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Genetic Analysis of Yellowtail (Seriola lalandi) for Aquaculture in Maine

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GENETIC ANALYSIS OF YELLOWTAIL (*SERIOLA LALANDI*) FOR AQUACULTURE IN MAINE

by

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ABSTRACT

Aquaculture is a growing industry in Maine, and identifying new species with potential for aquaculture is crucial for its continuing success. The University of Maine's Center for Cooperative Aquaculture Research (CCAR) currently holds a stock of California yellowtail (*Seriola lalandi*). Similar in taste to tuna or mackerel, yellowtail are wild-caught as well as aquacultured in net pens and recirculating aquaculture systems worldwide. The fish presently at CCAR originally came from Great Bay Aquaculture and their genetic makeup is unknown. Recent research on *S. lalandi* suggests that this circumglobal fish has enough genetic difference between populations in the Northeast Pacific, Northwest Pacific, and the Southern hemisphere to classify them as three distinct species. In this study, three fin clips and one muscle sample were taken from three individuals for DNA analysis. PCR amplification was performed for the mitochondrial control region and two nuclear genes (RAG2 and EHHADH). The results were sequenced and compared to published sequences for yellowtail stocks in the Northeast Pacific, Northwest Pacific, and the Southern hemisphere. This analysis found that there were at least two genetically distinct stocks in the population at CCAR, with two individuals matching published sequences from the Northeast Pacific clade and one individual matching published sequences from the Southern hemisphere clade, with higher variability in the mitochondrial haplotypes compared to the nuclear sequences. By understanding the genetic makeup of the yellowtail housed at CCAR, future genetic and physiological studies can be done to determine which stock of fish is better suited to aquaculture.

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INTRODUCTION

Background on Aquaculture

Aquaculture, or the "breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments" (NOAA 2019), has increased in recent years as global fish stocks have fallen due to fishing pressure. In 2018, world aquaculture fish production reached 82.1 million tonnes, with aquaculture making up 46 percent of global fish production, up from 25.7 percent in 2000 (FAO 2020). As global demand for seafood increases, aquaculture techniques and research have improved to expand the variety of fish, shellfish, and seaweed species that can be grown in captivity. In the United States, marine aquaculture includes oysters, clams, mussels, shrimp, seaweeds, and finfish such as salmon, black sea bass, sablefish, pompano, and yellowtail (NOAA 2019). Aquaculture's role in meeting the growing global demand for seafood means that species that may be traditionally wild-caught are now being considered as candidates for aquaculture. This requires research to develop better technology for sustainable and efficient aquaculture practices.

Marine finfish aquaculture is typically done in net pens in the water or on recirculating tanks on land. Net pen aquaculture has several advantages, notably it requires much less infrastructure than a recirculating aquaculture facility and is less expensive to operate. However, net pen aquaculture can pose many problems to both cultured fish and the environment. Fish raised in net pens are susceptible to parasites, which can spread rapidly through sea cages, harming the fish and resulting in significant economic consequences (Hutson et al. 2007). Cultured fish can also escape into the

surrounding environment if severe weather damages net pens. This can result in negative ecological and genetic interactions between cultured and wild fish populations, as well as impacts on the larger ecosystem (Lorenzen et al. 2012).

Recirculating aquaculture systems (RAS) are land-based tanks that are designed to grow cultured fish to market size. RAS have the benefit of being a controlled environment. In contrast, net pens can expose fish to highly variable climatic and environmental conditions as well as the potential for exposure to parasites, which can all negatively impact fish growth (Abbink et al. 2011). The ability to optimize environmental conditions such as temperature and pH along with increased biosecurity make RAS an appealing culture method for many finfish species (Abbink et al. 2011). Furthermore, closed RAS can operate with artificial seawater and less than 1% of water renewal per day and have better waste management capabilities than net pens (Orellana et al. 2014, Abbink et al. 2011). These benefits make RAS a sustainable alternative to net pen culture, with little to no environmental impacts. Finally, land-based RAS allows for species of interest to be grown globally and close to their intended market (Orellana et al. 2014).

The yellowtail or amberjack (*Seriola spp.*) is a fast-swimming, pelagic, carnivorous fish globally distributed in tropical and subtropical waters in the Atlantic, Pacific, and Indian oceans (Luna, n.d.). Similar in taste to tuna or mackerel, amberjack species have a large market worldwide and are a popular fish used in sushi, with an estimated value of $1.3 billion (USD) annually (Purcell et al. 2015). Yellowtail are fast-growing, hardy, and have a low feed conversion ratio, which makes them a good candidate for aquaculture. Yellowtail species have been cultured in Japan for decades,

with more recent production spreading to Australia, New Zealand, Chile, and Mexico (Purcell et al. 2015). Juveniles are produced in hatcheries from closed life cycle spawning, while growout is typically done in net pens (Abbink et al. 2011). In recent years, research has focused on optimizing conditions for yellowtail in land-based recirculating aquaculture systems, expanding the locations where aquaculture is possible. A study conducted by Abbink et al. (2011) found that *S. lalandi* growth in recirculating aquaculture systems was optimized at a water temperature of 26.5°C, finding reduced growth and a reduced feed conversion ratio at both higher and lower temperatures. Culture of yellowtail in RAS has the potential to provide this commercially valuable fish to markets that would not usually have access to *S. lalandi,* as well as to bolster supply to places where yellowtail is in high demand.

Physiology and Genomics

Physiology studies are an important part of aquaculture research, which contributes to the efficient growth of commercially valuable organisms in aquaculture systems. Selective breeding programs based on physiological and genetic information are used for a majority of aquacultured species because of high heritability estimates for desired traits, short generation times, and high fecundity (Peterson et al. 2020). Growth rate improvement of finfish using genetic and physiological data began in Europe with rainbow trout, common carp, and Atlantic salmon (Peterson et al. 2020). Today, Atlantic salmon are selectively bred from stocks in the North Atlantic for commercially valuable traits such as a fast growth rate, fillet color, and resistance to sea lice (Peterson et al. 2020). The combined use of physiology and genomics applied to aquaculture can result in

increased economic returns, a higher quality product, and contribute to increasing the sustainability of current culture methods.

Information on the reproduction of wild fish can act as a baseline for cultured fish, which is important in order to manage breeding of a stock (Poortenaar et al. 2001). Poortenaar et al. (2001) investigated the reproductive characteristics of yellowtail from northern New Zealand. They found that *S. lalandi* had the capacity for multiple spawns within a reproductive season by examining oocyte development in females as well as gamete development in males. Furthermore, this study found that the size at sexual maturity for *S. lalandi* was relatively large, indicating that large fish are required for successful spawning in aquaculture systems.

In order to optimize growth rates in fish intended for intensive culture, an understanding of the genetic makeup of the stock is also necessary in order to be able to select for traits that are desirable, such as length and weight, and to avoid genetic deformities. Premachandra et al. (2017) researched the genetic basis for weight, fork length, and the presence of deformities in broodstocks derived from wild S. *lalandi* and *S. lalandi* grown in both net pens and RAS. The researchers found a high heritability estimate of growth traits as well as a higher survival rate of the offspring of the selected broodstock than those of wild broodstock cultured in net pens. This study illustrates the need for a strong understanding of the genetic makeup of *S. lalandi* being considered for intensive aquaculture.

The yellowtail kingfish (*Seriola lalandi*) is a circumglobal fish, meaning that it is found worldwide in temperate and subtropical oceans. However, recent molecular research has suggested that many pelagic fish previously considered to be globally

distributed, such as bluefin tuna, albacore, and striped marlin, show significant genetic structure due to geographic separation (Martinez-Takeshita et al. 2015). Determining the level of genetic difference between stocks is important for aquaculture development of a species, because one stock could behave differently in culture systems than another. Features such as a faster growth rate, a higher rate of successful breeding, or resilience to disease and deformities may vary from one genetically distinct population to another. Therefore, there is a need for research in population genetics in order to optimize aquaculture of economically important species.

Genetic Markers

Several types of genetic markers, including both mitochondrial and nuclear, are used in population genetics to characterize genetic diversity within and between populations. Mitochondrial DNA, or mtDNA, has long been used as a marker of population divergence. One of the advantages of mtDNA is that it is usually maternally inherited without recombination, making the assessment of relatedness in a population fairly simple to determine (Dewoody 2005). The mitochondrial control region (mtCR), a non-coding region in the mitochondrial genome, has long been used in previous studies involving the population structure of globally distributed fish, including bluefin tuna (*Thunnus thynnus thynnus*) (Carlsson et al. 2004), the school shark *Galeorhinus galeus* (Chabot and Allen 2009), and tilapia, which are present worldwide in freshwater environments (Kofi et al. 2020).

Despite its widespread use, more recent research has suggested that mtDNA may not be as reliable of a measure of genetic diversity and relatedness as previously thought.

A review by Galtier et al. (2009) examined studies that were conducted using mtDNA and found that there was evidence of recombination of mitochondrial DNA in many groups of organisms, including fish. Recombination is the mechanism by which double strand breaks in the DNA strand are repaired, which involves the crossing over of DNA. This genetic exchange of information can change the DNA sequence in the mitochondrion, which is not useful for population genetics. For example, Hoarau et al. (2002) found evidence of recombination of the mitochondrial control region in the flatfish *Platichthys flesus.* Furthermore, mitochondrial DNA is located in a highly oxidative and metabolically active environment, subjecting its DNA to a mutation rate that is highly variable (Galtier et al. 2009). A variable rate of mutation is not desirable for studies that involve population genetics because it can provide incorrect information about the rate of evolution in a lineage of organisms. These reasons have brought many scientists to reconsider the usefulness of mitochondrial DNA as a marker for genetic research.

Since mitochondrial DNA can be incomplete as a marker of genetic relationships and diversity, nuclear genes can be used in conjunction with mtDNA to determine evolutionary relationships. Martinez-Takeshita and colleagues, in a study published in 2015, examined the genetic makeup of several different stocks of *Seriola lalandi* using information from mitochondrial and nuclear DNA. This study sampled *S. lalandi* from seven different geographic regions: South Africa, Japan, New Zealand, California, the coast of Baja California Mexico, the Mexico Gulf, and Chile, and sought to reevaluate the treatment of *S. lalandi* as a singular, circumglobal species. Their work points to the existence of three genetically distinct clades located in the Northeast Pacific, Northwest

Pacific, and the Southern Hemisphere (Martinez-Takeshita et al. 2015). From these findings, the authors proposed two new species: *Seriola aureovittata* for yellowtail found in the Northwest Pacific and *Seriola dorsalis* for yellowtail found in the Northeast Pacific, while *Seriola lalandi* remains the scientific name for yellowtail found in the Southern hemisphere. These discoveries in the *Seriola lalandi* species complex may help improve yellowtail aquaculture, as these genetically distinct species may react differently to growth in RAS. Future studies on genomics and physiology will add to our understanding of the physiological differences between these species and add to the growing body of knowledge that is needed for successfully growing yellowtail in RAS.

Aquaculture in Maine & Study Objectives

Aquaculture in Maine is a growing industry, with opportunities for development and expansion. Finfish aquaculture in Maine dates back to the early 1970s, where coho salmon and rainbow trout were raised in net pens in the Wiscasset River and off the coast of Vinalhaven Island (Maine DMR, n.d.). Today, the aquaculture sector contributes $137.6 million to the state of Maine's economy and employs 1,078 people in full- and part-time jobs (Cole et al., 2016). The three top species currently aquacultured in Maine are Atlantic salmon, blue mussels, and Eastern oysters (Cole et al., 2016). However, increasing the diversity of species grown in Maine will strengthen the resilience of the aquaculture industry. Industry partners are interested in yellowtail culture in recirculating aquaculture systems, such as Kingfish Maine, a company based in the Netherlands seeking to bring a recirculating aquaculture facility to Jonesport, Maine in order to

aquaculture yellowtail fish (White, 2020). Therefore, continued research on yellowtail in RAS is important for contributing to the growth of the aquaculture industry in Maine.

The University of Maine's Center for Cooperative Aquaculture Research (CCAR) in Franklin, ME holds a stock of yellowtail fish in RAS. These fish originally came from Great Bay Aquaculture, a now defunct hatchery, and their genetic makeup is unknown. The goal of this project is to run DNA analysis using the mitochondrial control region and the nuclear genes EHHADH and RAG2 on samples from the broodstock of yellowtail housed at CCAR in order to determine which stock the fish belong to. If there is a significant level of genetic difference observed in this stock of yellowtail, there may be potential for a selective breeding program to be developed.

METHODS

In order to determine the genetic origin of the F1 generation of *Seriola lalandi* present at CCAR, we performed a DNA extraction and polymerase chain reaction (PCR) amplification of mitochondrial and nuclear DNA. The genetic origin of the stock at CCAR was then determined by comparing the DNA sequences from individuals of *S. lalandi* at CCAR to published sequences of yellowtail from California, the Pacific coast of Baja California (Mexico), the Gulf of California (Mexico), New Zealand, Japan, South Africa, and Chile. In order to do this, DNA was extracted and analyzed from three fin clips and one muscle tissue sample collected from three adult yellowtail individuals (sample names F1, F2, and F3) housed at CCAR. DNA extraction was completed using the Qiagen QIAamp DNA Micro Kit for purification of Genomic DNA using the Isolation of Genomic DNA from Tissues protocol. Samples of approximately 7 mg in weight were placed in individual 1.5 ml centrifuge tubes with 180 µl of Buffer ATL. After the samples were equilibrated to room temperature, 20 µl of proteinase K was added and the samples were mixed thoroughly by using a pulse-vortex. The samples were then incubated overnight at 56 °C to completely lyse the samples.

After the samples were incubated, 200 µl of Buffer AL was added and the samples were mixed using a pulse vortex. 200 µl of 100% ethanol was then added to the samples and mixed using pulse-vortexing. After briefly centrifuging the samples to remove any drops from the lid, the samples were transferred into QiAmp MinElute columns in 2-mL collection tubes. These were centrifuged for one minute at 8000 rpm to concentrate the lysate on the membrane. Two wash steps were then carried out. 500 µl of

Buffer AW1 was added to the samples and centrifuged for 1 minute at 8000 rpm. This process was repeated with Buffer AW2. The samples were then centrifuged at full speed (14000 rpm) for 3 minutes to dry the membrane completely. Finally, we placed the MinElute columns in clean 1.5 ml microcentrifuge tubes and added 20 µl of Buffer AE. The samples were incubated at room temperature for five minutes before they were centrifuged at 14000 rpm for one minute to collect the eluate containing extracted genomic DNA in the microcentrifuge tube. 1.5 µl of extracted DNA from each sample was then tested for DNA yield (nucleic acid concentration) and quality (260/290 ratio) on a Nanodrop spectrophotometer.

Polymerase chain reaction (PCR) was then carried out on the DNA samples to amplify the control region of the mitochondrial genome and two regions of nuclear DNA: the nuclear recombination activation protein subunit 2 (RAG2) and peroxisomal enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase (EHHADH) genes. Each PCR procedure was completed with a negative control, using purified water in place of a DNA sample. This was done in order to ensure that no cross-contamination occurred during the procedure. Control region amplification was carried out in 20 µl volumes, each containing 12.5 µl purified water, 2 µl standard Taq buffer, 0.8 µl $MgCl_2$, 2 µl dNTPs, 0.8 µl of the forward primer CCR (5'-CCTGAAGTAGGAACCAGATG) and 0.8 µl of the reverse primer TRNA (5'-CACCACTAGCTCCCAAA), 0.1 µl NEB Taq, and 1 µl of DNA. PCR was performed according to Martinez-Takeshita et al. (2015) with an initial denaturing step of 95°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 48°C for 30 seconds, 72°C for 30 seconds, and a final extension step of 72°C for 10 minutes.

The RAG2 and EHHADH genes were amplified in 20 µl volumes each containing 12.5 µl purified water, 2 µl standard Taq buffer, 0.8 µl $MgCl_2$, 2 µl dNTPs, 0.8 µl of the forward primer and 0.8 µl of the reverse primer, 0.1 µl NEB Taq, and 1 µl of DNA. RAG2 was amplified using the RAG2 F2 primer (5'-CCTCTGTCTGGAAGCTCACC) and R2 primer (5'-ACGGCCTCCCAGAGAGTTAT). EHHADH was amplified using EHHADH forward (5'-TGTAAAACGACGGCCAGTACCACCTGGCCTCTCAAACTC) and reverse (5'-CAGGAAACAGCTATGACGCAGCTATTCCATCCTCCAAGATGC) primers with M13 sequences appended for ease of sequencing. PCR amplification was performed according to Martinez-Takeshita et al. (2015). PCR reaction conditions for the RAG2 gene consisted of an initial denaturing step of 94°C for 2 minutes, followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final extension step of 72°C for 10 minutes (Martinez-Takeshita et al. 2015). For the EHHADH gene, the PCR cycle consisted of an initial denaturing step of 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 68°C for 60 seconds, 72°C for 60 seconds, and a final extension step of 72°C for 5 minutes (Martinez-Takeshita et al. 2015).

After PCR was complete, all PCR products were visualized using gel electrophoresis. To prepare the samples for DNA sequencing, the PCR products were enzymatically cleaned through the addition of 0.2 µl Antarctic phosphatase buffer, 0.05 µl exonuclease I and 0.05 µl Antarctic phosphatase. The samples were incubated at 37°C for 60 minutes, 85°C for 15 minutes, and then held at 4°C. The samples were then sent to the UMaine DNA Sequencing Facility for sequencing in both the forward and reverse directions. All twelve sequences were aligned and edited using CodonCode. The

sequences were compared to *S. lalandi* DNA sequences for the mitochondrial control region (118 sequences) and the nuclear genes RAG2 (41 sequences) and EHHADH (41 sequences) published by Martinez-Takeshita et al. (2015) and available from GenBank.

RESULTS

The mitochondrial control region sequences for the three individuals of *Seriola lalandi* were between 373 and 400 base pairs long. This variation in base length was due to sequence quality after trimming the primers and flanking sequence. The mitochondrial control region sequences for F1M and F2 were identical in sequence and were most similar (within 1 base pair) to haplotypes sampled from New Zealand (Table 2). The mitochondrial control region sequences for the F1F and F3 samples were identical to each other and were most similar (within 1 base pair) to haplotypes from California and the Pacific side of Mexico (Table 2).

The RAG2 sequences were 715 base pairs in length. The F1M and F2 RAG2 sequences were identical to each other and had 3 polymorphic sites at positions 203, 211, and 302. These polymorphisms indicate that this sequence matches a mixture of sequences from Chile, South Africa, and New Zealand (Table 2). Only the F1M RAG2 reverse sequence was able to be compared to published sequences because of poor quality in the forward direction. The F3 RAG2 sequence was identical to published sequences from the Pacific coast of Mexico, the Gulf of Mexico, and California (Table 2). The F1F RAG2 sequence had one polymorphic site at position 211. This indicates that this sequence is a mix of published sequences from the Northeast Pacific clade (Pacific side of Mexico, the Gulf of Mexico, and California) and sequences from the Pacific side of Mexico, the Gulf of Mexico, California, and Japan (Table 2).

The obtained EHHADH sequences were 604 base pairs in length. The F1M and F2 EHHADH gene sequences were identical to each other and matched published

sequences from Chile, South Africa, and New Zealand, and the F3 and F1F EHHADH sequences were identical to each other and matched published sequences from California, the Gulf of Mexico, and the Pacific coast of Mexico (Table 2).

The results of the mitochondrial and nuclear sequencing suggest that there are at least two genetically distinct lineages of yellowtail at CCAR. Two samples (F1M and F2) matched published sequences from the Southern hemisphere clade (Chile, South Africa, and New Zealand) and two samples (F1F and F3) matched published sequences from the Northeast Pacific clade (California, the Pacific coast of Mexico, and the Gulf of Mexico) (Table 1, Figure 1).

Furthermore, the samples F1F and F1M were originally a fin clip and a muscle clip from the same individual. The results of this DNA sequencing shows that there was a mislabeling of the samples at some point during the DNA extraction, as the sequences of F1F and F1M should be identical. Due to this mislabeling, DNA extraction of the fin and muscle clips should be performed again to confirm the results of this study. However, these results show that the samples F1M and F2 produced identical sequences in all three genetic regions, which would be consistent with samples labeled as F1M and F2 originating from the same individual. Therefore, we can deduce that out of the three yellowtail individuals studied, two individuals are from the Northeast Pacific clade and one individual is from the Southern hemisphere clade.

DISCUSSION

The results of this study indicate that the broodstock of yellowtail located at CCAR are from at least two different stocks: The Southern hemisphere clade, referred to as *Seriola lalandi*, and the Northeast Pacific clade, with the proposed species name of *Seriola dorsalis,* as proposed by Martinez-Takeshita et al. (2015) (Figure 1, Table 1). The genetic divergence of these species due to geographic separation is similar to what has been observed in other circumglobal species that had previously been considered to be one species with little genetic variation. Furthermore, these results show polymorphic sites in two sequences. The sequencing of the RAG2 gene in samples F1M and F2 resulted in three polymorphic sites, and the sample F1F contained one polymorphic site. This indicates that there may be single nucleotide polymorphisms (SNPs) present in the population at CCAR. Lastly, the mitochondrial control region sequences obtained from this study had a large amount of variation between sequences when compared to published *S. lalandi* sequences. Mutations in the mitochondrial genome will be an important area of study in the future, as literature suggests that mutations and variation within species in the mitochondrial genome has functional effects on physiology.

Genetic Divergence in Globally Distributed Species

Several factors can influence the genetic divergence of highly migratory species of fish, resulting in genetically distinct lineages. The equatorial region could be acting as a dispersal boundary for yellowtail, due to warmer water temperatures that exceed the preferred thermal range for both juvenile and adult yellowtail (Martinez-Takeshita et al.,

2015). A similar phenomenon has been documented in the school shark *Garleorhinus galeus* and the wreckfish *Polyprion americanus* (Chabot & Allen, 2009; Sedberry et al., 1996). The genetic difference found between the Northeast and Northwest Pacific stocks of yellowtail may be due to physical conditions such as oceanic circulation patterns and thermal preferences, and migratory patterns that favor near-shore movements instead of cross-basin travel (Martinez-Takeshita et al., 2015). Oceanographic conditions have been hypothesized as the cause of the genetic difference found in bluefin tuna populations in the eastern and western basins of the Mediterranean Sea, limiting the transport of eggs and larvae (Carlsson et al. 2004). A combination of these factors may have driven the separation of yellowtail populations into distinct species, further emphasizing the need for genetic and physiological studies comparing members of the *Seriola* species complex with respect to fitness in recirculating aquaculture systems.

Single Nucleotide Polymorphisms

The results of the sequencing of the RAG2 nuclear gene in this study showed that there were single nucleotide polymorphisms (SNPs) present in two individuals. SNPs are a single difference in the base pairs of a DNA sequence between two alleles of a gene (De Woody, 2005). A polymorphism at a specific site can be used to differentiate between populations of organisms in genetic studies (Alberts et al., 2019). One organism can have two different alleles of one gene, which are differentiated by these single changes in the sequence of nucleotides. In this study, one polymorphic site in the RAG2 gene of sample F1F caused the sample to match multiple published RAG2 sequences from the Northwest and Northeast Pacific (Table 1). When conducting DNA analysis

studies, it is important to include multiple genetic markers to confirm the results of each marker. Since the two other markers matched sequences from the Northeast Pacific, we can conclude that this individual is from the Northeast Pacific clade.

For our study, two nuclear markers were used: RAG2 and EHHADH, which are protein coding genes and therefore, SNPs found in these genes may have a functional effect on an organism's physiological performance. SNPs have been found to be associated with desirable traits in several commercially valuable fish that are grown in aquaculture systems. By mapping the SNPs found in an organism's genome that may have functional differences on desired traits, selective breeding programs can be established to encourage these traits in a population. For example, increased fillet yield and fillet weight have been attributed to SNPs found in the rainbow trout (*Oncorhynchus mykiss*) genome (Gonzalez-Pena et al., 2016). Resistance to sea lice in the Atlantic salmon (*Salmo salar*) is a polygenic trait but is influenced by the presence of an SNP in chromosome 21 (Correa et al., 2017). Research on *Seriola lalandi* also indicates that SNPs have functional importance for economically valuable traits such as body weight and presence of deformities (Nguyen et al., 2018). Further research is needed on how SNPs may influence body weight and other economically important characteristics between the *Seriola* species complex of *Seriola lalandi, Seriola dorsalis,* and *Seriola aureovittata*.

Variation in the Mitochondrial Genome

In this study, we sequenced one mitochondrial genetic marker, the mitochondrial control region. The results of the comparison between the three individuals from CCAR

and published mtDNA haplotypes showed high variability in the sequences, meaning that there was a greater number of locations where base pairs differed from other sequences. The length of each mtCR sequence obtained from this study varied from 373 to 400 base pairs, which is consistent with the findings of Martinez-Takeshita et al. (2015). Mitochondrial DNA is known to evolve faster than nuclear DNA (Kofi et al., 2020), which may explain the high level of variability found in these sequences. Mitochondrial DNA evolves at a relatively fast rate for several reasons. Mitochondrial DNA has a faster rate of turnover compared to nuclear DNA, which increases the occurrence of mutations (Brown et al., 1979). Furthermore, the mitochondrial replication process lacks an efficient method of editing when mutations or substitutions occur in the DNA sequence (Vasemägi et al., 2017).

The mitochondrial control region is a noncoding region and the most variable part of the mitochondrial genome (Bronstein et al. 2018). The level of variation present in functional parts of the mitochondrial genome can influence physical traits of organisms. Understanding the diversity and variation present in the mitochondrial genome can improve selective breeding programs for aquaculture. Vasemägi et al. (2017) examined mutations in the mitochondrial oxidative phosphorylation (OXPHOS) genes and their connection to osmoregulation in the nine-spined stickleback *Pungitius pungitius*. This study found that mutations in the OXPHOS genes affected osmoregulation in freshwater and brackish water sticklebacks. Strohm et al. (2015) compared OXPHOS genes in a variety of sedentary and highly active fish to find if sedentary fish accumulated more mutations in their mitochondrial genome. This would be due to sedentary fish (such as anglerfish and eels) being less active, therefore requiring less energy and reducing the

need for high production of ATP. In this analysis, sedentary fish had more disadvantageous mutations in their mitochondrial genome compared to highly active fish such as tunas and kingfish (Strohm et al. 2015). The variation within the mitochondrial genome with implications for energy requirements and other important physiological traits is an area of research that will need to be explored further for efficient aquaculture of commercially valuable fish species.

Future Directions of Research

Preliminary genetic studies on species of interest are important for determining the genetic lineages of broodstock to optimize breeding programs for aquaculture. This study had a small sample size of only three individuals, so a more complete sequencing of the broodstock housed at CCAR would be an important next step. Additionally, more nuclear and genetic markers can be used to improve the reliability of results, and to track relatedness in this yellowtail broodstock. By understanding the genetic makeup of the yellowtail housed at CCAR, physiology studies can be conducted, such as running hatchery fitness tests on embryos to determine which genetic lineage of yellowtail is more suited to intensive aquaculture. This study provides the groundwork for future research to be done on the yellowtail broodstock held at CCAR, with exciting potential for diversifying the land-based aquaculture sector in Maine.

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APPENDIX

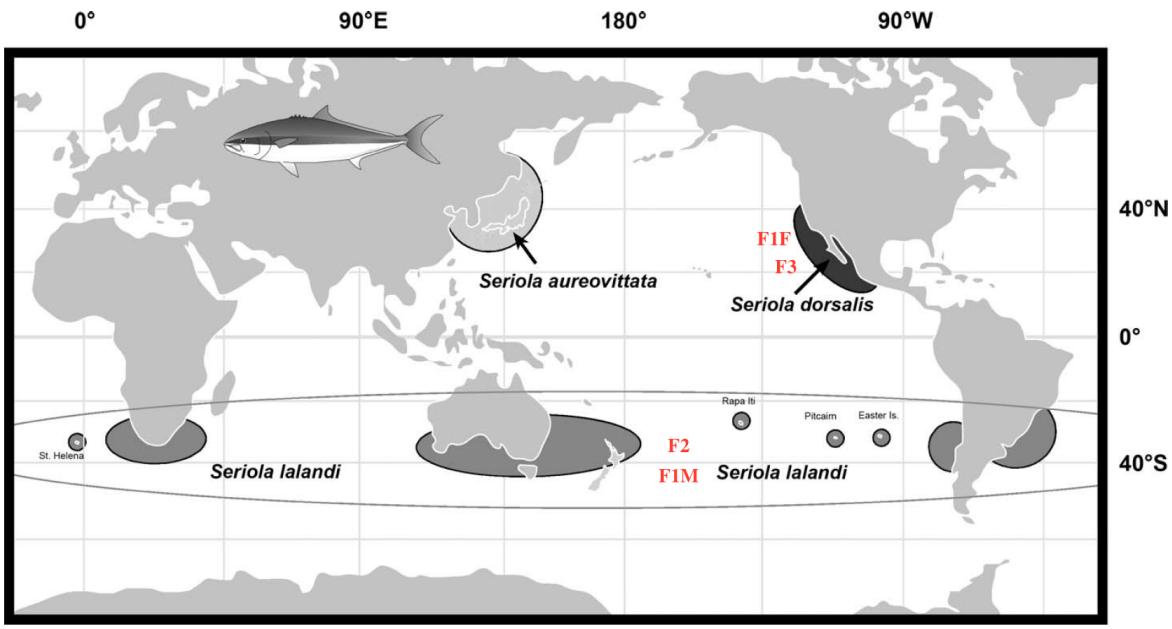

Figure 1: Map showing the distribution of the three clades of the fish formerly known as *Seriola lalandi* with new proposed species names. Sample names indicate the geographic origin of the individuals sampled from CCAR. Figure adapted from Martinez-Takeshita et al. (2015).

Table 1: Results of the sequences from each genetic marker (mitochondrial control region and nuclear genes RAG2 and EHHADH) when compared to published yellowtail sequences from the Northwest Pacific (*S. aureovittata*), Northeast Pacific (*S. dorsalis*), and the Southern hemisphere (*S. lalandi*).

Sample Name	Species Name		
	S. aureovittata	*S. dorsalis*	*S. lalandi*
F1M			✓ ✓ ✓
F1F	✓*	✓ ✓*✓	
F2			✓ ✓ ✓
F3		✓ ✓ ✓	

✓ mtCR
✓ RAG2
✓ EHHADH

*Sequence contained a polymorphism that matched multiple RAG2 sequences from the Northwest and Northeast Pacific clades.

Table 2: Comparison of nuclear and mitochondrial DNA sequences from three *Seriola lalandi* individuals housed at CCAR and published *Seriola lalandi* sequences.

Individual	**Sample Name**	**Tissue Type**	**Genetic Region**	**Putative geographic origin**	**Sequence match from GenBank**
F1	F1M-CCR	Muscle	Mitochondrial control region	New Zealand	KC242051 (1 bp difference) - NZ KC242036 (1 bp difference) - NZ
	F1M-RAG2	Muscle	Nuclear RAG2 gene	Chile, South Africa, New Zealand	3 polymorphic sites; mix of: KM877820, KM877818, KM877787, KM877788, and KM877783-86, 817, 819
	F1M-EHHADH	Muscle	Nuclear EHHADH gene	Chile, South Africa, New Zealand	KM877679 - KM877687
	F1F-CCR	Fin	Mitochondrial control region	California/Mexico Pacific	KC242099 (1 bp difference) - MP KC242080 (1 bp difference) - CA KC242095 (1 bp difference) - CA KC242091 (1 bp difference) - MP

Individual	Sample Name	Tissue Type	Genetic Region	Putative geographic origin	Sequence match from GenBank
	F1F-RAG2	Fin	Nuclear RAG2 gene	Mexico Pacific, Mexico Gulf, California, and Japan	1 polymorphic site; mix of: KM877789, KM877790, KM877794, KM877795, KM877796, KM877798 - KM877806 and KM877791-93, 797, 800, 807-816, 822, 824
	F1F-EHHADH	Fin	Nuclear EHHADH gene	California, Mexico Gulf, Mexico Pacific	KM877657-KM877678
F2	F2-CCR	Fin	Mitochondrial control region	New Zealand	KC242051 (1 bp difference) KC242036 (1 bp difference)
	F2-RAG2	Fin	Nuclear RAG2 gene	Chile, South Africa, New Zealand	3 polymorphic sites; mix of: KM877820, KM877818, KM877787, KM877788, and KM877783-86, 817, 819
	F2-EHHADH	Fin	Nuclear EHHADH gene	Chile, South Africa, New Zealand	KM877679 - KM877687

Individual	Sample Name	Tissue Type	Genetic Region	Putative geographic origin	Sequence match from GenBank
F3	F3-CCR	Fin	Mitochondrial control region	California/Mexico Pacific	KC242099 (1 bp difference) - MP KC242080 (1 bp difference) - CA KC242095 (1 bp difference) - CA KC242091 (1 bp difference) - MP
	F3-RAG2	Fin	Nuclear RAG2 gene	Mexico Pacific, Mexico Gulf, and California	KM877789, KM877790, KM877794-96, KM877798, 99, 801-806, 821, 823
	F3-EHHADH	Fin	Nuclear EHHADH gene	California, Mexico Gulf, Mexico Pacific	KM877657-KM877678

AUTHOR'S BIOGRAPHY

Sydney Avena is from East Lyme, Connecticut and came to the University of Maine in 2017 to pursue her passion for marine science. Sydney is interested in developing sustainable methods for advancing marine aquaculture, and after graduation she plans to take a research assistant position with the Maine Aquaculture Innovation Center to research the social license to operate for aquaculture in coastal waters and to investigate the effect of different aquaculture gear types on sea scallop growth. In the future, she hopes to pursue a master's degree in aquaculture. In her free time, Sydney enjoys skiing, hiking, reading, baking, playing violin, and exploring Maine!