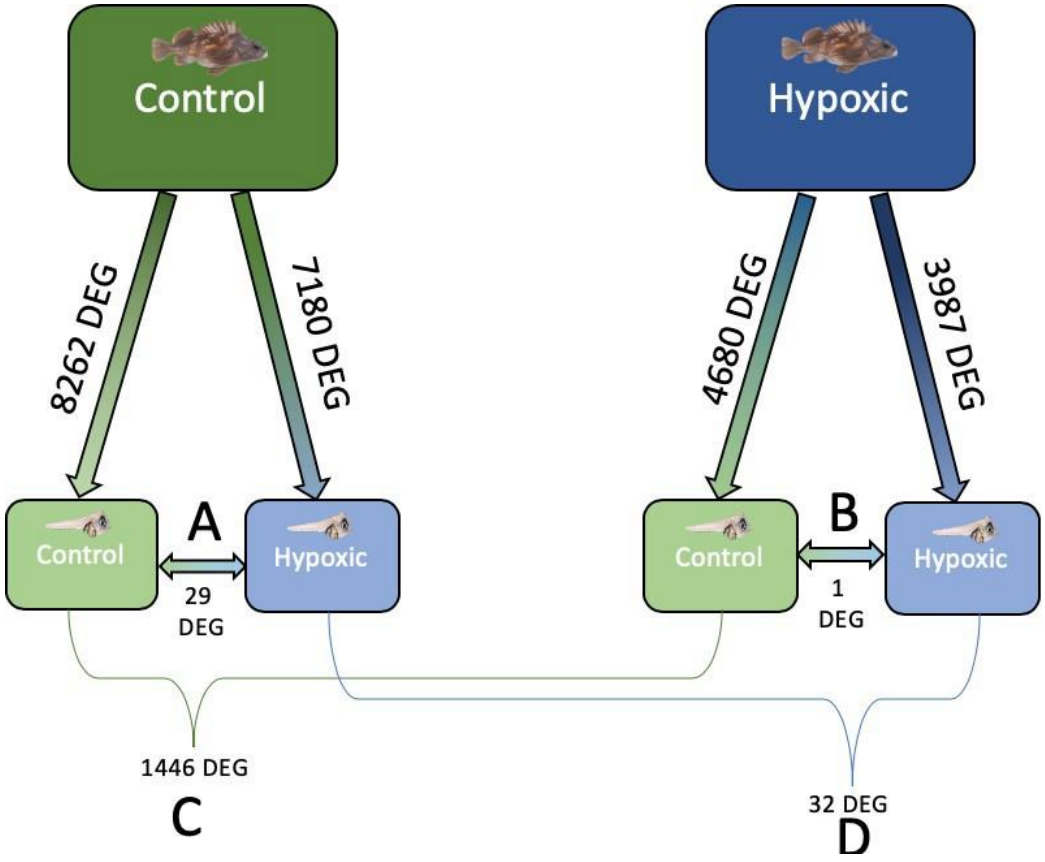

Control
Hypoxic
8262 DEG
7180 DEG
4680 DEG
3987 DEG
Control
A
Hypoxic
29
DEG
Control
B
Hypoxic
1
DEG
1446 DEG
C
32 DEG
D

Figure 4.

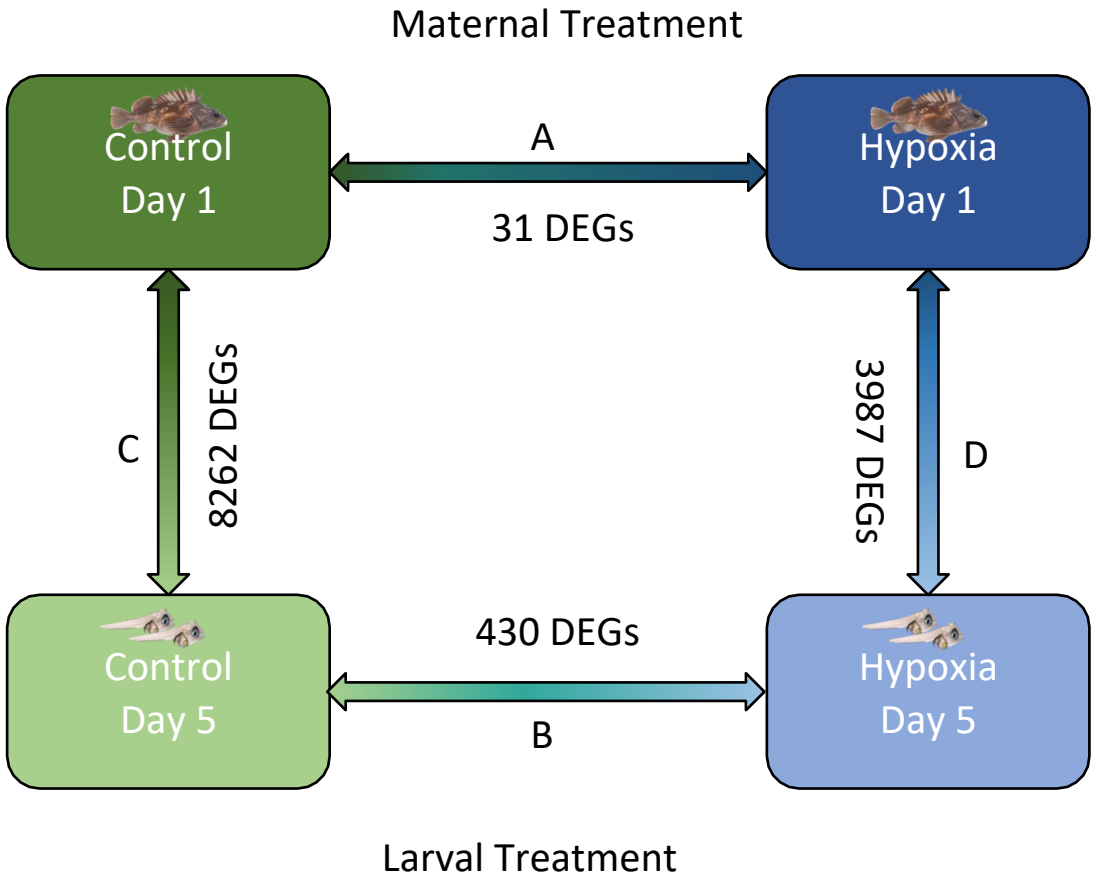

Maternal Treatment
Control
Day 1
A
31 DEGs
Hypoxia
Day 1
C
8262 DEGs
3987 DEGs
D
Control
Day 5
430 DEGs
B
Hypoxia
Day 5
Larval Treatment

Figure 5.

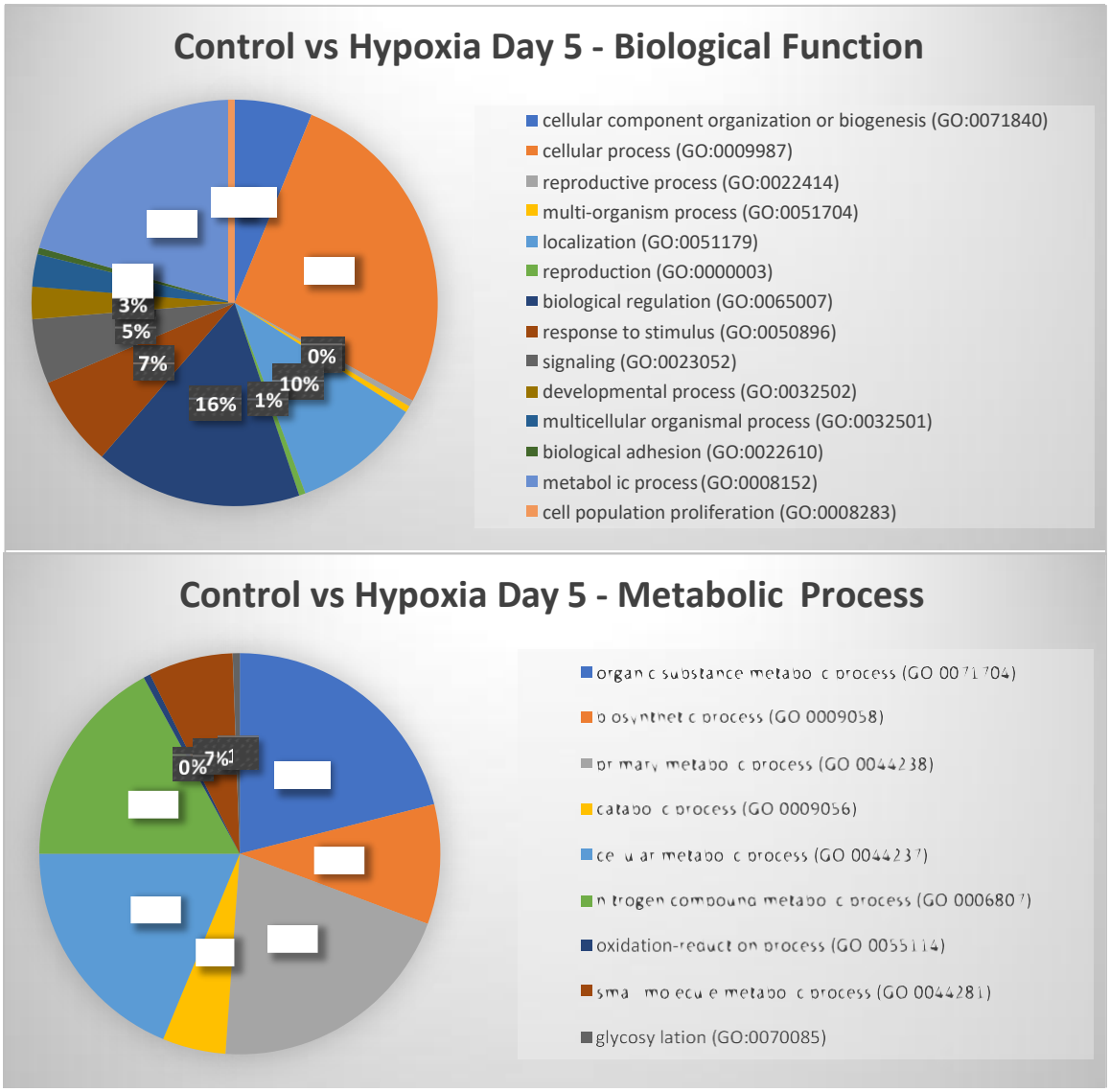

Control vs Hypoxia Day 5 - Biological Function
3%
5%
7%
16%
1%
10%
0%
cellular component organization or biogenesis (GO:0071840)
cellular process (GO:0009987)
reproductive process (GO:0022414)
multi-organism process (GO:0051704)
localization (GO:0051179)
reproduction (GO:0000003)
biological regulation (GO:0065007)
response to stimulus (GO:0050896)
signaling (GO:0023052)
developmental process (GO:0032502)
multicellular organismal process (GO:0032501)
biological adhesion (GO:0022610)
metabol ic process (GO:0008152)
cell population proliferation (GO:0008283)
Control vs Hypoxia Day 5 - Metabolic Process
0%
7%
organ c substance metabo c process (GO 00 71 704)
b osy nthet c process (GO 0009058)
pr mary metabo c process (GO 0044238)
catabo c process (GO 0009056)
ce u ar metabo c process (GO 0044237)
n trogen compound metabo c process (GO 000680)
oxidation-reduct on process (GO 0055114)
sma mo ecu e metabo c process (GO 0044281)
glycosy lation (GO:0070085)

Figure 6.

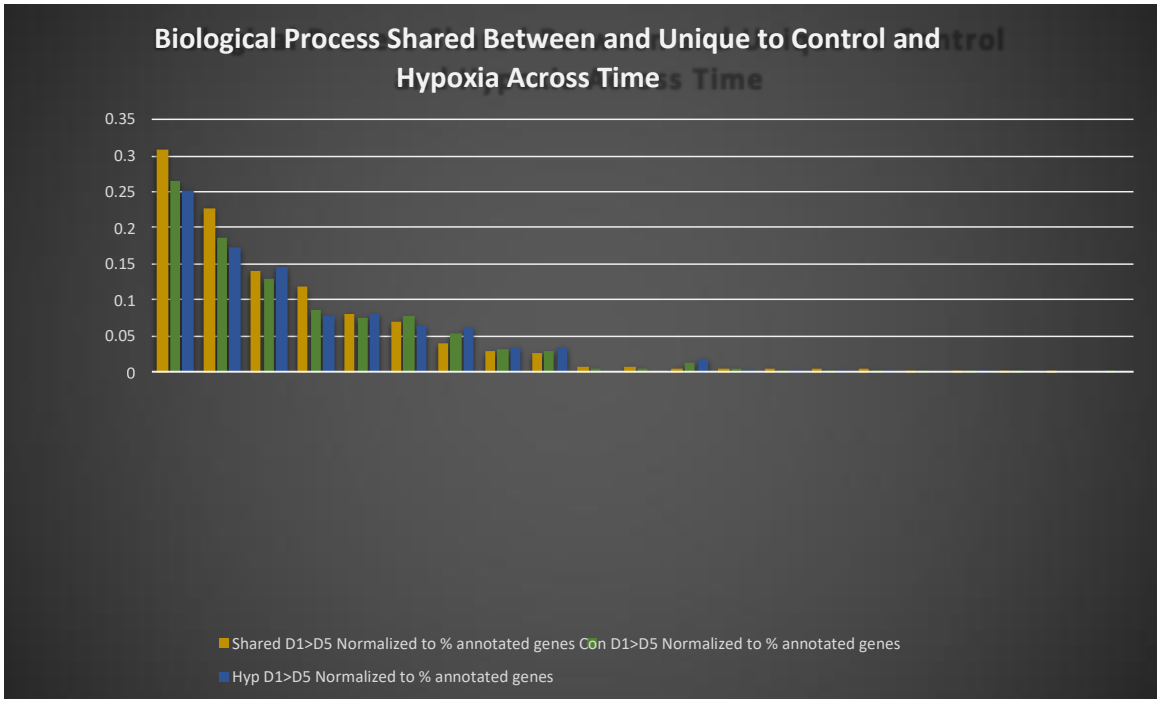

Biological Process Shared Between and Unique to Control and Hypoxia Across Time
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Shared D1>D5 Normalized to % annotated genes
Con D1>D5 Normalized to % annotated genes
Hyp D1>D5 Normalized to % annotated genes

Figure 7.

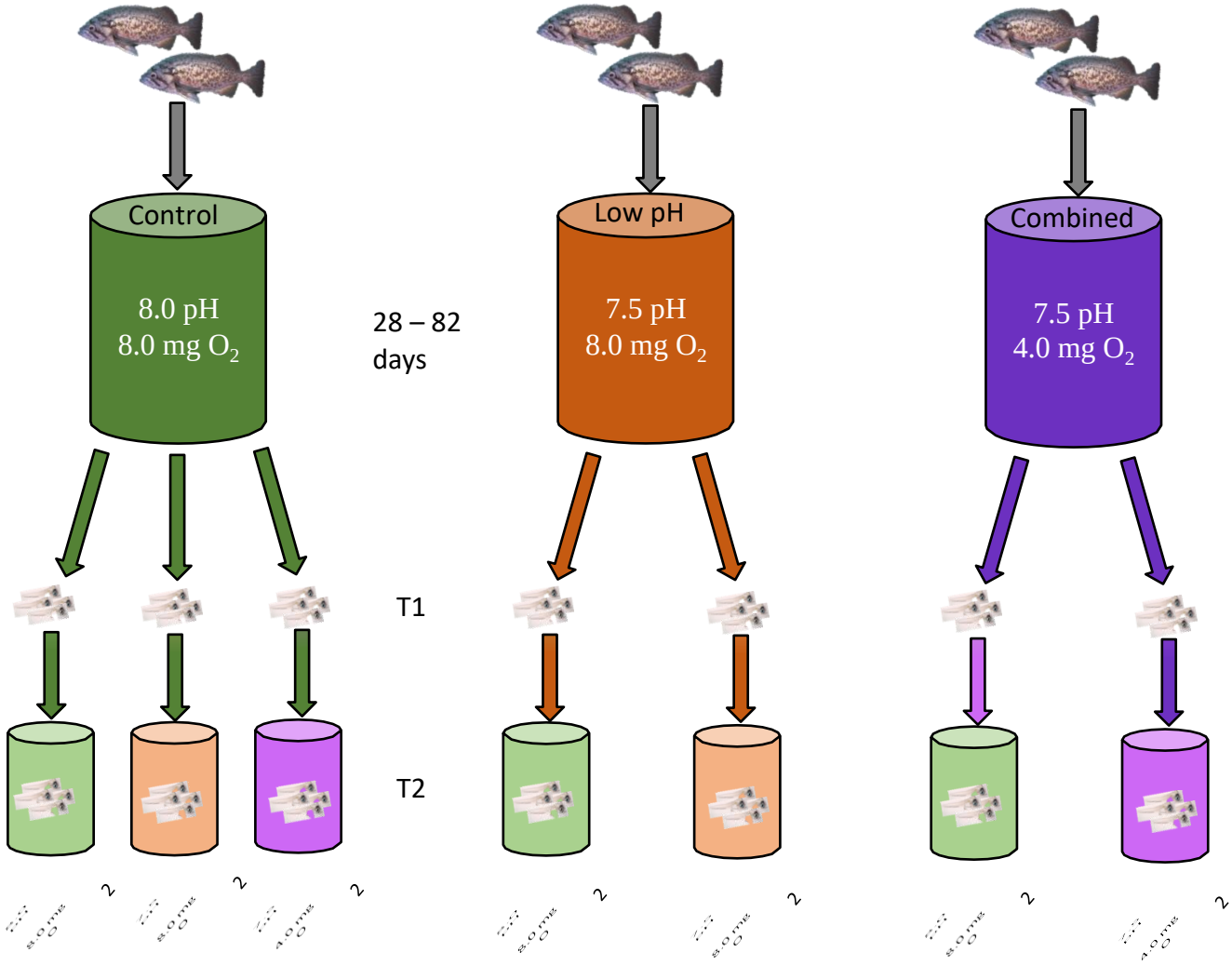

Control
8.0 pH
8.0 mg O2
28 – 82
days
Low pH
7.5 pH
8.0 mg O2
Combined
7.5 pH
4.0 mg O2
T1
T2

Figure 8.

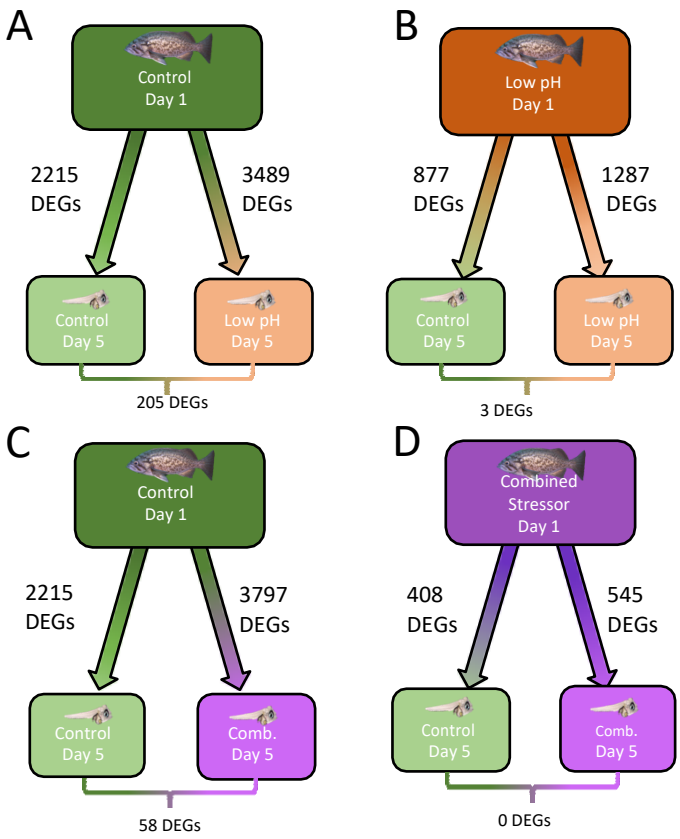

A
Control
Day 1
2215
DEGs
3489
DEGs
Control
Day 5
Low pH
Day 5
205 DEGs
B
Low pH
Day 1
877
DEGs
1287
DEGs
Control
Day 5
Low pH
Day 5
3 DEGs
C
Control
Day 1
2215
DEGs
3797
DEGs
Control
Day 5
Comb.
Day 5
58 DEGs
D
Combined
Stressor
Day 1
408
DEGs
545
DEGs
Control
Day 5
Comb.
Day 5
0 DEGs

Figure 9.

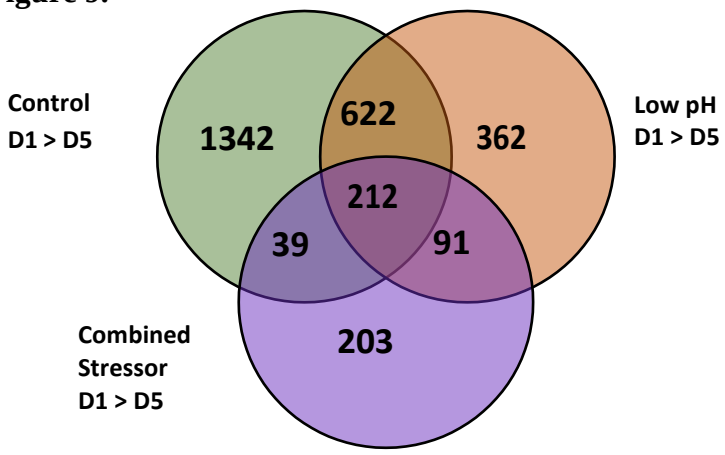

Control
D1 > D5
1342
622
362
Low pH
D1 > D5
212
39
91
Combined
Stressor
D1 > D5
203

Figure 10.

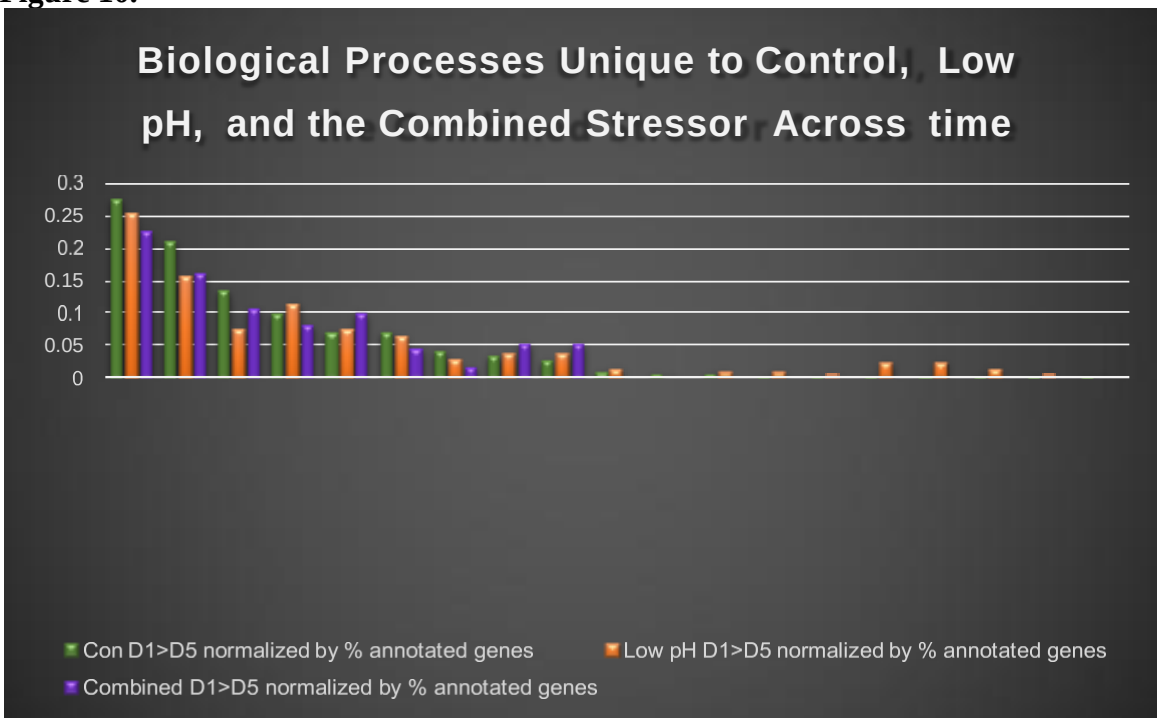
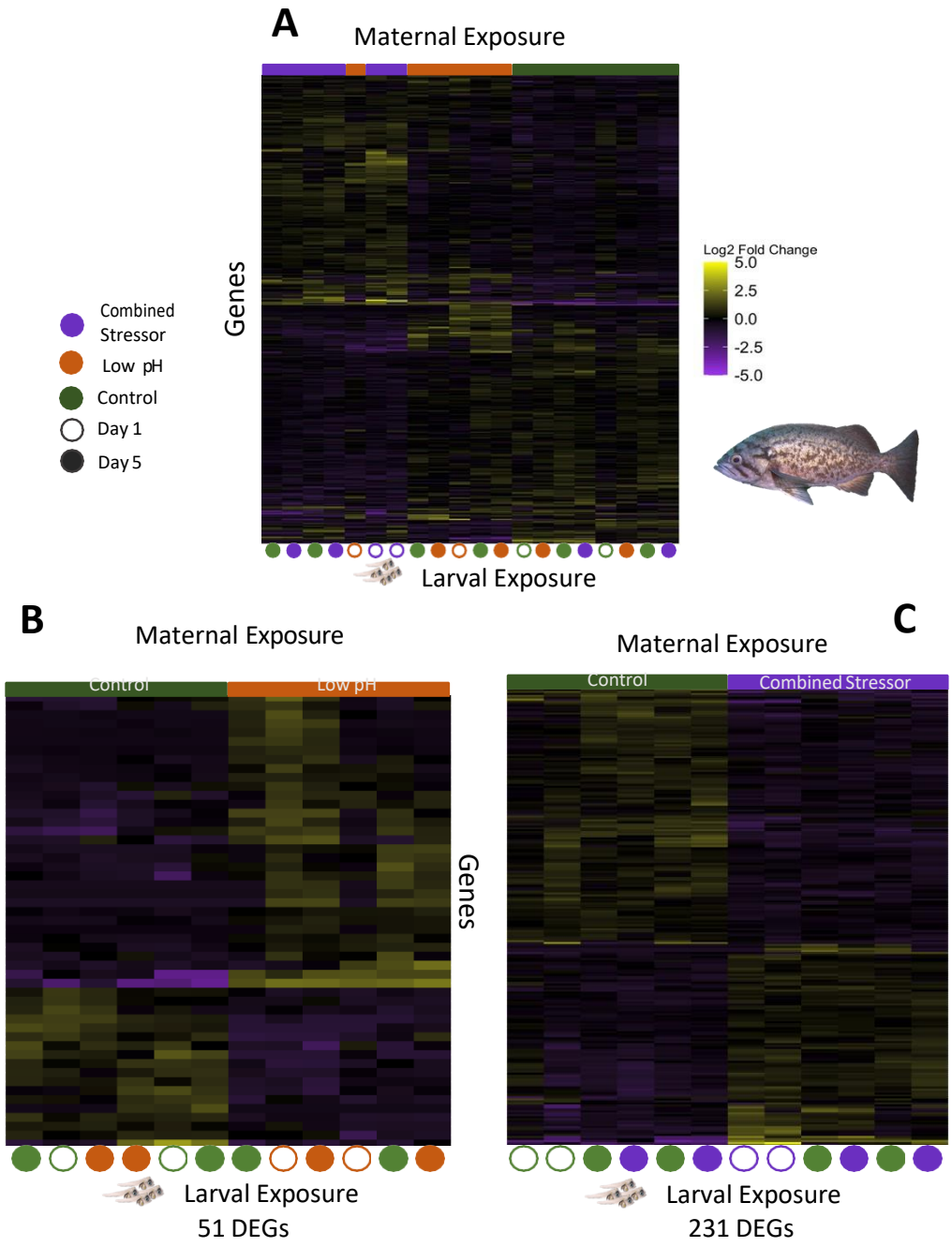

Biological Processes Unique to Control, Low pH, and the Combined Stressor Across time
0.3
0.25
0.2
0.15
0.1
0.05
0
Con D1>D5 normalized by % annotated genes
Low pH D1>D5 normalized by % annotated genes
Combined D1>D5 normalized by % annotated genes

Figure 11.


A
Maternal Exposure
Genes
Log2 Fold Change
5.0
2.5
0.0
-2.5
-5.0
Combined Stressor
Low pH
Control
Day 1
Day 5
Larval Exposure
B
Maternal Exposure
Control
Low pH
Genes
Larval Exposure
51 DEGs
C
Maternal Exposure
Control
Combined Stressor
Larval Exposure
231 DEGs

APPENDIX A

SUPPLEMENTAL TABLES

Supplemental Table 1 – top 10 enriched genes in larvae that gestated in either environment but were exposed to the control treatment for the larval exposure.

Enrichment FDR	Genes in list	Total genes	Functional Category	Genes
0.00044298	51	4312	Biosynthetic process	CERS5 GYS2 GNMT HNF1A ASAH2 SFR1 NSD2 GMDS PGP PPARG XBP1 RPA2 EGR1 DEGS2 COASY JUN CH25H IRAK3 KTI12 REL GARS PTGIS ENY2 ELF3 TCF20 PIGW EGR3 IRAK4 NR5A2 PEMT FOXN4 RIPK2 ADA IMPA1 ATF3 KMO STAT6 DGAT2 DTL ATF2 NFKB2 SFRP5 RX2 FOSL2 FOSB RX1 MALT1 MYCBP FUT11 LBH DAB2
0.00044298	37	2817	Heterocycle biosynthetic process	HNF1A SFR1 NSD2 GMDS PPARG XBP1 RPA2 EGR1 COASY JUN IRAK3 KTI12 REL ENY2 ELF3 TCF20 EGR3 IRAK4 NR5A2 FOXN4 RIPK2 ADA ATF3 KMO STAT6 ATF2 NFKB2 SFRP5 RX2 FOSL2 FOSB RX1 MALT1 MYCBP LBH DAB2 DTL
0.00044298	34	2443	Regulation of nucleobase-containing compound metabolic process	HNF1A MSH2 SFR1 NSD2 PPARG XBP1 EGR1 JUN IRAK3 KTI12 REL ENY2 ELF3 TCF20 EGR3 IRAK4 NR5A2 FOXN4 RIPK2 ATF3 STAT6 ATF2 NFKB2 SFRP5 RX2 FOSL2 FOSB DFFA RX1 MALT1 MYCBP LBH DAB2 ESRP1
0.00044298	46	3727	Regulation of metabolic process	A1CF GNMT HNF1A BCAR3 MSH2 SFR1 NSD2 PPARG PSME2 XBP1 EGR1 JUN DRAM1 IRAK3 FETUB KTI12 REL ENY2 ELF3 TCF20 EGR3 IRAK4 NR5A2 RIPK2 FOXN4 ATF3 STAT6 DTL ATF2 NFKB2 SFRP5 RX2 FOSL2 ITIH2 FOSB DFFA DOK7 CD109 SOGA1 RX1 MALT1 MYCBP LBH DAB2 ESRP1 CHAC1
0.00044298	37	2810	Aromatic compound biosynthetic process	HNF1A SFR1 NSD2 GMDS PPARG XBP1 RPA2 EGR1 COASY JUN IRAK3 KTI12 REL ENY2 ELF3 TCF20 EGR3 IRAK4 NR5A2 FOXN4 RIPK2 ADA ATF3 KMO STAT6 ATF2 NFKB2 SFRP5

				RX2 FOSL2 FOSB RX1 MALT1 MYCBP LBH DAB2 DTL
0.00044298	45	3539	Regulation of cellular metabolic process	GNMT HNF1A BCAR3 MSH2 SFR1 NSD2 PPARG PSME2 XBP1 EGR1 JUN DRAM1 IRAK3 FETUB KTI12 REL ENY2 ELF3 TCF20 EGR3 IRAK4 NR5A2 RIPK2 FOXN4 ATF3 STAT6 DTL ATF2 NFKB2 SFRP5 RX2 FOSL2 ITIH2 FOSB DFFA DOK7 CD109 SOGA1 RX1 MALT1 MYCBP LBH DAB2 ESRP1 CHAC1
0.00044298	52	4620	Developmental process	PDE3A ABL2 XKR9 FRK PPARG RAC2 EPHA3 JUN CAV2 AGGF1 RHOV ELF3 NHSL2 NR5A2 SFRP5 CSF3R CRYAA HNF1A FOXN4 ASAH2 FZD5 RX2 CHAC1 RX1 CNTN4 SDC2 DAB2 MMP9 RGS2 SVEP1 PTGES NSD2 GMDS XBP1 EGR1 NFKB2 FOSL2 MACC1 AFP4 TECTB STRA6 IRAK3 REL DOK7 ADAP2 ES1 IL1B LSR PIM1 PDE6C NDRG4 LBH
0.00044298	54	4854	Cellular nitrogen compound metabolic process	CERS5 A1CF GNMT HNF1A ASAH2 GDPD1 MSH2 PTGES SFR1 NSD2 GMDS PPARG XBP1 RPA2 EGR1 DEGS2 COASY JUN IRAK3 KTI12 XDH REL GARS ENY2 CHAC1 ELF3 TCF20 EGR3 IRAK4 NR5A2 FOXN4 RIPK2 ADA ATF3 KMO ACER1 ESRP1 STAT6 NOVA1 DTL ATF2 NFKB2 SFRP5 RX2 FOSL2 OSGEP FOSB DFFA RX1 MALT1 MYCBP MBD4 LBH DAB2
0.00044298	37	2731	Nucleobase-containing compound biosynthetic process	HNF1A SFR1 NSD2 GMDS PPARG XBP1 RPA2 EGR1 COASY JUN IRAK3 KTI12 REL ENY2 ELF3 TCF20 EGR3 IRAK4 NR5A2 FOXN4 RIPK2 ADA ATF3 KMO STAT6 ATF2 NFKB2 SFRP5 RX2 FOSL2 FOSB RX1 MALT1 MYCBP LBH DAB2 DTL
0.00044298	49	4136	Cellular biosynthetic process	CERS5 GYS2 HNF1A ASAH2 SFR1 NSD2 GMDS PGP PPARG

		XBP1 RPA2 EGR1 DEGS2 COASY JUN IRAK3 KTI12 REL GARS PTGIS ENY2 ELF3 TCF20 PIGW EGR3 IRAK4 NR5A2 PEMT FOXN4 RIPK2 ADA IMPA1 ATF3 KMO STAT6 DGAT2 DTL ATF2 NFKB2 SFRP5 RX2 FOSL2 FOSB RX1 MALT1 MYCBP FUT11 LBH DAB2

Supplemental Table 2 – Differentially expressed genes related to development in larvae that gestated in either environment but were exposed to the control treatment for the larval exposure.

Gene ID	Mapped IDs	Gene Name Gene Symbol Ortholog	PANTHER GO-Slim Biological Process	GP Database Biological Process Complete
DANRE|ZFIN=ZDB-GENE-060503-114|UniProtKB=A8BAW4	AMIGO1	Adhesion molecule with Ig-like domain 1 (Fragment); amigo1; ortholog	brain development(GO:0007420)	cell adhesion(GO:0007155); axonal fasciculation(GO:0007413); brain development(GO:0007420); myelination(GO:0042552)
DANRE|ZFIN=ZDB-GENE-990415-87|UniProtKB=Q6DG93	gap43	Gap43 protein; gap43; ortholog	wound healing(GO:0042060); axon guidance(GO:0007411); cellular response to stress(GO:0033554); developmental growth(GO:0048589); tissue development(GO:0009888)	multicellular organism development(GO:0007275); nervous system development(GO:0007399); axon choice point recognition(GO:0016198); cell differentiation(GO:0030154); axon regeneration(GO:0031103); regulation of growth(GO:0040008); tissue regeneration(GO:0042246)
DANRE|ZFIN=ZDB-GENE-040923-2|UniProtKB=Q7ZT82	rbfox1l	RNA binding protein fox-1 homolog 1-like; rbfox1l; ortholog	nervous system development(GO:0007399)	regulation of alternative mRNA splicing, via spliceosome(GO:0000381); heart process(GO:0003015); mRNA processing(GO:0006397); nervous system development(GO:0007399); RNA splicing(GO:0008380); regulation of RNA splicing(GO:0043484); cardiac muscle fiber development(GO:0048739); skeletal muscle fiber development(GO:0048741)
DANRE|ZFIN=ZDB-GENE-990415-213|UniProtKB=A0A140LGM0	PPARG	Peroxisome proliferator-activated receptor gamma; pparg; ortholog	negative regulation of transcription by RNA polymerase II(GO:0000122); cellular response to lipopolysaccharide(GO:0071222); multicellular organism development(GO:0007275); lipid metabolic process(GO:0006629); cell differentiation(GO:0030154); transcription by RNA polymerase II(GO:0006366); positive regulation of transcription by RNA polymerase II(GO:0045944); cholesterol homeostasis(GO:0042632)	negative regulation of transcription by RNA polymerase II(GO:0000122); regulation of transcription, DNA-templated(GO:0006355); cell differentiation(GO:0030154); positive regulation of transcription by RNA polymerase II(GO:0045944); rhythmic process(GO:0048511); triglyceride homeostasis(GO:0070328)
DANRE|ZFIN=ZDB-GENE-060421-6224|UniProtKB=B0S6I2	xkr9	XK-related protein; xkr9; ortholog	vesicle budding from membrane(GO:0006900); anatomical structure development(GO:0048856); lipid translocation(GO:0034204); phagocytosis(GO:0006909); membrane invagination(GO:0010324);	engulfment of apoptotic cell(GO:0043652); phosphatidylserine exposure on apoptotic cell surface(GO:0070782); apoptotic process involved in development(GO:1902742)

			phospholipid transport(GO:0015914); endocytosis(GO:0006897); execution phase of apoptosis(GO:0097194); plasma membrane bounded cell projection assembly(GO:0120031); endomembrane system organization(GO:0010256)	
DANRE|ZFIN=ZDB-GENE-110411-7|UniProtKB=E7F1M5	Frk	Tyrosine-protein kinase; frk; ortholog	cell population proliferation(GO:0008283); peptidyl-tyrosine phosphorylation(GO:0018108); cell differentiation(GO:0030154); transmembrane receptor protein tyrosine kinase signaling pathway(GO:0007169); regulation of cell population proliferation(GO:0042127)	protein phosphorylation(GO:0006468); transmembrane receptor protein tyrosine kinase signaling pathway(GO:0007169); phosphorylation(GO:0016310); cell differentiation(GO:0030154); peptidyl-tyrosine autophosphorylation(GO:0038083); regulation of cell population proliferation(GO:0042127); innate immune response(GO:0045087)
DANRE|ZFIN=ZDB-GENE-040625-27|UniProtKB=A2BHI1	Rac2	Rac family small GTPase 2; rac2; ortholog	regulation of cell shape(GO:0008360); motor neuron axon guidance(GO:0008045); cell projection assembly(GO:0030031); establishment or maintenance of cell polarity(GO:0007163); actin filament organization(GO:0007015); Rho protein signal transduction(GO:0007266); endocytosis(GO:0006897); regulation of leukocyte migration(GO:0002685); cortical cytoskeleton organization(GO:0030865); regulation of actin cytoskeleton organization(GO:0032956); vesicle budding from membrane(GO:0006900); phagocytosis(GO:0006909); membrane invagination(GO:0010324); myeloid leukocyte migration(GO:0097529)	neutrophil homeostasis(GO:0001780); neutrophil mediated immunity(GO:0002446); regulation of leukocyte migration(GO:0002685); actin filament organization(GO:0007015); establishment or maintenance of cell polarity(GO:0007163); small GTPase mediated signal transduction(GO:0007264); motor neuron axon guidance(GO:0008045); regulation of cell shape(GO:0008360); response to wounding(GO:0009611); Rac protein signal transduction(GO:0016601); cell projection assembly(GO:0030031); neutrophil chemotaxis(GO:0030593); cortical cytoskeleton organization(GO:0030865); regulation of establishment or maintenance of cell polarity(GO:0032878); regulation of actin cytoskeleton organization(GO:0032956); defense response to bacterium(GO:0042742); engulfment of apoptotic cell(GO:0043652); macrophage chemotaxis(GO:0048246); defense response to fungus(GO:0050832); regulation of neutrophil chemotaxis(GO:0090022);

				regulation of neutrophil migration(GO:1902622); macrophage migration(GO:1905517); neutrophil migration(GO:1990266); positive regulation of neutrophil extravasation(GO:2000391)
DANRE|ZFIN=ZDB-GENE-990415-79|UniProtKB=Q90YL6	NR5A2	Nr5a2 protein; nr5a2; ortholog	transcription by RNA polymerase II(GO:0006366); positive regulation of transcription by RNA polymerase II(GO:0045944); tissue development(GO:0009888); hormone-mediated signaling pathway(GO:0009755)	liver development(GO:0001889); regulation of transcription, DNA-templated(GO:0006355); regulation of transcription by RNA polymerase II(GO:0006357); hormone-mediated signaling pathway(GO:0009755); tissue development(GO:0009888); exocrine pancreas development(GO:0031017); digestive tract development(GO:0048565); cartilage development(GO:0051216); hepatoblast differentiation(GO:0061017)
DANRE|ZFIN=ZDB-GENE-090311-6|UniProtKB=A0A2R8QPJ6	PLXNA2	Plexin A2; plxna2; ortholog	regulation of cell shape(GO:0008360); positive regulation of axonogenesis(GO:0050772); negative regulation of cell adhesion(GO:0007162); regulation of GTPase activity(GO:0043087); axon guidance(GO:0007411); cell adhesion(GO:0007155); cell surface receptor signaling pathway(GO:0007166); regulation of cell migration(GO:0030334); cell migration(GO:0016477)	eye development(GO:0001654); optic vesicle formation(GO:0003403); negative regulation of cell adhesion(GO:0007162); signal transduction(GO:0007165); regulation of cell shape(GO:0008360); regulation of cell migration(GO:0030334); regulation of GTPase activity(GO:0043087); positive regulation of axonogenesis(GO:0050772); semaphorin-plexin signaling pathway(GO:0071526); semaphorin-plexin signaling pathway involved in axon guidance(GO:1902287)
DANRE|ZFIN=ZDB-GENE-040927-11|UniProtKB=Q642J5	rbfox1	RNA binding protein fox-1 homolog 1; rbfox1; ortholog	nervous system development(GO:0007399)	regulation of alternative mRNA splicing, via spliceosome(GO:0000381); mRNA processing(GO:0006397); nervous system development(GO:0007399); RNA splicing(GO:0008380); hypothalamus development(GO:0021854); regulation of RNA splicing(GO:0043484); heart contraction(GO:0060047)
DANRE|ZFIN=ZDB-GENE-030616-15|UniProtKB=A8WHU6	SCEL	Sciellin; scel; ortholog	epidermis development(GO:0008544)	epidermis development(GO:0008544)

DANRE|ZFIN=ZDB-GENE-041210-23|UniProtKB=F6P4K3	PDE3A	Phosphodiesterase; pde3a; ortholog	germ cell development(GO:0007281); cGMP-mediated signaling(GO:0019934); positive regulation of multi-organism process(GO:0043902); positive regulation of multicellular organismal process(GO:0051240); positive regulation of cell development(GO:0010720); negative regulation of cAMP-mediated signaling(GO:0043951); cAMP-mediated signaling(GO:0019933)	oocyte maturation(GO:0001556); signal transduction(GO:0007165); cAMP-mediated signaling(GO:0019933); cGMP-mediated signaling(GO:0019934); negative regulation of cAMP-mediated signaling(GO:0043951); positive regulation of oocyte development(GO:0060282)
DANRE|ZFIN=ZDB-GENE-040912-89|UniProtKB=Q66HU1	CDC42EP3	CDC42 effector protein (Rho GTPase-binding) 3; cdc42ep3; ortholog	regulation of cell shape(GO:0008360); regulation of plasma membrane bounded cell projection organization(GO:0120035); regulation of cell projection assembly(GO:0060491); cell morphogenesis(GO:0000902); actin filament polymerization(GO:0030041); positive regulation of actin filament polymerization(GO:0030838); Rho protein signal transduction(GO:0007266); plasma membrane bounded cell projection assembly(GO:0120031); positive regulation of cell projection organization(GO:0031346)	Rho protein signal transduction(GO:0007266); regulation of cell shape(GO:0008360); positive regulation of actin filament polymerization(GO:0030838); positive regulation of pseudopodium assembly(GO:0031274)
DANRE|ZFIN=ZDB-GENE-040426-2018|UniProtKB=A0A0R4IS50	DUSP1	Dual specificity protein phosphatase; dusp1; ortholog	inactivation of MAPK activity(GO:0000188); MAPK cascade(GO:0000165); mesoderm formation(GO:0001707)	inactivation of MAPK activity(GO:0000188); endoderm formation(GO:0001706); protein dephosphorylation(GO:0006470); dephosphorylation(GO:0016311); peptidyl-tyrosine dephosphorylation(GO:0035335); peptidyl-threonine dephosphorylation(GO:0035970); negative regulation of p38MAPK cascade(GO:1903753)
DANRE|ZFIN=ZDB-GENE-040516-11|UniProtKB=A0A0R4IBL7	TCF12	Transcription factor 12; tcf12; ortholog	neuron differentiation(GO:0030182); positive regulation of neuron differentiation(GO:0045666); transcription by RNA polymerase II(GO:0006366); cell development(GO:0048468); positive regulation of transcription by RNA polymerase II(GO:0045944)	regulation of transcription by RNA polymerase II(GO:0006357)
DANRE|ZFIN=ZDB-GENE-010724-10|UniProtKB=F1Q9D9	epha3	Ephrin type-A receptor 3; epha3; ortholog	axon guidance(GO:0007411)	somitogenesis(GO:0001756); protein phosphorylation(GO:000646

				8); transmembrane receptor protein tyrosine kinase signaling pathway(GO:0007169); multicellular organism development(GO:0007275); axon guidance(GO:0007411); mesoderm migration involved in gastrulation(GO:0007509); phosphorylation(GO:0016310); positive regulation of kinase activity(GO:0033674)
DANRE|ZFIN=ZDB-GENE-041111-216|UniProtKB=B0UY61	NEB	Nebulin; neb; ortholog	myofibril assembly(GO:0030239); actin filament organization(GO:0007015); cardiac muscle cell differentiation(GO:0055007); muscle fiber development(GO:0048747)	muscle fiber development(GO:0048747); cardiac muscle thin filament assembly(GO:0071691)
DANRE|ZFIN=ZDB-GENE-990415-58|UniProtKB=O13146	epha3	Ephrin type-A receptor 3; epha3; ortholog	axon guidance(GO:0007411)	protein phosphorylation(GO:0006468); transmembrane receptor protein tyrosine kinase signaling pathway(GO:0007169); multicellular organism development(GO:0007275); axon guidance(GO:0007411); regulation of epithelial to mesenchymal transition(GO:0010717); phosphorylation(GO:0016310); cell migration(GO:0016477); regulation of actin cytoskeleton organization(GO:0032956); positive regulation of kinase activity(GO:0033674); regulation of GTPase activity(GO:0043087); regulation of focal adhesion assembly(GO:0051893); regulation of microtubule cytoskeleton organization(GO:0070507); fasciculation of sensory neuron axon(GO:0097155); fasciculation of motor neuron axon(GO:0097156)
DANRE|ZFIN=ZDB-GENE-030131-8760|UniProtKB=E7FB26	Elf3	E74-like factor 3 (ets domain transcription factor, epithelial-specific); elf3; ortholog	cell differentiation(GO:0030154); transcription by RNA polymerase II(GO:0006366); regulation of transcription by RNA polymerase II(GO:0006357)	regulation of transcription, DNA-templated(GO:0006355); regulation of transcription by RNA polymerase II(GO:0006357); transcription by RNA polymerase II(GO:0006366); inflammatory response(GO:0006954); cell differentiation(GO:0030154); epithelial cell differentiation(GO:0030855)

DANRE|ZFIN=ZDB-GENE-050522-73|UniProtKB=Q503L1	phlda2	Pleckstrin homology-like domain family A member 2; phlda2; ortholog	reproductive structure development(GO:0048608); animal organ development(GO:0048513)	positive regulation of apoptotic process(GO:0043065)
DANRE|ZFIN=ZDB-GENE-040625-164|UniProtKB=A0M8V6	CAV2	Caveolin; cav2; ortholog	lipid transport(GO:0006869); membrane organization(GO:0061024); cellular component assembly(GO:0022607); cell differentiation(GO:0030154); endomembrane system organization(GO:0010256)	negative regulation of endothelial cell proliferation(GO:0001937); insulin receptor signaling pathway(GO:0008286); cell differentiation(GO:0030154); positive regulation of MAPK cascade(GO:0043410); regulation of cytosolic calcium ion concentration(GO:0051480); caveola assembly(GO:0070836)
DANRE|ZFIN=ZDB-GENE-030131-9|UniProtKB=Q6PVV8	SPARC	SPARC; sparc; ortholog	anatomical structure development(GO:0048856)	erythrocyte differentiation(GO:0030218); otic vesicle formation(GO:0030916); otolith morphogenesis(GO:0032474); semicircular canal morphogenesis(GO:0048752); anatomical structure development(GO:0048856); cartilage development(GO:0051216); pharyngeal system development(GO:0060037)
DANRE|Ensembl=ENSDARG00000089066|UniProtKB=A0A1L1QZ88	Nhsl2	NHS-like 2; nhsl2; ortholog	cell differentiation(GO:0030154)	cell differentiation(GO:0030154)

Supplemental Table 3: Differentially expressed genes related to muscle fiber development, muscle contraction, and muscle differentiation between larvae sampled at day five between the control and hypoxic treatment.

Gene ID	Mapped ID	Gene name Gene symbol	PANTHER GO - Slim Biological Process	GO database Biological Process Complete
DANRE|ZFIN=ZDB-GENE-040923-2|UniProtKB=Q7ZT82	RBFOX1L	RNA binding protein fox-1 homolog 1-like; rbfox1l; ortholog	nervous system development(GO:0007399)	regulation of alternative mRNA splicing, via spliceosome(GO:0000381); heart process(GO:0003015); mRNA processing(GO:0006397); nervous system development(GO:0007399); RNA splicing(GO:0008380); regulation of RNA splicing(GO:0043484); cardiac muscle fiber development(GO:0048739); skeletal muscle fiber development(GO:0048741)
DANRE|ZFIN=ZDB-GENE-030131-55|UniProtKB=Q6XNL8	ACTA1B	Actin; acta1b; ortholog	establishment of mitotic spindle orientation(GO:0000132); organelle transport along microtubule(GO:0072384); nuclear migration(GO:0007097); mitotic nuclear division(GO:0140014)	embryonic heart tube development(GO:0035050); skeletal muscle fiber development(GO:0048741)
DANRE|ZFIN=ZDB-GENE-040303-1|UniProtKB=B8A566	DAB2	DAB adaptor protein 2; dab2; ortholog	negative regulation of canonical Wnt signaling pathway(GO:0090090); vesicle budding from membrane(GO:0006900); positive regulation of cellular component organization(GO:0051130); canonical Wnt signaling pathway(GO:0060070); membrane invagination(GO:0010324); positive regulation of transport(GO:0051050); regulation of endocytosis(GO:0030100); receptor-mediated endocytosis(GO:0006898)	angiogenesis(GO:0001525); vasculature development(GO:0001944); receptor-mediated endocytosis(GO:0006898); Notch signaling pathway(GO:0007219); regulation of BMP signaling pathway(GO:0030510); positive regulation of endocytosis(GO:0045807); negative regulation of canonical Wnt signaling pathway(GO:0090090); regulation of cardiac muscle cell differentiation(GO:2000725)
DANRE|ZFIN=ZDB-GENE-030131-73|UniProtKB=Q503K1	TGFBI	Transforming growth factor, beta-induced; tgfbi; ortholog	extracellular matrix organization(GO:0030198); cell adhesion(GO:0007155)	cell adhesion(GO:0007155); cell population proliferation(GO:0008283); extracellular matrix organization(GO:0030198); muscle fiber development(GO:0048747); cellular response to xenobiotic stimulus(GO:0071466)
DANRE|ZFIN=ZDB-GENE-050302-78|UniProtKB=A0A0R4IBT9	SCUBE1	Signal peptide, CUB domain, EGF-like 1; scube1; ortholog	signal transduction(GO:0007165)	signal transduction(GO:0007165); smoothened signaling pathway(GO:0007224); BMP signaling pathway(GO:0030509); muscle fiber development(GO:0048747); primitive hemopoiesis(GO:0060215)
DANRE|ZFIN=ZDB-GENE-041111-216|UniProtKB=B0UY61	NEB	Nebulin; neb; ortholog	myofibril assembly(GO:0030239); actin filament organization(GO:0007015); cardiac muscle cell differentiation(GO:0055007); muscle fiber development(GO:0048747)	muscle fiber development(GO:0048747); cardiac muscle thin filament assembly(GO:0071691)
DANRE|ZFIN=ZDB-GENE-020905-1|UniProtKB=Q5U3A4	atp2a1	Calcium-transporting ATPase; atp2a1; ortholog	calcium ion transmembrane transport(GO:0070588); cellular calcium ion homeostasis(GO:0006874)	ion transport(GO:0006811); calcium ion transport(GO:0006816); cellular calcium ion homeostasis(GO:0006874); regulation of muscle contraction(GO:0006937); regulation of striated muscle contraction(GO:0006942); response to mechanical stimulus(GO:0009612); positive regulation of fast-twitch skeletal muscle fiber contraction(GO:0031448); negative regulation of muscle contraction(GO:0045932); negative regulation of striated muscle contraction(GO:0045988); calcium ion transmembrane transport(GO:0070588); relaxation of skeletal muscle(GO:0090076)

Supplemental Table 4 – Developmental genes differentially expressed unique to the hypoxic treatment between birth and the 5-day exposure:

Gene ID	Mapped IDs	Gene Name Gene Symbol Ortholog	PANTHER GO-Slim Biological Process	GO Database Biological Process Complete	Regulation
DANRE|ZFIN=ZDB-GENE-990415-121|UniProtKB=Q9PWM6	hoxd9a	Homeobox protein Hox-D9a;hoxd9a;ortholog	chordate embryonic development(GO:0043009);skeletal system development(GO:0001501);embryonic morphogenesis(GO:0048598);anterior/posterior pattern specification(GO:0009952);transcription by RNA polymerase II(GO:0006366);embryonic organ development(GO:0048568);positive regulation of transcription by RNA polymerase II(GO:0045944);animal organ morphogenesis(GO:0009887)	transcription, DNA-templated(GO:0006351);regulation of transcription, DNA-templated(GO:0006355);regulation of transcription by RNA polymerase II(GO:0006357);multicellular organism development(GO:0007275);anterior/posterior pattern specification(GO:0009952);proximal/distal pattern formation(GO:0009954);embryonic skeletal system morphogenesis(GO:0048704)	Up
DANRE|Ensembl=ENSDARG00000068288|UniProtKB=F1QIJ3	LAMC2	Laminin, gamma 2;lamc2;ortholog	tissue development(GO:0009888);animal organ morphogenesis(GO:0009887)	neuromuscular junction development(GO:0007528);animal organ morphogenesis(GO:0009887);tissue development(GO:0009888);receptor clustering(GO:0043113)	Up
DANRE|ZFIN=ZDB-GENE-040426-1758|UniProtKB=A0A0R4IXA9	Cap2	Adenylyl cyclase-associated protein;cap2;ortholog	actin polymerization or depolymerization(GO:0008154);regulation of adenylate cyclase activity(GO:0045761);cell morphogenesis(GO:0000902);establishment or maintenance of cell polarity(GO:0007163)	cell morphogenesis(GO:0000902);cytoskeleton organization(GO:0007010);cAMP-mediated signaling(GO:0019933);regulation of adenylate cyclase activity(GO:0045761)	Up
DANRE|ZFIN=ZDB-GENE-040426-2089|UniProtKB=A0A2R8PZI6	Hapln3	Hyaluronan and proteoglycan link protein 3;hapln3;ortholog	central nervous system development(GO:0007417);skeletal system development(GO:0001501)	skeletal system development(GO:0001501);cell adhesion(GO:0007155);central nervous system development(GO:0007417)	Up
DANRE|Ensembl=ENSDARG00000078302|UniProtKB=E7FFP8	BRINP1	Bone morphogenetic protein/retinoic acid-inducible neural-specific 1;brinp1;ortholog	response to acid chemical(GO:0001101); neuron differentiation(GO:0030182); cellular response to lipid(GO:0071396); mitotic nuclear division(GO:0140014); negative regulation of mitotic cell cycle(GO:0045930); positive regulation of neuron differentiation(GO:0045666); cell development(GO:0048468); cellular response to oxygen-containing compound(GO:1901701)	cell cycle(GO:0007049); cell cycle arrest(GO:0007050); positive regulation of neuron differentiation(GO:0045666); negative regulation of mitotic cell cycle(GO:0045930); cellular response to retinoic acid(GO:0071300)	Down
DANRE|ZFIN=ZDB-GENE-080327-7|UniProtKB=F1QE14	Slitrk2	SLIT and NTRK-like family, member 2;slitrk2;ortholog	axonogenesis(GO:0007409)	axonogenesis(GO:0007409); glial cell development(GO:0021782); retina development in camera-type eye(GO:0060041); regulation of presynapse assembly(GO:1905606)	Down
DANRE|ZFIN=ZDB-GENE-980526-	ihhb	Indian hedgehog B protein;ihhb;ortholog	regulation of gene expression(GO:0010468); gene expression(GO:0010467); smoothened signaling pathway(GO:0007224); cell fate specification(GO:0001708)	cell fate specification(GO:0001708); proteolysis(GO:0006508); smoothened signaling pathway(GO:0007224); cell-cell signaling(GO:0007267); multicellular organism development(GO:0007275); regulation of gene	Down

135|UniProtKB=Q98862				expression(GO:0010468); intein-mediated protein splicing(GO:0016539); protein autoprocessing(GO:0016540); oligodendrocyte differentiation(GO:0048709); striated muscle cell development(GO:0055002); regulation of oligodendrocyte progenitor proliferation(GO:0070445)	
DANRE|ZFIN=ZDB-GENE-050523-3|UniProtKB=Q5EGE1	ism1	Isthmin-1;ism1;ortholog	angiogenesis(GO:0001525); negative regulation of multicellular organismal process(GO:0051241); regulation of anatomical structure morphogenesis(GO:0022603); regulation of multicellular organismal development(GO:2000026)	angiogenesis(GO:0001525); negative regulation of angiogenesis(GO:0016525); erythrocyte differentiation(GO:0030218); embryonic hemopoiesis(GO:0035162); hematopoietic stem cell proliferation(GO:0071425)	Down
DANRE|ZFIN=ZDB-GENE-980526-437|UniProtKB=Q07998	tbxta	T-box transcription factor T-A;tbxta;ortholog	negative regulation of transcription by RNA polymerase II(GO:0000122); heart morphogenesis(GO:0003007); somitogenesis(GO:0001756); transcription by RNA polymerase II(GO:0006366); mesoderm formation(GO:0001707); cell fate specification(GO:0001708); positive regulation of transcription by RNA polymerase II(GO:0045944)	mesoderm formation(GO:0001707); cell fate specification(GO:0001708); somitogenesis(GO:0001756); liver development(GO:0001889); heart looping(GO:0001947); heart morphogenesis(GO:0003007); determination of left/right asymmetry in lateral mesoderm(GO:0003140); embryonic heart tube morphogenesis(GO:0003143); regulation of transcription, DNA-templated(GO:0006355); regulation of transcription by RNA polymerase II(GO:0006357); multicellular organism development(GO:0007275); determination of left/right symmetry(GO:0007368); mesoderm development(GO:0007498); specification of animal organ position(GO:0010159); notochord formation(GO:0014028); Wnt signaling pathway(GO:0016055); notochord development(GO:0030903); pancreas development(GO:0031016); determination of left/right asymmetry in diencephalon(GO:0035462); pronephric glomerulus morphogenesis(GO:0035775); post-anal tail morphogenesis(GO:0036342); regulation of endodermal cell fate specification(GO:0042663); digestive tract development(GO:0048565); convergent extension involved in axis elongation(GO:0060028); notochord cell differentiation(GO:0060034); somite development(GO:0061053); determination of heart left/right asymmetry(GO:0061371); Kupffer's vesicle development(GO:0070121); convergent extension involved in nephron morphogenesis(GO:0072045); regulation of BMP signaling pathway involved in heart jogging(GO:2000223)	Down
DANRE|ZFIN=ZDB-GENE-030131-2427|UniProtKB=E7FA40	Col7a1	Collagen, type VII, alpha 1;col7a1;ortholog	tissue morphogenesis(GO:0048729); cell morphogenesis involved in differentiation(GO:0000904); bone morphogenesis(GO:0060349); chondrocyte differentiation(GO:0002062); developmental growth(GO:0048589)	cell adhesion(GO:0007155)	Down
DANRE|ZFIN=ZDB-GENE-040426-1130|UniProtKB=F1Q7R2	CHAD	Chondroadherin;chad;ortholog	axon guidance(GO:0007411)	axon guidance(GO:0007411); negative chemotaxis(GO:0050919)	Down

Supplemental Table 5 – Gene enrichment analysis for gopher rockfish Hypoxia Day 1 > Day 5 exposure with zebrafish as the reference species.

Enrichment FDR	Genes in list	Total genes	Functional Category	Genes
0.037068779	2	4	Thiosulfate transport	SLC25A10 SLC25A14
0.037068779	2	4	Oxaloacetate transport	SLC25A10 SLC25A14
0.037068779	2	4	Malate transport	SLC25A10 SLC25A14
0.037068779	2	4	Malate transmembrane transport	SLC25A10 SLC25A14
0.037068779	2	4	Oxaloacetate(2-) transmembrane transport	SLC25A10 SLC25A14

Supplemental Table 6 – Gene enrichment analysis of DEGs in the control treatment from day 1 to day 5: Enrichment for zebrafish

Enrichment FDR	Genes in list	Total genes	Functional Category	Genes
1.53E-10	112	1084	Oxidation-reduction process	ECHS1 GYS2 ACO2 PTGES GSTO1 NNT NDUFAF1 ACO1 CYP2U1 CPT2 ACOX3 AUH LOXL2A LOXL2B STEAP4 UQCRH SDHAF2 EHHADH STEAP3 RB1CC1 ACADL SLC25A12 HSD17B8 CYP11A1 HSDL2 MIOX CRYL1 DHFR SUCLA2 ETHE1 CYP20A1 FAR1 RDH5 KMO UEVLD P4HA2 CP MICAL1 RNLS ALDH3B1 LBR PYROXD2 PGD DLAT CRYZ DHTKD1 PNPO CYBA ALDH7A1 GSR MICAL2B FAXDC2 IDH1 AGMO PRXL2B PHYHD1 IDH3A HADH HIF1AN HSPBAP1 GLDC AKR1A1A HSD17B12B OGFOD1 ALDH8A1 HSD3B7 ALDH9A1B NDUFB6 ALDH4A1 ASPDH TYR AGPS IVD PRDX6 TXN IDH3B SUCLG2 CH25H DECR2 PTGS1 FDX2 BDH2 CYP8B1 SORD ALDH6A1 ALDH1A2 RPE65C XDH ASPH GSTK1 AIFM1 SELENOT1A FOXRED2 NOS1 CYP1B1 P3H1 TDO2A DHRS7CB FH P4HTM NDUFB3 ALDH1L1 CYP26B1 PHKB SH3BGRL3 SDR42E1 FOXRED1 PAM ME2 HSD17B4 NDUFC2 CAT
2.24E-10	343	4854	Cellular nitrogen compound metabolic process	DHFR ZMAT2 RPE MTFMT ETHE1 EXOSC9 GNMT FAR1 RECQL MEOX1 SART3 TRMT61A HSF1 MYOG HNF1A XRCC1 CCNC SOX19A SOX5 PAPOLG RGMA METTL3 ASAH2 ESCO2 RBFOX1 POLA2 PRPF19 HDAC1 FXR2 DKC1 RBM8A PRX HAL PNPO SEPHS2 SF3B2 NEIL1 WDR33 SF3B4 GCLM NLN GSR PTGES SF3A2 ORC5 SFR1 TRAF3 NNT LSM4 IDH1 NDUFAF1 UBE2V2 TFCP2L1 SGMS1 MYF6 DNMT1 MYBL1 LEF1 HIF1AN DIS3L2 MRRF CPSF1 OGFOD1 NFATC1 RBM4.1 POP7 TBPL1 ST6GALNAC6 TTR TAF11 TPK1 RPRD1B ASCL1A SULF1 MAGOH RAD50 XPC SYNCRIP RSL24D1 INTS14 UBE2V1 PEBP1 RFC4 ALLC GTF3C6 RBBP8 PPIL4 PARS2 PCF11 ERI1 CH25H MCM10 MED20 RBFOX2 PCGF5B PHMT1 ALDH6A1 FANCD2 TRUB1 FBL PGK1 MRPL1 XDH MCMBP KAT6B MTO1 RFC3 HELT RPIA NSUN2 ITPA NR1H4 ENDOG UQCRH HNRNPA3 MYEF2 OGG1 MTRF1L DIS3 NUDT14 MRPL2 SDHAF2 MSH3 GFM1 MT-ATP6 PRPF4 PPARGC1A KLF9 NOS1 MPG DEK CHAC1 GCH1 E2F3 CENPS HEY1 GAMT TDO2A RRP8 TDP1 TFB2M CRTC2 SALL2 PMS2 MRPL47 ASXL2 METTL1 RAI1 ELK4 ZNF296 DPH2 TCF20 DNA2 MRPL58 DHX9 ACADL EGR3 MRPL52 CSTF2 AEBP1 POLD2 SLIRP SMPD3 PAM ALAD LNPEP SMG6 GART POLRMT MYRFL TAF2 SARS2 TRMT13 RRN3 ZFHX3 RRP1 TUFM NOP10 LTO1 MSH6 DR1 SRSF4 PARK7 CBFB BHLHE40 FOXN4 HES6 SCUBE1 BPTF RXRBA MEN1 CRY2 RIPK2 AFF4 DBX1B RTCA DMBX1B TBX15 RUVBL1 NEUROD4 RPL3 POLB NELFE PPAT KMT2A TCEA3 GFM2 MLLT3 KMO SREK1 SMPDL3B IGF2BP3 UMPS EIF3JA ATF6 MED22 ATP5PF PGD QTRT2 ATIC NR2E1 EIF5A LARP7 MICAL2B HNF4A ARNT ALG5 UCK2B PRKCBB STAT3 ATF2 GMPPAA ETS1 L3MBTL3 KHSRP APTX CHCHD1 PARPBP NUDT1 PRIMPOL MDM2 SEPSECS RSRC1 EEF2K CTDSP2 NDUFB6 MTAP ATOH8 ACSS2 ASPDH VGLL3 ZFYVE26 SOX19B RX2 DRAP1 DMAP1 TRMT6 PTBP3 ACSS1 MAFAA ATF1 SUCLG2 RNF8 IRF5 GLI3 FAM172A PNRC2 GEMIN8 HOXB5B HOXB1B FOSB NR4A3 HOXB13A NEUROG1 SF3B1 CREB3L3A NT5C3A MRPL4 NHEJ1 EXOSC1 USP16 NELFA GBA2 EIF2AK3 MRPS5 MRPL9 GTF3C5 EIF2AK2 FANCE BCL6B PITX3 HOXC5A HOXC8A NT5E OLIG3 MRM2 MRPL17 MRI1 MRPL43 GAR1 ALDH1L1 YEATS2 GEMIN5 AFF3 RNASEH2C SMC6 MRPL34 PANK4 SRSF11 POLR2B VDRA TYMP RPS27L MCM7 ORC2 MRPS7 XRN2 NDUFC2 NOTCH1A ACTR8 ARID3B MRPS30 AKAP17A DLAT SLC33A1 DAB2 ZNF703 TNPO3 PPP4CB SLC22A5 ZDHHC13 ILF2 MYSM1 LOXL2A LOXL2B INSM1B MOV10B.2 SPX COPS5
9.66E-09	310	4426	Organic cyclic compound metabolic process	CYP11A1 DHFR ZMAT2 RPE MTFMT EXOSC9 GNMT FAR1 RECQL MEOX1 SART3 TRMT61A HSF1 MYOG HNF1A XRCC1 CCNC SOX19A SOX5 PAPOLG RGMA METTL3 LBR ESCO2 RBFOX1 POLA2 PRPF19 HDAC1 FXR2 DKC1 RBM8A PRX HAL PNPO SEPHS2 SF3B2 NEIL1 WDR33 SF3B4 SF3A2 ORC5 SFR1 TRAF3 NNT FAXDC2 LSM4 IDH1 NDUFAF1 TTPA UBE2V2 TFCP2L1 CLYBL MYF6 DNMT1 MYBL1 LEF1 HIF1AN DIS3L2 CPSF1 NFATC1 RBM4.1 POP7 TBPL1 TTR TAF11 TPK1 RPRD1B ASCL1A SULF1 MAGOH RAD50 TYR XPC SYNCRIP INTS14 UBE2V1 RFC4 ALLC GTF3C6 RBBP8 PPIL4 PARS2 PCF11 ERI1 CH25H MCM10 MED20 RBFOX2 PCGF5B SHMT1 ALDH6A1 FANCD2 TRUB1 FBL PGK1 MRPL1 XDH MCMBP KAT6B MTO1 RFC3 HELT RPIA NSUN2 ITPA NR1H4 ENDOG UQCRH HNRNPA3 MYEF2 OGG1 DIS3 NUDT14 SDHAF2 MSH3 MT-ATP6 PRPF4 PPARGC1A KLF9 NOS1 MPG DEK GCH1 E2F3 CENPS HEY1 GAMT TDO2A RRP8 TDP1 TFB2M ARV1 CRTC2 SALL2 PMS2 ASXL2 METTL1 RAI1 ELK4 ZNF296 TCF20 OAT DNA2 DHX9 EGR3 CSTF2 POLD2 SLIRP ALAD SMG6 FDFT1 GART POLRMT MYRFL TAF2 SARS2 TRMT13 RRN3 ZFHX3 RRP1 NOP10 LTO1 MSH6 DR1 SRSF4 PARK7 CBFB BHLHE40 FOXN4 HES6 SCUBE1 BPTF RXRBA MEN1 CRY2 RIPK2 AFF4 DBX1B RTCA DMBX1B TBX15 RUVBL1 NEUROD4 POLB NELFE PPAT KMT2A TCEA3 MMADHC MLLT3 KMO SREK1 UMPS ATF6 MED22 ATP5PF PGD QTRT2 ATIC NR2E1 LARP7 MICAL2B HNF4A ARNT ALG5 UCK2B PRKCBB STAT3 ATF2 GMPPAA ETS1 L3MBTL3 KHSRP APTX PARPBP NUDT1 PRIMPOL MDM2 SEPSECS RSRC1 HSD17B12B CTDSP2 ALDH8A1 HSD3B7 NDUFB6 MTAP ATOH8 ACSS2 ALDH4A1 ASPDH VGLL3 ZFYVE26 SOX19B RX2 DRAP1 DMAP1 TRMT6 PTBP3 ACSS1 MAFAA ATF1 SUCLG2 RNF8 IRF5 GLI3 FAM172A PNRC2 GEMIN8 HOXB5B HOXB1B FOSB NR4A3 HOXB13A NEUROG1 SF3B1 CREB3L3A NT5C3A NHEJ1 EXOSC1 USP16 NELFA GTF3C5 FANCE BCL6B PITX3 HOXC5A HOXC8A NT5E OLIG3 MRM2 MRI1 GAR1 ALDH1L1 YEATS2 AFF3 RNASEH2C AEBP1 SMC6 PANK4 SRSF11 POLR2B SDR42E1 VDRA TYMP MCM7 ORC2 XRN2 NDUFC2 NOTCH1A ACTR8 ARID3B AKAP17A DLAT SLC33A1 FLVCR1 DAB2 ZNF703 TNPO3 BDH2 PPP4CB SLC22A5 ZDHHC13 ILF2 MYSM1 LOXL2A LOXL2B INSM1B MOV10B.2 SPX COPS5
2.66E-08	299	4290	Heterocycle metabolic process	DHFR ZMAT2 RPE MTFMT EXOSC9 GNMT FAR1 RECQL MEOX1 SART3 TRMT61A HSF1 MYOG HNF1A XRCC1 CCNC SOX19A SOX5 PAPOLG RGMA METTL3 ESCO2 RBFOX1 POLA2 PRPF19 HDAC1 FXR2 DKC1 RBM8A PRX HAL PNPO SEPHS2 SF3B2 NEIL1 WDR33 SF3B4 SF3A2 ORC5 SFR1 TRAF3 NNT LSM4 IDH1 NDUFAF1 TTPA UBE2V2 TFCP2L1 CLYBL MYF6 DNMT1 MYBL1 LEF1 HIF1AN DIS3L2 CPSF1 NFATC1 RBM4.1 POP7 TBPL1 TTR TAF11 TPK1 RPRD1B ASCL1A SULF1 MAGOH RAD50 XPC SYNCRIP INTS14 UBE2V1 RFC4 ALLC GTF3C6 RBBP8 PPIL4 PARS2 PCF11 ERI1 MCM10 MED20 RBFOX2 PCGF5B SHMT1 ALDH6A1 FANCD2 TRUB1 FBL PGK1 MRPL1 XDH MCMBP KAT6B MTO1 RFC3 HELT RPIA NSUN2 ITPA NR1H4 ENDOG UQCRH HNRNPA3 MYEF2 OGG1 DIS3 NUDT14 SDHAF2 MSH3 MT-ATP6 PRPF4 PPARGC1A KLF9 NOS1 MPG DEK GCH1 E2F3 CENPS HEY1 GAMT TDO2A RRP8 TDP1 TFB2M CRTC2 SALL2 PMS2 ASXL2 METTL1 RAI1 ELK4 ZNF296 TCF20 OAT DNA2 DHX9 EGR3 CSTF2 POLD2 SLIRP ALAD SMG6 GART POLRMT MYRFL TAF2 SARS2 TRMT13 RRN3 ZFHX3 RRP1 NOP10 LTO1 MSH6 DR1 SRSF4 PARK7 CBFB BHLHE40 FOXN4 HES6 SCUBE1 BPTF RXRBA MEN1 CRY2 RIPK2 AFF4 DBX1B RTCA DMBX1B TBX15 RUVBL1 NEUROD4 POLB NELFE PPAT KMT2A TCEA3 MMADHC MLLT3 KMO SREK1 UMPS ATF6 MED22 ATP5PF PGD QTRT2 ATIC NR2E1 LARP7 MICAL2B HNF4A ARNT ALG5 UCK2B PRKCBB STAT3 ATF2 GMPPAA ETS1 L3MBTL3 KHSRP APTX PARPBP NUDT1 PRIMPOL MDM2 SEPSECS RSRC1 CTDSP2 NDUFB6 MTAP ATOH8 ACSS2 ALDH4A1 ASPDH VGLL3 ZFYVE26 SOX19B RX2 DRAP1 DMAP1 TRMT6 PTBP3 ACSS1 MAFAA ATF1 SUCLG2 RNF8 IRF5 GLI3 FAM172A PNRC2 GEMIN8 HOXB5B HOXB1B FOSB NR4A3 HOXB13A NEUROG1 SF3B1 CREB3L3A NT5C3A NHEJ1 EXOSC1 USP16 NELFA GTF3C5 FANCE BCL6B PITX3 HOXC5A HOXC8A NT5E OLIG3 MRM2 MRI1 GAR1 ALDH1L1 YEATS2 AFF3 RNASEH2C AEBP1 SMC6 PANK4 SRSF11 POLR2B VDRA TYMP MCM7 ORC2 XRN2 NDUFC2 NOTCH1A ACTR8 ARID3B AKAP17A DLAT SLC33A1 FLVCR1 DAB2 ZNF703 TNPO3 BDH2 PPP4CB SLC22A5 ZDHHC13 ILF2 MYSM1 LOXL2A LOXL2B INSM1B MOV10B.2 SPX COPS5
2.66E-08	195	2506	Animal organ development	LGSN MYOG MYBPC3 CACYBP MATN4 COL7A1 PKP2 MYF6 FLVCR1 LEF1 NEB ASCL1A SULF1 KLHL40A TAZ PEBP1 TCTA MYBPC1 HELT POPDC3 HEY1 TACC1 MYORG ASXL2 SEMA7A RFLNB SGCD FZD4 BHLHA15 CRYAA NEUROG1 CDKN1A HNF1A HNF4A GLI3 SGCB ANO6 RIPOR2 KLHL41A OLIG3 SLC25A38B MYMK ZNF703 MYH7 DMBX1B NEUROD4 DLB DHFR KMT2A NEK2 FOXN4 HDAC1 DAG1 DKC1 ALDH7A1 SPARC FZD5 NUMB DNMT1 ATOH8 OTOMP TNPO3 RBFOX2 ALDH1A2 ILK TECTA MAP3K3 PLXNA2 TRIM69 HOXC8A GEMIN5 AP1M1 RGS2 LAMA2 SDC2 DHPS STX16 RPL3 GALNT2 CASP9 NELFE TRAPPC11 CDH17 KATNB1 MEOX1 SART3 RDH5 HSF1 CHRNA1 CCNC KRAS FGFR1A SCUBE3 RCC2 DCN METTL3 ESCO2 RBFOX1 ZC4H2 DDC HAL CELSR2 MICAL2B NONO TAB2 PRKCBB STAT3 ATG5 NOLC1 IDH1 SLC48A1B ERBB2 PODXL RBCK1 DIS3L2 CPSF1 MPPED2 ALDH8A1 TSEN54 SLC2A12 LFNG MACC1 CBFB TNFRSF19 DMAP1 RFC4 NDUFB11 CXADR ANGPTL3 TTC4 TOMM22 PTGS1 SMARCA5 BDH2 GPATCH3 AP2A1 FRAS1 SLC7A7 PRSS23 SF3B1 COPS5 MYEF2 OGG1 AGGF1 BMP3 LRSAM1 ABCC5 XYLT1 HAUS3 EDARADD SCUBE1 RASA3 PRPF4 PPARGC1A SLC39A6 KLF9 NOS1 CYP1B1 BTBD9 VPS4B PDIA5 PITX3 SLC41A1 VPS18 CRIP2 HPS5 P4HTM ALDH1L1 ADAMTS9 METTL22 NOSTRIN SMOC1 CRISPLD2 CLPB WNT16 TBCD PIM1 CAPZB PDE6C PTPRO GART COQ8B HSD17B4 GAS8 PEPD LAMA1 CIB2 ATG7 NOP10 SEC23A PEF1 LOXL2A LOXL2B INSM1B TDRD7B
3.50E-08	307	4449	Anatomical structure development	BHLHE40 CDH17 TENM3 LGSN MEOX1 MYOG SOX19A FGFR1A MYBPC3 CACYBP RBFOX1 ROR1 MATN4 PARVB HES6 TMOD4 COL7A1 PKP2 ERBB2 XKR9 MYF6 FLVCR1 LEF1 NEB PLXNB3 ASCL1A SULF1 KLHL40A INHBB TAZ PEBP1 LOXL2A LOXL2B TCTA MYBPC1 RBFOX2 CDC42EP3 CDC42EP2 RHOF MAP2 HELT ILK NR1H4 POPDC3 AGGF1 PLXNA2 STK26 BMP3 GBA2 AMOTL2A PITX3 RHOV HEY1 RND2 TACC1 MYORG ASXL2 SEMA7A DAGLB RFLNB WNT16 SLIRP SGCD CAPZB TRAK2 CDH1 FZD4 HIF1AN BHLHA15 CRYAA PGRMC1 NEUROG1 CDKN1A DMBX1B DPYSL3 NEUROD4 DLB WNT11 VANGL1 HNF1A FOXN4 DLA RGMA ASAH2 ZC4H2 DLD HNF4A FZD5 ZNF703 BZW2 TBPL1 ATOH8 LFNG RX2 NIFK MAFAA SIAH2L GLI3 SGCB INSM1B HOXB5B HOXB13A TBCCD1 ANO6 RIPOR2 FZD10 KLHL41A HOXC5A HOXC8A CHAC1 VPS18 OLIG3 SLC25A38B PLPPR1 LAMA1 NOTCH1A MYMK NDEL1B VDAC2 PRPH MYH7 SDC2 LATS1 DHFR CASP9 KMT2A NEK2 SCUBE3 ESCO2 HDAC1 RASL11B DAG1 DKC1 ALDH7A1 SPARC CELSR2 STAT3 ETS1 NUMB DNMT1 DAB2 EEF2K CTDSP2 CLDN7A ZFYVE26 OTOMP RAB11A TNFRSF19 MMP9 TNPO3 ALDH1A2 TECTA MAP3K3 TRIM69 SCUBE1 SH3KBP1 PPP4CB TMEM67 BPTF GEMIN5 AP1M1 RGS2 LAMA2 GAS8 CCDC80 CYP11A1 DHPS STX16 RPL3 GALNT2 NELFE PANX3 TRAPPC11 KATNB1 XPNPEP3 SART3 RDH5 MTTP HSF1 CHRNA1 CCNC MSI1 KRAS RCC2 DCN METTL3 LBR BBS12 FAM53B DDC HAL SLC33A1 PTGES MICAL2B NONO TAB2 PRKCBB ATG5 NOLC1 CD63 IDH1 SLC48A1B SUSD4 TTPA TNFAIP3 CNTFR HSPA12B PODXL ACHE RBCK1 DIS3L2 CPSF1 MPPED2 MYSM1 ALDH8A1 TSEN54 SLC2A12 GAB1 SESTD1 MACC1 CBFB DMAP1 RFC4 NDUFB11 CXADR STAU1 PRRC1 ANGPTL3 TTC4 TOMM22 STRA6 IST1 COL4A6 COL4A5 PTGS1 SMARCA5 BDH2 GPATCH3 AP2A1 FRAS1 SLC7A7 PRSS23 SF3B1 COPS5 HOMER2 MYEF2 SDC4 OGG1 SYBU LRRN1 LRSAM1 CHD2 ABCC5 XYLT1 HAUS3 ATP13A2 EDARADD RASA3 PRPF4 PPARGC1A SLC39A6 KLF9 NOS1 CYP1B1 BTBD9 VPS4B PDIA5 SLC41A1 CRIP2 HPS5 TGFBI P4HTM COL19A1 CEP70 ALDH1L1 ASCC1 ADAMTS9 METTL22 NOSTRIN SMOC1 CRISPLD2 CLPB MPC1 LAMC3 SMPD3 IL1B TBCD PIM1 PDE6C PTPRO FKRP SMG6 GART COQ8B ZDHHC13 HSD17B4 PEPD CIB2 ATG7 UCHL5 VIPAS39 NOP10 SEC23A GIPC1 PEF1 AMER2 TDRD7B
4.02E-08	299	4319	Cellular aromatic compound metabolic process	DHFR ZMAT2 RPE MTFMT EXOSC9 GNMT FAR1 RECQL MEOX1 SART3 TRMT61A HSF1 MYOG HNF1A XRCC1 CCNC SOX19A SOX5 PAPOLG RGMA METTL3 ESCO2 RBFOX1 POLA2 PRPF19 HDAC1 FXR2 DKC1 RBM8A PRX HAL PNPO SEPHS2 SF3B2 NEIL1 WDR33 SF3B4 SF3A2 ORC5 SFR1 TRAF3 NNT LSM4 IDH1 NDUFAF1 UBE2V2 TFCP2L1 CLYBL MYF6 DNMT1 MYBL1 LEF1 HIF1AN DIS3L2 CPSF1 NFATC1 RBM4.1 POP7 TBPL1 TTR TAF11 TPK1 RPRD1B ASCL1A SULF1 MAGOH RAD50 TYR XPC SYNCRIP INTS14 UBE2V1 RFC4 GTF3C6 RBBP8 PPIL4 PARS2 PCF11 ERI1 MCM10 MED20 RBFOX2 PCGF5B SHMT1 ALDH6A1 FANCD2 TRUB1 FBL PGK1 MRPL1 XDH MCMBP KAT6B MTO1 RFC3 HELT RPIA NSUN2 ITPA NR1H4 ENDOG UQCRH HNRNPA3 MYEF2 OGG1 DIS3 NUDT14 SDHAF2 MSH3 MT-ATP6 PRPF4 PPARGC1A KLF9 NOS1 MPG DEK GCH1 E2F3 CENPS HEY1 GAMT TDO2A RRP8 TDP1 TFB2M CRTC2 SALL2 PMS2 ASXL2 METTL1 RAI1 ELK4 ZNF296 TCF20 DNA2 DHX9 EGR3 CSTF2 POLD2 SLIRP ALAD SMG6 GART POLRMT MYRFL TAF2 SARS2 TRMT13 RRN3 ZFHX3 RRP1 NOP10 LTO1 MSH6 DR1 SRSF4 PARK7 CBFB BHLHE40 FOXN4 HES6 SCUBE1 BPTF RXRBA MEN1 CRY2 RIPK2 AFF4 DBX1B RTCA DMBX1B TBX15 RUVBL1 NEUROD4 POLB NELFE PPAT KMT2A TCEA3 MMADHC MLLT3 KMO SREK1 UMPS ATF6 MED22 ATP5PF PGD QTRT2 ATIC NR2E1 LARP7 MICAL2B HNF4A ARNT ALG5 UCK2B PRKCBB STAT3 ATF2 GMPPAA ETS1 L3MBTL3 KHSRP APTX PARPBP NUDT1 PRIMPOL MDM2 SEPSECS RSRC1 CTDSP2 ALDH8A1 NDUFB6 MTAP ATOH8 ACSS2 ASPDH VGLL3 ZFYVE26 SOX19B RX2 DRAP1 DMAP1 TRMT6 ALLC EPHX1 PTBP3 ACSS1 MAFAA ATF1 SUCLG2 RNF8 IRF5 GLI3 FAM172A PNRC2 GEMIN8 HOXB5B HOXB1B FOSB NR4A3 HOXB13A NEUROG1 SF3B1 CREB3L3A NT5C3A NHEJ1 EXOSC1 USP16 NELFA GTF3C5 FANCE BCL6B PITX3 HOXC5A HOXC8A NT5E OLIG3 MRM2 MRI1 GAR1 ALDH1L1 YEATS2 AFF3 RNASEH2C AEBP1 SMC6 PANK4 SRSF11 POLR2B VDRA TYMP MCM7 ORC2 XRN2 NDUFC2 NOTCH1A ACTR8 ARID3B AKAP17A DLAT SLC33A1 FLVCR1 DAB2 ZNF703 TNPO3 BDH2 PPP4CB SLC22A5 ZDHHC13 ILF2 MYSM1 LOXL2A LOXL2B INSM1B MOV10B.2 SPX COPS5

5.39E-08	315	4620	Developmental process	LATS1 BHLHE40 CDH17 TENM3 LGSN MEOX1 MYOG SOX19A FGFR1A SOX5 MYBPC3 ABL2 CACYBP RBFOX1 ROR1 MATN4 PARVB HES6 TMOD4 COL7A1 PKP2 ERBB2 XKR9 FRK MYF6 FLVCR1 LEF1 NEB PLXNB3 ASCL1A SULF1 KLHL40A INHBB TAZ PEBP1 LOXL2A LOXL2B TCTA MYBPC1 RBFOX2 CDC42EP3 CDC42EP2 RHOF MAP2 HELT ILK NR1H4 POPDC3 AGGF1 PLXNA2 STK26 BMP3 GBA2 SI:CH211-194E15.5 AMOTL2A PITX3 RHOV HEY1 RND2 TACC1 MYORG ASXL2 ELK4 SEMA7A DAGLB RFLNB WNT16 SLIRP SGCD CAPZB TRAK2 CDH1 FZD4 HIF1AN BHLHA15 CRYAA PGRMC1 NEUROG1 CDKN1A DMBX1B DPYSL3 NEUROD4 DLB WNT11 VANGL1 HNF1A FOXN4 DLA RGMA METTL3 ASAH2 ZC4H2 DLD HNF4A FZD5 ZNF703 BZW2 TBPL1 ATOH8 LFNG RX2 NIFK MAFAA SIAH2L GLI3 SGCB INSM1B HOXB5B HOXB13A TBCCD1 ANO6 RIPOR2 PPARGC1A FZD10 KLHL41A HOXC5A HOXC8A CHAC1 VPS18 OLIG3 SLC25A38B TDRD7B FLNB PLPPR1 LAMA1 NOTCH1A VIPAS39 MYMK NDEL1B VDAC2 PRPH MYH7 SDC2 DHFR CASP9 KMT2A NEK2 SCUBE3 ESCO2 HDAC1 RASL11B DAG1 DKC1 ALDH7A1 SPARC CELSR2 STAT3 ETS1 NUMB DNMT1 DAB2 EEF2K CTDSP2 CLDN7A ZFYVE26 OTOMP RAB11A TNFRSF19 MMP9 TNPO3 ALDH1A2 SF3B1 TECTA MAP3K3 TRIM69 SCUBE1 SH3KBP1 PPP4CB TMEM67 BPTF GEMIN5 AP1M1 RGS2 LAMA2 GAS8 CCDC80 CYP11A1 DHPS STX16 RPL3 GALNT2 NELFE PANX3 TRAPPC11 KATNB1 XPNPEP3 SART3 RDH5 MTTP HSF1 CHRNA1 CCNC MSI1 KRAS RCC2 DCN LBR BBS12 FAM53B DDC HAL SLC33A1 PTGES MICAL2B NONO TAB2 PRKCBB ATG5 NOLC1 CD63 IDH1 SLC48A1B SUSD4 TTPA TNFAIP3 CNTFR HSPA12B PODXL ACHE RBCK1 DIS3L2 CPSF1 MPPED2 MYSM1 ALDH8A1 TSEN54 SLC2A12 GAB1 TYR SESTD1 MACC1 CBFB DMAP1 RFC4 NDUFB11 CXADR STAU1 PRRC1 ANGPTL3 TTC4 TOMM22 STRA6 IST1 COL4A6 COL4A5 PTGS1 SMARCA5 BDH2 GPATCH3 AP2A1 FRAS1 SLC7A7 PRSS23 COPS5 HOMER2 MYEF2 SDC4 OGG1 SYBU LRRN1 LRSAM1 CHD2 ABCC5 XYLT1 HAUS3 ATP13A2 EDARADD RASA3 PRPF4 SLC39A6 KLF9 NOS1 CYP1B1 BTBD9 VPS4B PDIA5 SLC41A1 CRIP2 HPS5 TGFBI P4HTM COL19A1 CEP70 ALDH1L1 ASCC1 ADAMTS9 METTL22 NOSTRIN SMOC1 CRISPLD2 CLPB MPC1 LAMC3 SMPD3 IL1B TBCD PIM1 PDE6C PTPRO FKRP SMG6 GART COQ8B ZDHHC13 HSD17B4 PEPD CIB2 ATG7 UCHL5 NOP10 SEC23A GIPC1 IGSF11 PEF1 AMER2
8.15E-08	281	4039	Multicellular organism development	BHLHE40 TENM3 LGSN MEOX1 MYOG SOX19A FGFR1A MYBPC3 CACYBP RBFOX1 MATN4 HES6 COL7A1 PKP2 ERBB2 MYF6 FLVCR1 LEF1 NEB PLXNB3 ASCL1A SULF1 KLHL40A TAZ PEBP1 LOXL2A LOXL2B TCTA MYBPC1 RBFOX2 MAP2 HELT NR1H4 POPDC3 AGGF1 PLXNA2 STK26 GBA2 AMOTL2A HEY1 TACC1 MYORG ASXL2 SEMA7A DAGLB RFLNB WNT16 SGCD TRAK2 FZD4 HIF1AN BHLHA15 CRYAA NEUROG1 CDKN1A DMBX1B DPYSL3 NEUROD4 DLB WNT11 VANGL1 HNF1A FOXN4 DLA RGMA ASAH2 ZC4H2 DLD HNF4A FZD5 ZNF703 BZW2 TBPL1 ATOH8 LFNG RX2 NIFK MAFAA SIAH2L GLI3 SGCB INSM1B HOXB5B HOXB13A ANO6 RIPOR2 FZD10 KLHL41A PITX3 HOXC5A HOXC8A CHAC1 VPS18 OLIG3 SLC25A38B PLPPR1 LAMA1 NOTCH1A MYMK NDEL1B MYH7 LATS1 DHFR CASP9 KMT2A NEK2 HDAC1 DAG1 DKC1 ALDH7A1 SPARC CELSR2 ETS1 NUMB DNMT1 DAB2 EEF2K CTDSP2 ZFYVE26 OTOMP TNFRSF19 TNPO3 ALDH1A2 ILK TECTA MAP3K3 TRIM69 SH3KBP1 PPP4CB TMEM67 BPTF GEMIN5 AP1M1 RGS2 LAMA2 CDH1 CCDC80 SDC2 DHPS STX16 RPL3 GALNT2 NELFE TRAPPC11 CDH17 KATNB1 XPNPEP3 SART3 RDH5 MTTP HSF1 CHRNA1 CCNC MSI1 KRAS SCUBE3 RCC2 DCN METTL3 LBR ESCO2 BBS12 FAM53B DDC HAL SLC33A1 PTGES MICAL2B NONO TAB2 PRKCBB STAT3 ATG5 NOLC1 CD63 IDH1 SLC48A1B SUSD4 TTPA CNTFR HSPA12B PODXL ACHE RBCK1 DIS3L2 CPSF1 MPPED2 ALDH8A1 TSEN54 SLC2A12 SESTD1 MACC1 CBFB RAB11A DMAP1 RFC4 MMP9 NDUFB11 CXADR PRRC1 ANGPTL3 TTC4 TOMM22 STRA6 IST1 COL4A6 COL4A5 PTGS1 SMARCA5 BDH2 GPATCH3 AP2A1 FRAS1 SLC7A7 PRSS23 SF3B1 COPS5 HOMER2 MYEF2 SDC4 OGG1 SYBU LRRN1 BMP3 LRSAM1 CHD2 ABCC5 XYLT1 HAUS3 ATP13A2 EDARADD SCUBE1 RASA3 PRPF4 PPARGC1A SLC39A6 KLF9 NOS1 CYP1B1 BTBD9 VPS4B PDIA5 SLC41A1 RHOV CRIP2 HPS5 P4HTM COL19A1 CEP70 ALDH1L1 ASCC1 ADAMTS9 METTL22 NOSTRIN SMOC1 CRISPLD2 CLPB MPC1 LAMC3 SMPD3 TBCD PIM1 CAPZB PDE6C PTPRO FKRP SMG6 GART COQ8B ZDHHC13 HSD17B4 GAS8 PEPD CIB2 ATG7 VIPAS39 NOP10 SEC23A GIPC1 PEF1 TDRD7B
1.08E-07	121	1386	Small molecule metabolic process	ECHS1 CYP11A1 GALK2 DHFR RPE GNMT FAR1 ACO2 P4HA2 ASAH2 BNIP2 SLC23A2 HAL MCCC2 PNPO SEPHS2 ABHD3 NNT IDH1 NDUFAF1 ACO1 CYP2U1 TTPA CLYBL TTR MTAP CPT2 ACOX3 GOT1 AUH PARS2 CH25H SHMT1 ALDH6A1 PGK1 XDH RPIA ITPA UQCRH BNIPL NUDT14 SDHAF2 MT-ATP6 NOS1 EHHADH GCH1 GAMT TDO2A GNPAT SDSL P4HTM MRI1 OAT PPIP5K2 ACADL DAGLB ME2 FDFT1 GART COQ8B SARS2 PARK7 RDH5 PFKFB3 MIOX CRYL1 PPAT SRCAP SUCLA2 MMADHC LGSN KMO UEVLD UMPS ATP5PF PGD DLAT DHTKD1 DDC ATIC CRTAP DGAT2 ESD RBKS UCK2B GMPPAA IDH3A HADH GLDC HSD17B12B ALDH8A1 NDUFB6 ACSS2 TPK1 ALDH4A1 ASPDH RAD50 MAT1A ALLC AGPS TXN ACSS1 IDH3B SUCLG2 POFUT2 DECR2 PTGS1 RPE65C HIBCH NT5C3A NT5E FH ALDH1L1 ACACA PANK4 TYMP NDUFC2 LEF1 ALDH1A2 TYR DBT
1.78E-07	70	654	Oxoacid metabolic process	ECHS1 DHFR GNMT ACO2 P4HA2 ASAH2 BNIP2 SLC23A2 HAL MCCC2 SEPHS2 ABHD3 IDH1 ACO1 MTAP CPT2 ACOX3 GOT1 AUH PARS2 SHMT1 ALDH6A1 PGK1 BNIPL SDHAF2 NOS1 EHHADH GAMT TDO2A GNPAT SDSL P4HTM MRI1 OAT ACADL DAGLB ME2 SARS2 PARK7 CRYL1 SRCAP SUCLA2 LGSN KMO UEVLD PGD DLAT DHTKD1 DDC CRTAP IDH3A HADH GLDC HSD17B12B ALDH8A1 ACSS2 ALDH4A1 ACSS1 IDH3B SUCLG2 DECR2 PTGS1 HIBCH GCH1 FH ALDH1L1 ACACA LEF1 TYR DBT
1.88E-07	130	1543	Organic substance transport	NUP155 NUP88 STX16 EXOC4 GRPEL1 NUP35 ANXA13 SLC7A1 SGTB SLC23A2 SYS1 VPS13D SLC6A9 ZDHHC7 SLC25A48 SLC16A7 USP6NL MPC2 PEX14 MFSD2B FLVCR1 SLC38A9 NDFIP2 ZDHHC14 SLC25A33 SLC38A11 ADPRH RABL3 RAB11A IPO4 ARL4D SLC13A1 TNPO3 SLC38A2 STRA6 GOSR2 RAB24 PRELID3A NUTF2 IPO8 SLC19A2 SLC32A1 LRSAM1 SLC28A1 ATP8B1 TBC1D1 SLC6A15 ARL14 RAB8B SLC13A3 SELENOS SLC25A38B ARV1 ATP8A2 ATP10D MPC1 SEC16B CHMP1B RAB21 SLC25A12 TRAK2 SLC26A5 SLC22A5 PANX3 OSBP STAM2 SLC7A9 MTTP UEVLD SLC35E4 HNF1A IGF2BP3 SLC5A1 BCAP29 RBM8A COPE SEC61G SLC33A1 HGS HNF4A SNX4 SLC48A1B TTPA SLC26A1 TIMM50 SLC15A2 SLC19A1 BET1 SPNS3 SLC2A12 SLC7A2 MAGOH SLC6A8 PCF11 TOMM22 CLTA IST1 SNX11 AP2A1 SLC7A7 SLC26A6 ABCC5 SYTL5 SLC35A4 ABCG8 ABCG5 VPS41 OSBPL6 VPS18 PLA2G12A SLC45A3 OSBPL11 SLC29A3 SLC25A5 AP1M1 SERP1 XPOT MON2 SYTL4 GGA3 VIPAS39 SEC23A OSBPL1A TRAPPC11 FAM53B RHAG SLC25A24 AGK SPX PARK7
2.21E-07	293	4312	Biosynthetic process	CYP11A1 DHPS DHFR GYS2 GNMT FAR1 MEOX1 HSF1 MYOG HNF1A CCNC SOX19A SOX5 PIGO RGMA ASAH2 LBR ESCO2 POLA2 HDAC1 FXR2 PNPO SEPHS2 PIK3CG ABHD3 GCLM ZDHHC7 ORC5 ALG5 SFR1 TRAF3 ATG5 FAXDC2 IDH1 TFCP2L1 SGMS1 MYF6 MYBL1 LEF1 HIF1AN MRRF OGFOD1 NFATC1 C1GALT1C1 TBPL1 ST6GALNAC6 ZDHHC14 MTAP ST3GAL5 TAF11 LFNG TPK1 ASCL1A SULF1 RAD50 TYR GOT1 RSL24D1 RFC4 GTF3C6 PPIL4 PARS2 PCF11 CH25H MCM10 MED20 PCGF5B PISD BDH2 SHMT1 PGK1 RPE65C MCMBP KAT6B RFC3 EEF1G HELT ITPA NR1H4 OLAH MTRF1L MRPL2 MSH3 GFM1 MT-ATP6 PPARGC1A KLF9 NOS1 GCH1 E2F3 CENPS HEY1 GAMT RRP8 GNPAT TFB2M CRTC2 SALL2 MRPL47 MRI1 ASXL2 RAI1 DOLK ELK4 ZNF296 DPH2 TCF20 PPIP5K2 DNA2 MRPL58 DHX9 EGR3 DAGLB MRPL52 POLD2 SERP1 PIK3C2G ALAD SMG6 FDFT1 GART COQ8B POLRMT MYRFL B3GALT2 TAF2 SARS2 ATG7 RRN3 ZFHX3 PEMT TUFM MSH6 HDHD5 DR1 PARK7 CBFB BHLHE40 RDH5 FOXN4 HES6 SCUBE1 BPTF RXRBA MEN1 CRY2 RIPK2 AFF4 DBX1B DMBX1B TBX15 NEUROD4 RPL3 POLB GALNT2 NELFE PPAT KMT2A TCEA3 MTFMT GFM2 MLLT3 RECQL LGSN KMO IGF2BP3 LPCAT1 UMPS PDSS2 EIF3JA ATF6 MED22 ATP5PF RBM8A PBLD ATIC NR2E1 EIF5A APMAP PDSS1 DGAT2 GPT2L MICAL2B HNF4A ARNT UCK2B PRKCBB STAT3 GGPS1 ATF2 GMPPAA ETS1 AGMO L3MBTL3 KHSRP CHCHD1 PRIMPOL MDM2 SEPSECS CHST11 EEF2K HSD17B12B CTDSP2 HSD3B7 ATOH8 ACSS2 PIGQ ASPDH VGLL3 MAGOH MAT1A SOX19B RX2 DRAP1 DMAP1 AGPS PCYT2 ACSS1 MAFAA ATF1 POFUT2 IRF5 GLI3 PTGS1 GALNT16 HOXB5B HOXB1B FOSB NR4A3 HOXB13A NEUROG1 CREB3L3A MRPL4 MYEF2 USP16 XYLT1 NELFA EIF2AK3 MRPS5 MRPL9 GTF3C5 EIF2AK2 BCL6B PITX3 HOXC5A HOXC8A NT5E OLIG3 MRPL17 MRPL43 ALDH1L1 OAT ACACA YEATS2 GEMIN5 AFF3 GAL3ST4 AEBP1 MRPL34 PANK4 GCHFR POLR2B SDR42E1 VDRA RPS27L EXT1B MCM7 ORC2 MRPS7 FUT11 NOTCH1A ARID3B MRPS30 SLC25A38B FKRP TRAPPC11 MTTP DLAT SLC33A1 DAB2 ZNF703 PPP4CB SLC22A5 ZDHHC13 METTL3 ILF2 MYSM1 LOXL2A LOXL2B INSM1B SPX COPS5 NOP10
2.21E-07	68	634	Carboxylic acid metabolic process	ECHS1 DHFR GNMT ACO2 P4HA2 ASAH2 SLC23A2 HAL MCCC2 SEPHS2 ABHD3 IDH1 ACO1 MTAP CPT2 ACOX3 GOT1 AUH PARS2 SHMT1 ALDH6A1 PGK1 SDHAF2 NOS1 EHHADH GAMT TDO2A GNPAT SDSL P4HTM MRI1 OAT ACADL DAGLB ME2 SARS2 PARK7 CRYL1 SRCAP SUCLA2 LGSN KMO UEVLD PGD DLAT DHTKD1 DDC CRTAP IDH3A HADH GLDC HSD17B12B ALDH8A1 ACSS2 ALDH4A1 ACSS1 IDH3B SUCLG2 DECR2 PTGS1 HIBCH GCH1 FH ALDH1L1 ACACA LEF1 TYR DBT
2.21E-07	80	801	Cellular response to stress	RECQL HSF1 XRCC1 SGTB NEIL1 PARP16 GSR SFR1 ATG5 UBE2V2 HIF1AN RNF185 MAP4K5 XPC DUSP22B HSPA13 TNFRSF19 UBE2V1 ANKZF1 RBBP8 PRDX6 FANCD2 DNAJC18 DUSP22A MYEF2 OGG1 MAP3K3 STK26 FOXRED2 MAP3K8 MSH3 MPG SELENOS DEK RRP8 TDP1 PMS2 MAP3K19 SERP1 HIC2 ATG7 RIPK2 MSH6 MAP3K4 RUVBL1 POLB RGMA ATF6 METTL3 SLC12A4 PRPF19 APTX PARPBP NUDT1 PRIMPOL TMUB1 RAD50 ZFYVE26 DMAP1 RNF8 MCM10 CREB3L3A NHEJ1 EIF2AK3 EIF2AK2 FANCE CENPS SMC6 ACTR8 TRAPPC11 DGAT2 CNTFR ASCL1A NEUROG1 CHCHD6A SLC25A24 PHLDA3 USP16 DNA2 PARK7
3.70E-07	284	4176	Nucleobase-containing compound metabolic process	ZMAT2 RPE MTFMT EXOSC9 GNMT FAR1 RECQL MEOX1 SART3 TRMT61A HSF1 MYOG HNF1A XRCC1 CCNC SOX19A SOX5 PAPOLG RGMA METTL3 ESCO2 RBFOX1 POLA2 PRPF19 HDAC1 FXR2 DKC1 RBM8A PRX SEPHS2 SF3B2 NEIL1 WDR33 SF3B4 SF3A2 ORC5 SFR1 TRAF3 NNT LSM4 IDH1 NDUFAF1 UBE2V2 TFCP2L1 MYF6 DNMT1 MYBL1 LEF1 HIF1AN DIS3L2 CPSF1 NFATC1 RBM4.1 POP7 TBPL1 TTR TAF11 RPRD1B ASCL1A SULF1 MAGOH RAD50 XPC SYNCRIP INTS14 UBE2V1 RFC4 GTF3C6 RBBP8 PPIL4 PARS2 PCF11 ERI1 MCM10 MED20 RBFOX2 PCGF5B SHMT1 ALDH6A1 FANCD2 TRUB1 FBL PGK1 MRPL1 XDH MCMBP KAT6B MTO1 RFC3 HELT RPIA NSUN2 ITPA NR1H4 ENDOG UQCRH HNRNPA3 MYEF2 OGG1 DIS3 NUDT14 SDHAF2 MSH3 MT-ATP6 PRPF4 PPARGC1A KLF9 NOS1 MPG DEK E2F3 CENPS HEY1 GAMT TDO2A RRP8 TDP1 TFB2M CRTC2 SALL2 PMS2 ASXL2 METTL1 RAI1 ELK4 ZNF296 TCF20 DNA2 DHX9 EGR3 CSTF2 POLD2 SLIRP SMG6 GART POLRMT MYRFL TAF2 SARS2 TRMT13 RRN3 ZFHX3 RRP1 NOP10 LTO1 MSH6 DR1 SRSF4 PARK7 CBFB BHLHE40 FOXN4 HES6 SCUBE1 BPTF RXRBA MEN1 CRY2 RIPK2 AFF4 DBX1B RTCA DMBX1B TBX15 RUVBL1 NEUROD4 POLB NELFE PPAT KMT2A TCEA3 MLLT3 KMO SREK1 UMPS ATF6 MED22 ATP5PF PGD QTRT2 ATIC NR2E1 LARP7 MICAL2B HNF4A ARNT ALG5 UCK2B PRKCBB STAT3 ATF2 GMPPAA ETS1 L3MBTL3 KHSRP APTX PARPBP NUDT1 PRIMPOL MDM2 SEPSECS RSRC1 CTDSP2 NDUFB6 MTAP ATOH8 ACSS2 ASPDH VGLL3 ZFYVE26 SOX19B RX2 DRAP1 DMAP1 TRMT6 ALLC PTBP3 ACSS1 MAFAA ATF1 SUCLG2 RNF8 IRF5 GLI3 FAM172A PNRC2 GEMIN8 HOXB5B HOXB1B FOSB NR4A3 HOXB13A NEUROG1 SF3B1 CREB3L3A NT5C3A NHEJ1 EXOSC1 USP16 NELFA GTF3C5 FANCE BCL6B PITX3 HOXC5A HOXC8A NT5E OLIG3 MRM2 MRI1 GAR1 YEATS2 AFF3 RNASEH2C AEBP1 SMC6 PANK4 SRSF11 POLR2B VDRA TYMP MCM7 ORC2 XRN2 NDUFC2 NOTCH1A ACTR8 ARID3B AKAP17A DLAT SLC33A1 DAB2 ZNF703 TNPO3 PPP4CB ZDHHC13 ILF2 MYSM1 LOXL2A LOXL2B INSM1B MOV10B.2 SPX COPS5
1.20E-06	71	706	Organic acid metabolic process	ECHS1 DHFR GNMT ACO2 P4HA2 ASAH2 BNIP2 SLC23A2 HAL MCCC2 SEPHS2 ABHD3 IDH1 ACO1 CYP2U1 MTAP CPT2 ACOX3 GOT1 AUH PARS2 SHMT1 ALDH6A1 PGK1 BNIPL SDHAF2 NOS1 EHHADH GAMT TDO2A GNPAT SDSL P4HTM MRI1 OAT ACADL DAGLB ME2 SARS2 PARK7 CRYL1 SRCAP SUCLA2 LGSN KMO UEVLD PGD DLAT DHTKD1 DDC CRTAP IDH3A HADH GLDC HSD17B12B ALDH8A1 ACSS2 ALDH4A1 ACSS1 IDH3B SUCLG2 DECR2 PTGS1 HIBCH GCH1 FH ALDH1L1 ACACA LEF1 TYR DBT
1.20E-06	285	4249	Organic substance biosynthetic process	CYP11A1 DHFR GYS2 GNMT FAR1 MEOX1 HSF1 MYOG HNF1A CCNC SOX19A SOX5 PIGO RGMA ASAH2 LBR ESCO2 POLA2 HDAC1 FXR2 PNPO SEPHS2 PIK3CG ABHD3 GCLM ZDHHC7 ORC5 ALG5 SFR1 TRAF3 ATG5 FAXDC2 IDH1 TFCP2L1 SGMS1 MYF6 MYBL1 LEF1 HIF1AN MRRF OGFOD1 NFATC1 C1GALT1C1 TBPL1 ST6GALNAC6 ZDHHC14 MTAP ST3GAL5 TAF11 LFNG TPK1 ASCL1A SULF1 RAD50 TYR GOT1 RSL24D1 RFC4 GTF3C6 PPIL4 PARS2 PCF11 CH25H MCM10 MED20 PCGF5B PISD BDH2 SHMT1 PGK1 RPE65C MCMBP KAT6B RFC3 EEF1G HELT ITPA NR1H4 OLAH MTRF1L MRPL2 MSH3 GFM1 MT-ATP6 PPARGC1A KLF9 NOS1 GCH1 E2F3 CENPS HEY1 GAMT RRP8 GNPAT TFB2M CRTC2 SALL2 MRPL47 MRI1 ASXL2 RAI1 DOLK ELK4 ZNF296 DPH2 TCF20 PPIP5K2 DNA2 MRPL58 DHX9 EGR3 MRPL52 POLD2 SERP1 PIK3C2G ALAD SMG6 FDFT1 GART COQ8B POLRMT MYRFL B3GALT2 TAF2 SARS2 ATG7 RRN3 ZFHX3 PEMT TUFM MSH6 HDHD5 DR1 PARK7 CBFB BHLHE40 RDH5 FOXN4 HES6 SCUBE1 BPTF RXRBA MEN1 CRY2 RIPK2 AFF4 DBX1B DMBX1B TBX15 NEUROD4 RPL3 POLB GALNT2 NELFE PPAT KMT2A TCEA3 MTFMT GFM2 MLLT3 RECQL LGSN KMO IGF2BP3 LPCAT1 UMPS PDSS2 EIF3JA ATF6 MED22 ATP5PF RBM8A ATIC NR2E1 EIF5A PDSS1 DGAT2 MICAL2B HNF4A ARNT UCK2B PRKCBB STAT3 GGPS1 ATF2 GMPPAA ETS1 AGMO L3MBTL3 KHSRP CHCHD1 PRIMPOL MDM2 SEPSECS CHST11 EEF2K HSD17B12B CTDSP2 HSD3B7 ATOH8 ACSS2 PIGQ ASPDH VGLL3 MAGOH MAT1A SOX19B RX2 DRAP1 DMAP1 AGPS ACSS1 MAFAA ATF1 POFUT2 IRF5 GLI3 PTGS1 GALNT16 HOXB5B HOXB1B FOSB NR4A3 HOXB13A NEUROG1 CREB3L3A MRPL4 MYEF2 USP16 XYLT1 NELFA EIF2AK3 MRPS5 MRPL9 GTF3C5 EIF2AK2 BCL6B PITX3 HOXC5A HOXC8A NT5E OLIG3 MRPL17 MRPL43 OAT ACACA YEATS2 GEMIN5 AFF3 GAL3ST4 AEBP1 MRPL34 PANK4 POLR2B SDR42E1 VDRA RPS27L EXT1B MCM7 ORC2 MRPS7 FUT11 NOTCH1A ARID3B MRPS30 SLC25A38B FKRP TRAPPC11 MTTP DLAT SLC33A1 DAB2 ZNF703 PPP4CB SLC22A5 ZDHHC13 METTL3 ILF2 MYSM1 LOXL2A LOXL2B INSM1B SPX COPS5 NOP10

1.92E-06	243	3526	System development	BHLHE40 TENM3 LGSN MYOG SOX19A FGFR1A MYBPC3 CACYBP RBFOX1 MATN4 HES6 COL7A1 PKP2 ERBB2 MYF6 FLVCR1 LEF1 NEB PLXNB3 ASCL1A SULF1 KLHL40A TAZ PEBP1 LOXL2A LOXL2B TCTA MYBPC1 RBFOX2 MAP2 HELT POPDC3 AGGF1 PLXNA2 STK26 GBA2 AMOTL2A HEY1 TACC1 MYORG ASXL2 SEMA7A DAGLB RFLNB WNT16 SGCD TRAK2 FZD4 HIF1AN BHLHA15 CRYAA NEUROG1 CDKN1A DPYSL3 NEUROD4 DLB HNF1A FOXN4 RGMA HNF4A BZW2 ATOH8 LFNG GLI3 SGCB INSM1B ANO6 RIPOR2 KLHL41A CHAC1 OLIG3 SLC25A38B PLPPR1 MYMK NDEL1B ZNF703 MYH7 DMBX1B DHFR CASP9 KMT2A NEK2 HDAC1 DAG1 DKC1 ALDH7A1 SPARC ETS1 FZD5 NUMB DNMT1 DAB2 EEF2K CTDSP2 ZFYVE26 OTOMP TNFRSF19 TNPO3 ALDH1A2 ILK TECTA MAP3K3 TRIM69 HOXC8A SH3KBP1 GEMIN5 AP1M1 RGS2 LAMA2 LAMA1 SDC2 DHPS STX16 RPL3 GALNT2 NELFE TRAPPC11 CDH17 KATNB1 MEOX1 SART3 RDH5 MTTP HSF1 CHRNA1 CCNC MSI1 KRAS SCUBE3 RCC2 DCN METTL3 ESCO2 ZC4H2 FAM53B DDC HAL CELSR2 SLC33A1 MICAL2B NONO TAB2 PRKCBB STAT3 ATG5 NOLC1 IDH1 SLC48A1B TTPA CNTFR HSPA12B PODXL ACHE RBCK1 DIS3L2 CPSF1 MPPED2 ALDH8A1 TSEN54 SLC2A12 SESTD1 MACC1 CBFB RAB11A DMAP1 RFC4 NDUFB11 CXADR ANGPTL3 TTC4 TOMM22 IST1 COL4A6 COL4A5 PTGS1 SMARCA5 BDH2 GPATCH3 AP2A1 FRAS1 SLC7A7 PRSS23 SF3B1 COPS5 HOMER2 MYEF2 OGG1 LRRN1 BMP3 LRSAM1 ABCC5 XYLT1 HAUS3 EDARADD SCUBE1 RASA3 PRPF4 PPARGC1A SLC39A6 KLF9 NOS1 CYP1B1 BTBD9 VPS4B PDIA5 PITX3 SLC41A1 VPS18 CRIP2 HPS5 P4HTM COL19A1 ALDH1L1 ASCC1 ADAMTS9 METTL22 NOSTRIN SMOC1 CRISPLD2 CLPB MPC1 LAMC3 SMPD3 TBCD PIM1 CAPZB PDE6C PTPRO FKRP GART COQ8B HSD17B4 GAS8 PEPD CDH1 CIB2 ATG7 VIPAS39 NOP10 SEC23A GIPC1 PEF1 TDRD7B
4.79E-06	275	4136	Cellular biosynthetic process	CYP11A1 DHFR GYS2 MEOX1 HSF1 MYOG HNF1A CCNC SOX19A SOX5 PIGO RGMA ASAH2 ESCO2 POLA2 HDAC1 FXR2 PNPO SEPHS2 PIK3CG ABHD3 GCLM ZDHHC7 ORC5 ALG5 SFR1 TRAF3 ATG5 IDH1 TFCP2L1 SGMS1 MYF6 MYBL1 LEF1 HIF1AN MRRF OGFOD1 NFATC1 C1GALT1C1 TBPL1 ST6GALNAC6 ZDHHC14 MTAP ST3GAL5 TAF11 LFNG TPK1 ASCL1A SULF1 RAD50 TYR GOT1 RSL24D1 RFC4 GTF3C6 PPIL4 PARS2 PCF11 MCM10 MED20 PCGF5B PISD BDH2 SHMT1 PGK1 RPE65C MCMBP KAT6B RFC3 EEF1G HELT ITPA NR1H4 MTRF1L MRPL2 MSH3 GFM1 MT-ATP6 PPARGC1A KLF9 NOS1 GCH1 E2F3 CENPS HEY1 GAMT RRP8 GNPAT TFB2M CRTC2 SALL2 MRPL47 MRI1 ASXL2 RAI1 DOLK ELK4 ZNF296 DPH2 TCF20 DNA2 MRPL58 DHX9 EGR3 DAGLB MRPL52 POLD2 SERP1 PIK3C2G ALAD SMG6 GART COQ8B POLRMT MYRFL B3GALT2 TAF2 SARS2 ATG7 RRN3 ZFHX3 PEMT TUFM MSH6 HDHD5 DR1 PARK7 CBFB BHLHE40 RDH5 FOXN4 HES6 SCUBE1 BPTF RXRBA MEN1 CRY2 RIPK2 AFF4 DBX1B DMBX1B TBX15 NEUROD4 RPL3 POLB GALNT2 NELFE PPAT KMT2A TCEA3 MTFMT GFM2 MLLT3 RECQL LGSN KMO IGF2BP3 LPCAT1 UMPS PDSS2 EIF3JA ATF6 MED22 ATP5PF RBM8A ATIC NR2E1 EIF5A PDSS1 DGAT2 MICAL2B HNF4A ARNT UCK2B PRKCBB STAT3 GGPS1 ATF2 GMPPAA ETS1 L3MBTL3 KHSRP CHCHD1 PRIMPOL MDM2 SEPSECS EEF2K HSD17B12B CTDSP2 ATOH8 ACSS2 PIGQ ASPDH VGLL3 MAGOH MAT1A SOX19B RX2 DRAP1 DMAP1 AGPS ACSS1 MAFAA ATF1 POFUT2 IRF5 GLI3 PTGS1 GALNT16 HOXB5B HOXB1B FOSB NR4A3 HOXB13A NEUROG1 CREB3L3A MRPL4 MYEF2 USP16 NELFA EIF2AK3 MRPS5 MRPL9 GTF3C5 EIF2AK2 BCL6B PITX3 HOXC5A HOXC8A NT5E OLIG3 MRPL17 MRPL43 OAT ACACA YEATS2 GEMIN5 AFF3 GAL3ST4 AEBP1 MRPL34 PANK4 POLR2B VDRA RPS27L EXT1B FDFT1 MCM7 ORC2 MRPS7 FUT11 NOTCH1A ARID3B MRPS30 SLC25A38B FKRP TRAPPC11 MTTP DLAT SLC33A1 DAB2 ZNF703 XYLT1 PPP4CB SLC22A5 ZDHHC13 METTL3 ILF2 MYSM1 LOXL2A LOXL2B INSM1B SPX COPS5 NOP10

Supplementary Table 7 – Top 20 genes enriched unique to control across time

Enrichment FDR	Genes in list	Total genes	Functional Category	Genes
6.09E-10	144	4854	Cellular nitrogen compound metabolic process	RPA3 EXOSC9 TRMT61A SEC11A SNRPD1 RFC2 NCBP2 U2AF1 SOX6 WDHD1 FXR2 DKC1 SF3B2 LPIN1 CPSF3 POLD1 MYOD1 EIF4EBP2 SNRPE TOP2B PER2 NCOR1 RNASET2 LSM6 GGH MRPL12 PRIM1 SNRPD2 TFAP2C MORF4L1 MRPL51 UBE2V1 ELP3 LSM1 NSUN5 EIF4EBP1 NPEPPS PHF5A POLA1 DCP1B BDH2 DNAJB5 FBL RBM41 KAT6B PELO FOXO4 RFC3 MRPL13 UQCRH LIG1 PRPF4 JUND KLF9 E2F3 PMVK GAMT TMA7 CRTC2 MRPS11 LARS2 ATAD2 ELK4 SNRNP70 NAB2 TIMELESS GRHL3 FOXE1 TOP1 EGR3 CSTF2 CAMTA2 CC2D1B POLE2 SF3A3 THUMPD1 LNPEP HK2 BACE1 TK1 RRP1 EXOSC4 SF3B3 NOP10 PARK7 HES6 UCK2A MYCB FOXP1B ATP5PF DLX3B HELZ2 NPAS2 CMPK HNF4A ARNT SMAD9 UCK2B NUDT1 TBX1 ERH TBX18 PSMC3IP DUSP11 TSC22D1 POLR2H MCM4 RX2 EIF2B2 HP1BP3 APEX1 CARD11 MRPS17 STAT5B IRF8 HTATSF1 DPH1 LSM7 EXOSC1 TSFM MRPL9 IBA57 SREBF1 BPGM MRPS21 LSM5 POLR2K TSC22D3 MRPL43 HAAO GAR1 RNASEH2C WDR4 VDRA SNRPG RPS27L DUT ENDOUC MBD4 LBH ZNF703 IGFBP2A MOV10B.2 USP13
1.19E-08	127	4290	Heterocycle metabolic process	RPA3 EXOSC9 TRMT61A SNRPD1 RFC2 NCBP2 U2AF1 SOX6 WDHD1 FXR2 DKC1 SF3B2 LPIN1 CPSF3 POLD1 MYOD1 SNRPE TOP2B PER2 NCOR1 RNASET2 LSM6 GGH MRPL12 PRIM1 SNRPD2 TFAP2C MORF4L1 UBE2V1 ELP3 LSM1 NSUN5 PHF5A POLA1 DCP1B DNAJB5 FBL RBM41 KAT6B PELO FOXO4 RFC3 UQCRH LIG1 PRPF4 JUND KLF9 E2F3 PMVK GAMT CRTC2 MRPS11 LARS2 ATAD2 ELK4 SNRNP70 NAB2 TIMELESS GRHL3 FOXE1 TOP1 EGR3 CSTF2 CAMTA2 CC2D1B POLE2 SF3A3 THUMPD1 HK2 TK1 RRP1 EXOSC4 SF3B3 NOP10 PARK7 HES6 UCK2A MYCB FOXP1B ATP5PF DLX3B HELZ2 NPAS2 CMPK HNF4A ARNT SMAD9 UCK2B NUDT1 TBX1 ERH TBX18 PSMC3IP DUSP11 TSC22D1 POLR2H MCM4 RX2 HP1BP3 APEX1 CARD11 STAT5B IRF8 HTATSF1 LSM7 EXOSC1 IBA57 SREBF1 BPGM LSM5 POLR2K TSC22D3 HAAO GAR1 RNASEH2C WDR4 VDRA SNRPG DUT ENDOUC MBD4 LBH ZNF703 IGFBP2A BDH2 MOV10B.2 USP13
1.19E-08	124	4176	Nucleobase-containing compound metabolic process	RPA3 EXOSC9 TRMT61A SNRPD1 RFC2 NCBP2 U2AF1 SOX6 WDHD1 FXR2 DKC1 SF3B2 LPIN1 CPSF3 POLD1 MYOD1 SNRPE TOP2B PER2 NCOR1 RNASET2 LSM6 MRPL12 PRIM1 SNRPD2 TFAP2C MORF4L1 UBE2V1 ELP3 LSM1 NSUN5 PHF5A POLA1 DCP1B DNAJB5 FBL RBM41 KAT6B PELO FOXO4 RFC3 UQCRH LIG1 PRPF4 JUND KLF9 E2F3 PMVK GAMT CRTC2 MRPS11 LARS2 ATAD2 ELK4 SNRNP70 NAB2 TIMELESS GRHL3 FOXE1 TOP1 EGR3 CSTF2 CAMTA2 CC2D1B POLE2 SF3A3 THUMPD1 HK2 TK1 RRP1 EXOSC4 SF3B3 NOP10 PARK7 HES6 UCK2A MYCB FOXP1B ATP5PF DLX3B HELZ2 NPAS2 CMPK HNF4A ARNT SMAD9 UCK2B NUDT1 TBX1 ERH TBX18 PSMC3IP DUSP11 TSC22D1 POLR2H MCM4 RX2 HP1BP3 APEX1 CARD11 STAT5B IRF8 HTATSF1 LSM7 EXOSC1 SREBF1 BPGM LSM5 POLR2K TSC22D3 HAAO GAR1 RNASEH2C WDR4 VDRA SNRPG DUT ENDOUC MBD4 LBH ZNF703 IGFBP2A MOV10B.2 USP13
1.19E-08	127	4319	Cellular aromatic compound metabolic process	RPA3 EXOSC9 TRMT61A SNRPD1 RFC2 NCBP2 U2AF1 SOX6 WDHD1 FXR2 DKC1 SF3B2 LPIN1 CPSF3 POLD1 MYOD1 SNRPE TOP2B PER2 NCOR1 RNASET2 LSM6 GGH MRPL12 PRIM1 SNRPD2 TFAP2C MORF4L1 UBE2V1 ELP3 LSM1 NSUN5 PHF5A POLA1 DCP1B DNAJB5 FBL RBM41 KAT6B PELO FOXO4 RFC3 UQCRH LIG1 PRPF4 JUND KLF9 E2F3 PMVK GAMT CRTC2 MRPS11 LARS2 ATAD2 ELK4 SNRNP70 NAB2 TIMELESS GRHL3 FOXE1 TOP1 EGR3 CSTF2 CAMTA2 CC2D1B POLE2 SF3A3 THUMPD1 HK2 TK1 RRP1 EXOSC4 SF3B3 NOP10 PARK7 HES6 UCK2A MYCB FOXP1B ATP5PF DLX3B HELZ2 NPAS2 CMPK HNF4A ARNT SMAD9 UCK2B NUDT1 TBX1 ERH TBX18 PSMC3IP DUSP11 TSC22D1 POLR2H MCM4 RX2 HP1BP3 APEX1 CARD11 STAT5B IRF8 HTATSF1 LSM7 EXOSC1 IBA57 SREBF1 BPGM LSM5 POLR2K TSC22D3 HAAO GAR1 RNASEH2C WDR4 VDRA SNRPG DUT ENDOUC MBD4 LBH ZNF703 IGFBP2A BDH2 MOV10B.2 USP13
2.24E-08	111	3634	Nucleic acid metabolic process	RPA3 EXOSC9 TRMT61A SNRPD1 RFC2 NCBP2 U2AF1 SOX6 WDHD1 FXR2 DKC1 SF3B2 LPIN1 CPSF3 POLD1 MYOD1 SNRPE

				TOP2B PER2 NCOR1 RNASET2 LSM6 MRPL12 PRIM1 SNRPD2 TFAP2C MORF4L1 UBE2V1 ELP3 LSM1 NSUN5 PHF5A POLA1 DCP1B DNAJB5 FBL RBM41 KAT6B PELO FOXO4 RFC3 LIG1 PRPF4 JUND KLF9 E2F3 CRTC2 MRPS11 LARS2 ATAD2 ELK4 SNRNP70 NAB2 TIMELESS GRHL3 FOXE1 TOP1 EGR3 CSTF2 CAMTA2 CC2D1B POLE2 SF3A3 THUMPD1 TK1 RRP1 EXOSC4 SF3B3 NOP10 HES6 MYCB FOXP1B DLX3B HELZ2 NPAS2 HNF4A ARNT SMAD9 NUDT1 TBX1 TBX18 PSMC3IP TSC22D1 POLR2H MCM4 RX2 HP1BP3 APEX1 CARD11 STAT5B IRF8 HTATSF1 LSM7 EXOSC1 SREBF1 LSM5 POLR2K TSC22D3 GAR1 RNASEH2C WDR4 VDRA SNRPG ENDOUC MBD4 LBH ZNF703 IGFBP2A MOV10B.2 USP13 PARK7
2.24E-08	128	4426	Organic cyclic compound metabolic process	RPA3 EXOSC9 TRMT61A SNRPD1 RFC2 NCBP2 U2AF1 SOX6 WDHD1 FXR2 DKC1 SF3B2 LPIN1 CPSF3 POLD1 MYOD1 SNRPE TOP2B PER2 NCOR1 RNASET2 LSM6 GGH MRPL12 PRIM1 SNRPD2 TFAP2C MORF4L1 UBE2V1 ELP3 LSM1 NSUN5 PHF5A POLA1 DCP1B DNAJB5 FBL RBM41 KAT6B PELO FOXO4 RFC3 UQCRH LIG1 PRPF4 JUND KLF9 E2F3 PMVK GAMT CRTC2 MRPS11 LARS2 ATAD2 ELK4 SNRNP70 NAB2 TIMELESS GRHL3 FOXE1 TOP1 EGR3 CSTF2 CAMTA2 CC2D1B POLE2 SF3A3 THUMPD1 HK2 TK1 RRP1 EXOSC4 SF3B3 NOP10 PARK7 HES6 UCK2A MYCB FOXP1B ATP5PF DLX3B HELZ2 NPAS2 CMPK HNF4A ARNT SMAD9 UCK2B NUDT1 TBX1 ERH TBX18 PSMC3IP DUSP11 TSC22D1 POLR2H MCM4 RX2 HP1BP3 APEX1 CYB5R2 CARD11 STAT5B IRF8 HTATSF1 LSM7 EXOSC1 IBA57 SREBF1 BPGM LSM5 POLR2K TSC22D3 HAAO GAR1 RNASEH2C WDR4 VDRA SNRPG DUT ENDOUC MBD4 LBH ZNF703 IGFBP2A BDH2 MOV10B.2 USP13
3.50E-07	27	412	MRNA metabolic process	EXOSC9 SNRPD1 NCBP2 U2AF1 FXR2 DKC1 SF3B2 CPSF3 SNRPE SNRPD2 LSM1 PHF5A DCP1B RBM41 PELO PRPF4 SNRNP70 CSTF2 SF3A3 EXOSC4 SF3B3 LSM6 HTATSF1 LSM7 LSM5 SNRPG MOV10B.2
5.66E-07	34	640	RNA processing	EXOSC9 TRMT61A SNRPD1 NCBP2 U2AF1 DKC1 SF3B2 CPSF3 SNRPE LSM6 SNRPD2 ELP3 NSUN5 PHF5A FBL RBM41 PRPF4 MRPS11 SNRNP70 CSTF2 SF3A3 THUMPD1 RRP1 EXOSC4 SF3B3 NOP10 LSM1 HTATSF1 LSM7 EXOSC1 LSM5 GAR1 WDR4 SNRPG
1.46E-06	105	3649	Gene expression	NUP88 EXOSC9 TRMT61A SEC11A SNRPD1 NCBP2 U2AF1 SOX6 FXR2 DKC1 SF3B2 LPIN1 CPSF3 MYOD1 EIF4EBP2 SNRPE PER2 NCOR1 LSM6 MRPL12 SNRPD2 TFAP2C MORF4L1 MRPL51 ELP3 LSM1 NSUN5 EIF4EBP1 PHF5A DCP1B DNAJB5 FBL RBM41 KAT6B PELO FOXO4 MOV10B.2 THOC3 MRPL13 PRPF4 JUND KLF9 E2F3 TMA7 TYSND1 CRTC2 MRPS11 LARS2 ATAD2 ELK4 SNRNP70 NAB2 GRHL3 FOXE1 EGR3 CSTF2 CAMTA2 CC2D1B SF3A3 THUMPD1 RRP1 EXOSC4 SF3B3 NOP10 HES6 MYCB FOXP1B DLX3B NPAS2 HNF4A ARNT SMAD9 TBX1 TBX18 TSC22D1 POLR2H RX2 EIF2B2 HP1BP3 CARD11 MRPS17 STAT5B IRF8 HTATSF1 DPH1 LSM7 EXOSC1 TSFM MRPL9 SREBF1 MRPS21 LSM5 POLR2K TSC22D3 MRPL43 GAR1 WDR4 AGO1 VDRA SNRPG RPS27L LBH ZNF703 DDI2 USP13
4.00E-06	93	3160	RNA metabolic process	EXOSC9 TRMT61A SNRPD1 NCBP2 U2AF1 SOX6 FXR2 DKC1 SF3B2 LPIN1 CPSF3 MYOD1 SNRPE PER2 NCOR1 RNASET2 LSM6 MRPL12 PRIM1 SNRPD2 TFAP2C MORF4L1 ELP3 LSM1 NSUN5 PHF5A POLA1 DCP1B DNAJB5 FBL RBM41 KAT6B PELO FOXO4 LIG1 PRPF4 JUND KLF9 E2F3 CRTC2 MRPS11 LARS2 ATAD2 ELK4 SNRNP70 NAB2 GRHL3 FOXE1 EGR3 CSTF2 CAMTA2 CC2D1B SF3A3 THUMPD1 RRP1 EXOSC4 SF3B3 NOP10 HES6 MYCB FOXP1B DLX3B HELZ2 NPAS2 HNF4A ARNT SMAD9 TBX1 TBX18 TSC22D1 POLR2H RX2 HP1BP3 CARD11 STAT5B IRF8 HTATSF1 LSM7 EXOSC1 SREBF1 LSM5 POLR2K TSC22D3 GAR1 RNASEH2C WDR4 VDRA SNRPG ENDOUC LBH ZNF703 MOV10B.2 USP13
1.06E-05	15	162	Autophagy	GABARAPL2 ATG13 WIPI2 ATG9A ATG4B NPRL2 EPG5 LRSAM1 SESN2 ATG2B MAP1LC3B ATG4C TIGARA USP13 PARK7
1.06E-05	15	162	Process utilizing autophagic mechanism	GABARAPL2 ATG13 WIPI2 ATG9A ATG4B NPRL2 EPG5 LRSAM1 SESN2 ATG2B MAP1LC3B ATG4C TIGARA USP13 PARK7
1.14E-05	53	1473	Catabolic process	ECHS1 SKP2 EXOSC9 UBR4 FXR2 LPIN1 GABARAPL2 NOTUM1A UBE3C ATG13 RNASET2 WIPI2 MMP9 LSM1 PRDX6 ATG9A NPEPPS DCP1B ATG4B PELO CPVL PRDX1 NPRL2 EPG5 LRSAM1 TRIB2 SESN2 TYSND1 MMP19 ATG2B LNPEP MAP1LC3B HK2 BACE1 EXOSC4 USP25 NCBP2 PLA2G12B UCHL3 ATG4C SIAH2L TIGARA

				HIBCH LSM7 PLCB3 BPGM HAAO PNPLA8 USP13 LPL RNASEH2C PARK7 DDI2
1.14E-05	11	82	Macroautophagy	GABARAPL2 ATG13 WIPI2 ATG9A ATG4B NPRL2 EPG5 LRSAM1 SESN2 ATG2B MAP1LC3B
1.93E-05	9	54	Autophagosome assembly	GABARAPL2 ATG13 WIPI2 ATG9A ATG4B NPRL2 LRSAM1 ATG2B MAP1LC3B
1.93E-05	9	54	Autophagosome organization	GABARAPL2 ATG13 WIPI2 ATG9A ATG4B NPRL2 LRSAM1 ATG2B MAP1LC3B
1.93E-05	20	304	MRNA processing	SNRPD1 NCBP2 U2AF1 SF3B2 CPSF3 SNRPE SNRPD2 PHF5A RBM41 PRPF4 SNRNP70 CSTF2 SF3A3 SF3B3 LSM6 LSM1 HTATSF1 LSM7 LSM5 SNRPG
2.07E-05	16	202	RNA splicing, via transesterification reactions	SNRPD1 NCBP2 U2AF1 SF3B2 SNRPE SNRPD2 PHF5A RBM41 PRPF4 SNRNP70 SF3A3 SF3B3 LSM6 HTATSF1 LSM7 SNRPG
2.07E-05	16	202	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	SNRPD1 NCBP2 U2AF1 SF3B2 SNRPE SNRPD2 PHF5A RBM41 PRPF4 SNRNP70 SF3A3 SF3B3 LSM6 HTATSF1 LSM7 SNRPG
2.07E-05	16	202	MRNA splicing, via spliceosome	SNRPD1 NCBP2 U2AF1 SF3B2 SNRPE SNRPD2 PHF5A RBM41 PRPF4 SNRNP70 SF3A3 SF3B3 LSM6 HTATSF1 LSM7 SNRPG

Supplementary Table 8 – Response to stress and developmental process genes unique to low pH across time.

Gene ID	Mapped IDs	Gene Name Gene Symbol Ortholog	PANTHER GO-Slim Biological Process	GO Database Biological Process Complete	Category	Regulation at Day 5
DANRE|ZFIN=ZDB-GENE-050220-14|UniProtKB=F1R983	rbbp8	DNA endonuclease RBBP8;rbbp8;ortholog	nucleic acid phosphodiester bond hydrolysis(GO:0090305);DNA double-strand break processing(GO:0000729);non-recombinational repair(GO:0000726);double-strand break repair via homologous recombination(GO:0000724);nucleotide-excision repair(GO:0006289)	double-strand break repair via homologous recombination(GO:0000724);DNA repair(GO:0006281);cellular response to DNA damage stimulus(GO:0006974);cell cycle(GO:0007049);DNA double-strand break processing involved in repair via single-strand annealing(GO:0010792);cell division(GO:0051301);meiotic cell cycle(GO:0051321)	Response to Stress	Down
DANRE|ZFIN=ZDB-GENE-050419-19|UniProtKB=Q4VBJ6	GINS2	DNA replication complex GINS protein PSF2;gins2;ortholog	double-strand break repair via break-induced replication(GO:0000727)	double-strand break repair via break-induced replication(GO:0000727);DNA replication(GO:0006260)	Response to Stress	Down
DANRE|ZFIN=ZDB-GENE-061013-547|UniProtKB=Q08BH7	ube2t	Ubiquitin-conjugating enzyme E2 T;ube2t;ortholog	protein polyubiquitination(GO:0000209);cellular response to DNA damage stimulus(GO:0006974)	protein polyubiquitination(GO:0000209);DNA repair(GO:0006281);protein monoubiquitination(GO:0006513);cellular response to DNA damage stimulus(GO:0006974);protein autoubiquitination(GO:0051865)	Response to Stress	Down
DANRE|ZFIN=ZDB-GENE-030131-9008|UniProtKB=E7F4J7	PRKDC	Protein kinase, DNA-activated, catalytic subunit;prkdc;ortholog	telomere maintenance(GO:0000723);double-strand break repair(GO:0006302)	telomere maintenance(GO:0000723);DNA repair(GO:0006281);double-strand break repair(GO:0006302);double-strand break repair via nonhomologous end joining(GO:0006303);intrinsic apoptotic signaling pathway in response to DNA damage(GO:0008630);phosphorylation(GO:0016310);B cell differentiation(GO:0030183);T cell differentiation(GO:0030217);immunoglobulin V(D)J recombination(GO:0033152);T cell receptor V(D)J recombination(GO:0033153);immunoglobulin heavy chain V-D-J recombination(GO:0071707)	Response to Stress	Down
DANRE|ZFIN=ZDB-GENE-040426-2286|UniProtKB=Q5TYR1	RAD51	DNA repair protein RAD51 homolog;rad51;ortholog	reciprocal meiotic recombination(GO:0007131);protein-DNA complex assembly(GO:0065004);double-strand break repair via homologous recombination(GO:0000724);meiotic telophase I(GO:0007134);chromosome organization involved in meiotic cell cycle(GO:0070192);mitotic recombination(GO:0006312);response to ionizing radiation(GO:0010212)	double-strand break repair via homologous recombination(GO:0000724);DNA recombinase assembly(GO:0000730);DNA metabolic process(GO:0006259);DNA repair(GO:0006281);DNA recombination(GO:0006310);mitotic recombination(GO:0006312);cellular response to DNA damage stimulus(GO:0006974);reciprocal meiotic recombination(GO:0007131);response to ionizing radiation(GO:0010212);strand invasion(GO:0042148);response to	Response to Stress	Down

				cadmium ion(GO:0046686);response to methylmercury(GO:0051597);chromosome organization involved in meiotic cell cycle(GO:0070192);hematopoietic stem cell proliferation(GO:0071425);mitotic recombination-dependent replication fork processing(GO:1990426)		
DANRE|ZFIN=ZDB-GENE-070705-557|UniProtKB=B0V351	POLE	DNA polymerase epsilon catalytic subunit;pole;ortholog	base-excision repair(GO:0006284);DNA strand elongation involved in DNA replication(GO:0006271);DNA biosynthetic process(GO:0071897);mitotic nuclear division(GO:0140014);RNA biosynthetic process(GO:0032774);nucleotide-excision repair(GO:0006289);RNA catabolic process(GO:0006401)	mitotic cell cycle(GO:0000278);DNA replication(GO:0006260);leading strand elongation(GO:0006272);DNA repair(GO:0006281);base-excision repair, gap-filling(GO:0006287);nucleotide-excision repair, DNA gap filling(GO:0006297);DNA replication proofreading(GO:0045004)	Response to Stress	Down
DANRE|ZFIN=ZDB-GENE-021226-2|UniProtKB=Q8JHV6	lamb4	Laminin subunit beta-4;lamb4;ortholog	cellular component assembly(GO:0022607);extracellular matrix organization(GO:0030198);substrate adhesion-dependent cell spreading(GO:0034446);cell migration(GO:0016477);tissue development(GO:0009888);animal organ morphogenesis(GO:0009887)	cell adhesion(GO:0007155);animal organ morphogenesis(GO:0009887);tissue development(GO:0009888);cell migration(GO:0016477);glial cell development(GO:0021782);substrate adhesion-dependent cell spreading(GO:0034446);retina development in camera-type eye(GO:0060041);basement membrane assembly(GO:0070831)	Developmental Process	Down
DANRE|ZFIN=ZDB-GENE-070818-1|UniProtKB=F1QQC3	loxl2a	Lysyl oxidase homolog 2A;loxl2a;ortholog	sprouting angiogenesis(GO:0002040);oxidation-reduction process(GO:0055114);collagen fibril organization(GO:0030199);peptidyl-lysine modification(GO:0018205)	negative regulation of transcription by RNA polymerase II(GO:0000122);response to hypoxia(GO:0001666);epithelial to mesenchymal transition(GO:0001837);endothelial cell proliferation(GO:0001935);sprouting angiogenesis(GO:0002040);chromatin organization(GO:0006325);positive regulation of epithelial to mesenchymal transition(GO:0010718);peptidyl-lysine oxidation(GO:0018057);collagen fibril organization(GO:0030199);positive regulation of chondrocyte differentiation(GO:0032332);endothelial cell migration(GO:0043542);oxidation-reduction process(GO:0055114);heterochromatin organization(GO:0070828);negative regulation of stem cell population maintenance(GO:1902455)	Developmental Process	Down
DANRE|ZFIN=ZDB-GENE-040426-2089|UniProtK	Hapln3	Hyaluronan and proteoglycan link protein	central nervous system development(GO:0007417);skeletal system development(GO:0001501)	skeletal system development(GO:0001501);cell adhesion(GO:0007155);central nervous system development(GO:0007417)	Developmental Process	Up

B=A0A2R8PZI6		3;hapln3;ortholog				
DANRE|ZFIN=ZDB-GENE-040426-784|UniProtKB=Q802V6	abhd2a	Monoacylglycerol lipase ABHD2-A;abhd2a;ortholog	acylglycerol catabolic process(GO:0046464);spermatid development(GO:0007286);steroid hormone mediated signaling pathway(GO:0043401)	lipid metabolic process(GO:0006629);lipid catabolic process(GO:0016042);response to progesterone(GO:0032570);steroid hormone mediated signaling pathway(GO:0043401);cellular lipid metabolic process(GO:0044255);acylglycerol catabolic process(GO:0046464);sperm capacitation(GO:0048240);medium-chain fatty acid biosynthetic process(GO:0051792);medium-chain fatty acid catabolic process(GO:0051793)	Developmental Process	Up
DANRE|ZFIN=ZDB-GENE-070501-6|UniProtKB=F1Q6P3	COL6A1	Collagen, type VI, alpha 1;col6a1;ortholog	tissue morphogenesis(GO:0048729);cell morphogenesis involved in differentiation(GO:0000904);bone morphogenesis(GO:0060349);chondrocyte differentiation(GO:0002062);developmental growth(GO:0048589)	skeletal muscle tissue development(GO:0007519);locomotory behavior(GO:0007626);muscle structure development(GO:0061061)	Developmental Process	Down
DANRE|ZFIN=ZDB-GENE-070501-8|UniProtKB=F1Q4X1	COL6A3	Collagen, type VI, alpha 3;col6a3;ortholog	tissue morphogenesis(GO:0048729);cell morphogenesis involved in differentiation(GO:0000904);bone morphogenesis(GO:0060349);chondrocyte differentiation(GO:0002062);developmental growth(GO:0048589)	cell adhesion(GO:0007155);motor neuron axon guidance(GO:0008045);axon extension(GO:0048675)	Developmental Process	Down
DANRE|ZFIN=ZDB-GENE-030131-2427|UniProtKB=E7FA40	Col7a1	Collagen, type VII, alpha 1;col7a1;ortholog	tissue morphogenesis(GO:0048729);cell morphogenesis involved in differentiation(GO:0000904);bone morphogenesis(GO:0060349);chondrocyte differentiation(GO:0002062);developmental growth(GO:0048589)	cell adhesion(GO:0007155)	Developmental Process	Down
DANRE|ZFIN=ZDB-GENE-040625-164|UniProtKB=A0M8V6	CAV2	Caveolin;cav2;ortholog	lipid transport(GO:0006869);membrane organization(GO:0061024);cellular component assembly(GO:0022607);cell differentiation(GO:0030154);endomembrane system organization(GO:0010256)	negative regulation of endothelial cell proliferation(GO:0001937);insulin receptor signaling pathway(GO:0008286);cell differentiation(GO:0030154);positive regulation of MAPK cascade(GO:0043410);regulation of cytosolic calcium ion concentration(GO:0051480);caveola assembly(GO:0070836)	Developmental Process	Down

Supplementary Table 9 – DEGs related to response to stress and development in the combined stressor across time.

Gene ID	Mapped IDs	Gene Symbol Gene Symbol Ortholog	PANTHER GO-Slim Biological Process	GO Database Biological Process Complete	Category	Regulation at Day 5
DANRE|ZFIN=ZDB-GENE-080610-1|UniProtKB=A0A0R4IPV5	P4HB	Protein disulfide-isomerase;p4hb;ortholog	protein folding(GO:0006457);response to endoplasmic reticulum stress(GO:0034976)	protein folding(GO:0006457);response to endoplasmic reticulum stress(GO:0034976);cell redox homeostasis(GO:0045454)	Response to Stress	Down
DANRE|ZFIN=ZDB-GENE-030909-11|UniProtKB=F1QYH3	PCK1	Phosphoenolpyruvate carboxykinase 1 (soluble);pck1;ortholog	cellular chemical homeostasis(GO:0055082);fatty acid catabolic process(GO:0009062);response to starvation(GO:0042594);glyceroli pid biosynthetic process(GO:0045017);triglyceride metabolic process(GO:0006641);gland development(GO:0048732);respo nse to xenobiotic stimulus(GO:0009410);glucose metabolic process(GO:0006006);cellular response to insulin stimulus(GO:0032869);cellular response to steroid hormone stimulus(GO:0071383);epithelial cell differentiation(GO:0030855);alco hol biosynthetic process(GO:0046165);response to drug(GO:0042493);glucose homeostasis(GO:0042593);respon se to glucocorticoid(GO:0051384);drug metabolic process(GO:0017144);cellular carbohydrate biosynthetic process(GO:0034637)	gluconeogenesis(GO:0006094); response to glucose(GO:0009749);phospho rylation(GO:0016310);propiona te catabolic process(GO:0019543);cellular response to insulin stimulus(GO:0032869);respons e to lipid(GO:0033993);response to starvation(GO:0042594);glycer ol biosynthetic process from pyruvate(GO:0046327);ion homeostasis(GO:0050801);hep atocyte differentiation(GO:0070365);ce llular response to glucose stimulus(GO:0071333);cellular response to dexamethasone stimulus(GO:0071549)	Response to Stress	Up
DANRE|ZFIN=ZDB-GENE-980526-533|UniProtKB=P09074	hoxc5a	Homeobox protein Hox-C5a;hoxc5a;ortholog	anterior/posterior pattern specification(GO:0009952)	regulation of transcription, DNA-templated(GO:0006355);regula tion of transcription by RNA polymerase II(GO:0006357);multicellular organism development(GO:0007275);ant erior/posterior pattern specification(GO:0009952)	Develop mental Process	Down
DANRE|ZFIN=ZDB-GENE-030909-11|UniProtKB=F1QYH3	PCK1	Phosphoenolpyruvate carboxykinase 1 (soluble);pck1;ortholog	cellular chemical homeostasis(GO:0055082);fatty acid catabolic process(GO:0009062);response to starvation(GO:0042594);glyceroli pid biosynthetic process(GO:0045017);triglyceride metabolic process(GO:0006641);gland development(GO:0048732);respo nse to xenobiotic stimulus(GO:0009410);glucose metabolic process(GO:0006006);cellular response to insulin	gluconeogenesis(GO:0006094); response to glucose(GO:0009749);phospho rylation(GO:0016310);propiona te catabolic process(GO:0019543);cellular response to insulin stimulus(GO:0032869);respons e to lipid(GO:0033993);response to starvation(GO:0042594);glycer ol biosynthetic process from pyruvate(GO:0046327);ion homeostasis(GO:0050801);hep atocyte	Develop mental Process	Up

			stimulus(GO:0032869);cellular response to steroid hormone stimulus(GO:0071383);epithelial cell differentiation(GO:0030855);alcohol biosynthetic process(GO:0046165);response to drug(GO:0042493);glucose homeostasis(GO:0042593);response to glucocorticoid(GO:0051384);drug metabolic process(GO:0017144);cellular carbohydrate biosynthetic process(GO:0034637)	differentiation(GO:0070365);cellular response to glucose stimulus(GO:0071333);cellular response to dexamethasone stimulus(GO:0071549)		
DANRE|ZFIN=ZDB-GENE-990415-79|UniProtKB=Q90YL6	NR5A2	Nr5a2 protein;nr5a2;ortholog	transcription by RNA polymerase II(GO:0006366);positive regulation of transcription by RNA polymerase II(GO:0045944);tissue development(GO:0009888);hormone-mediated signaling pathway(GO:0009755)	liver development(GO:0001889);regulation of transcription, DNA-templated(GO:0006355);regulation of transcription by RNA polymerase II(GO:0006357);hormone-mediated signaling pathway(GO:0009755);tissue development(GO:0009888);exocrine pancreas development(GO:0031017);digestive tract development(GO:0048565);cartilage development(GO:0051216);hepatoblast differentiation(GO:0061017)	Developmental Process	Up
DANRE|ZFIN=ZDB-GENE-010813-1|UniProtKB=Q90WT1	CRYBB1	Beta B1-crystallin;crybb1;ortholog	lens development in camera-type eye(GO:0002088);sensory perception of light stimulus(GO:0050953)	lens development in camera-type eye(GO:0002088);visual perception(GO:0007601)	Developmental Process	Up
DANRE|ZFIN=ZDB-GENE-040801-16|UniProtKB=Q6DGY7	crygnb	Gamma-crystallin N-B;crygnb;ortholog	lens development in camera-type eye(GO:0002088);sensory perception of light stimulus(GO:0050953)	lens development in camera-type eye(GO:0002088);visual perception(GO:0007601)	Developmental Process	Up
DANRE|ZFIN=ZDB-GENE-030131-8760|UniProtKB=E7FB26	Elf3	E74-like factor 3 (ets domain transcription factor, epithelial-specific);elf3;ortholog	cell differentiation(GO:0030154);transcription by RNA polymerase II(GO:0006366);regulation of transcription by RNA polymerase II(GO:0006357)	regulation of transcription, DNA-templated(GO:0006355);regulation of transcription by RNA polymerase II(GO:0006357);transcription by RNA polymerase II(GO:0006366);inflammatory response(GO:0006954);cell differentiation(GO:0030154);epithelial cell differentiation(GO:0030855)	Developmental Process	Up