

Tracing Transgenerational Plasticity Through
Ova Fatty Acid Biomarkers in Giant Kōkopu
(*Galaxias argenteus*)

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Abstract

The domestication of native fish species is emerging as a vital approach to addressing New Zealand's unique challenges in biodiversity conservation, food security, and sustainable aquaculture. This thesis focuses on the giant kōkopu (*Galaxias argenteus*), one of New Zealand's five endemic galaxiid species and a cornerstone of the culturally and economically significant whitebait fishery. As a species classified as "vulnerable" by the IUCN, *G. argenteus* faces ongoing threats from habitat degradation, land-use changes, and overfishing. With traditional whitebait fisheries under increasing scrutiny for their sustainability, this research explores aquaculture as a viable pathway to secure the future of *G. argenteus* while preserving New Zealand's rich white baiting heritage.

Building on the domestication efforts of Manāki Whitebait, this study investigates the conditions that facilitated the first successful captive spawning of *G. argenteus* in 2012 and examines strategies for optimising broodstock management to improve reproductive success. A key focus of this research is the role of essential fatty acids (EFAs)—docosahexaenoic acid (DHA), arachidonic acid (ARA), and eicosapentaenoic acid (EPA)—in influencing fecundity, gamete quality, and offspring viability. These EFAs are explored in the context of their metabolic, structural, and signalling roles, revealing their critical impact on fish reproduction and larval development.

The thesis also investigates transgenerational plasticity in *G. argenteus*, analysing how lipid profiles shift across generations to adapt to captivity. This work demonstrates the potential for targeted dietary and environmental interventions to enhance reproductive outcomes and improve the sustainability of aquaculture systems. By integrating ecological, physiological, and aquacultural perspectives, the research highlights the capacity of aquaculture to alleviate pressure on wild populations while contributing to New Zealand's conservation goals and white baiting traditions.

This study serves as a foundational step toward developing a sustainable aquaculture model for *G. argenteus* and other native species, reinforcing the importance of indigenous biodiversity and the integration of mātauranga Māori in contemporary conservation practices. It calls for continued investment in aquaculture innovation and ecological research to ensure that New Zealand remains a global leader in the sustainable management of its aquatic resources.

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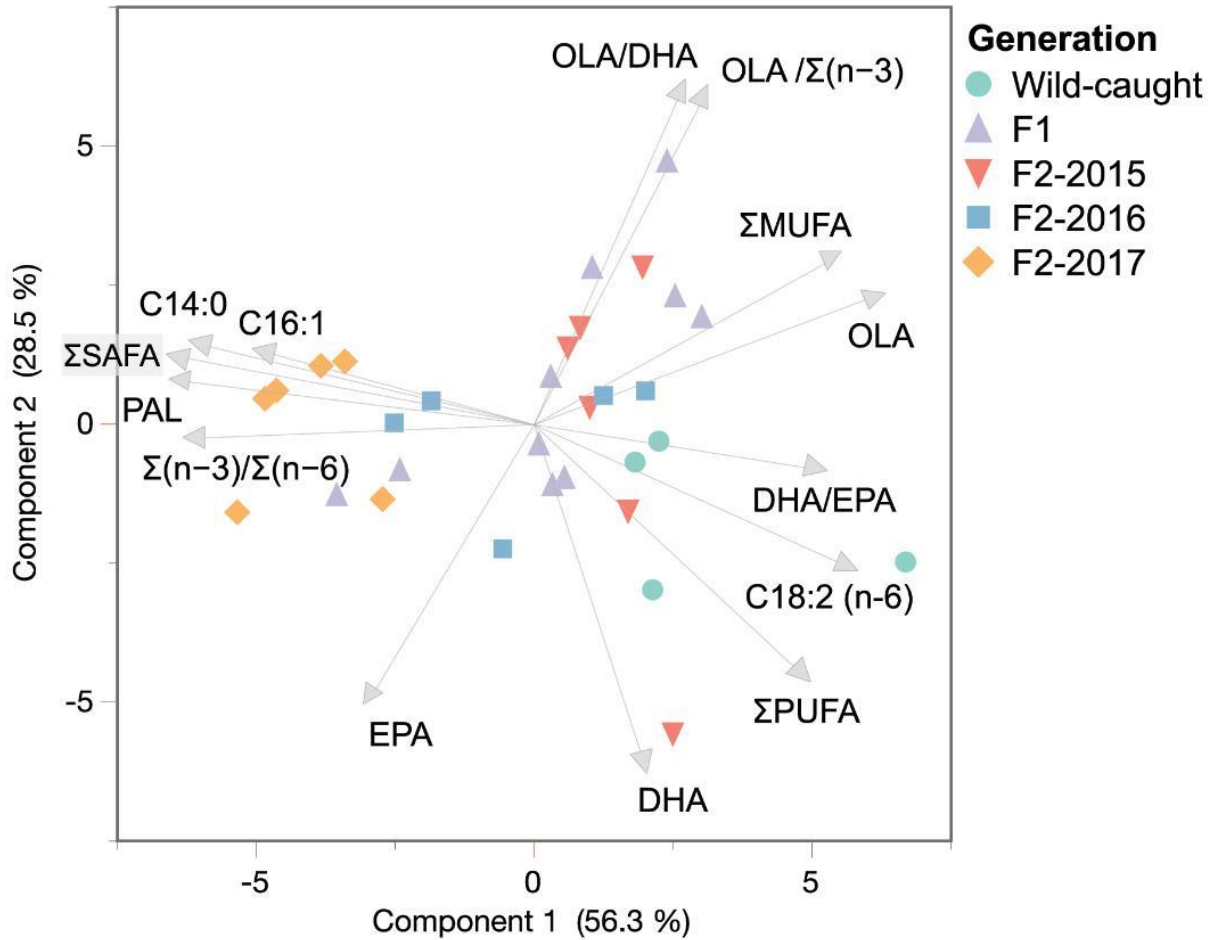


Figure 1. Biplot of the first two principal components of ova fatty acid profiles across different broodstock generations of Giant Kōkopu (*Galaxias argenteus*), highlighting the key fatty acids and ratios that contribute to the overall variance. DHA: C22:6 (n-3), Docosahexaenoic acid; EPA: C20:5 (n-3), Eicosapentaenoic acid; OLA: C18:1 (n-9), Oleic acid; PAL: C16:0, Palmitic acid.

Tables

Fatty Acid	Otohime Pellets			Meal worms		
Σ SAFA	29.90	±	1.04	76.03	±	0.50
14:0	6.66	±	0.28	9.95	±	0.37
16:0 (PAL)	14.86	±	0.08	40.65	±	1.20
18:0	4.96	±	0.54	19.82	±	1.33
Σ MUFA	31.42	±	0.27	12.07	±	0.38
14:1(n-5)	2.00	±	0.13	1.37	±	0.09
16:1(n-7)	8.03	±	0.10	9.12	±	0.16
18:1(n-7)	4.16	±	0.21	0	±	0
18:1(n-9)[OLA]	10.42	±	0.11	0	±	0
Σ PUFA	38.68	±	0.96	11.90	±	0.37
18:3(n-3)	2.00	±	0.04	10.23	±	0.50
20:4(n-6)[ARA]	1.78	±	0.04	0	±	0
20:5(n-3)[EPA]	9.73	±	0.36	0.07	±	0.00
22:5(n-3)	2.81	±	0.08	0.03	±	0.00
22:6(n-3)[DHA]	17.40	±	0.63	0.04	±	0.00
Σ (n-3)	34.7	±	1.15	10.77	±	0.43
Σ (n-6)	4.0	±	0.04	1.14	±	0.10
Σ (n-3) / Σ (n-6)	8.78	±	0.24	9.55	±	1.18
DHA / EPA	1.78	±	0.00	0.50	±	0.12
OLA/ Σ (n-3)	0.3	±	0.01	0	±	0
OLA/DHA	0.6	±	0.02	0	±	0
Other FA	7.90	±	0.50	6.01	±	0.36

Table 1. Mean (± SD) relative fatty acid content (%) of pellets (Otohime) and mealworms (*Tenebrio molitor*).

Fatty Acid	Wild-caught (n=4)	F1 (n=10)	F2-2015 (n=6)	F2-2016 (n=5)	F2-2017 (n=6)	<i>p</i>
Σ SAFA	48.319 \pm 6.003 ^B	54.099 \pm 4.121 ^{AB}	51.869 \pm 4.065 ^{AB}	55.943 \pm 4.920 ^A	61.839 \pm 3.218 ^A	0.0006*
C6:0	1.118 \pm 0.279 ^A	1.298 \pm 0.240 ^A	1.410 \pm 0.612 ^A	1.294 \pm 0.473 ^A	0.471 \pm 0.118 ^B	0.0011*
C14:0	3.278 \pm 0.392 ^B	4.352 \pm 0.628 ^A	3.996 \pm 0.323 ^{AB}	4.464 \pm 0.517 ^A	5.517 \pm 0.376 ^A	<.0001*
C16:0 (PAL)	33.525 \pm 4.362 ^B	38.240 \pm 3.612 ^{AB}	37.136 \pm 2.773 ^{AB}	39.936 \pm 3.862 ^A	44.637 \pm 2.578 ^A	0.0005*
C18:0	5.787 \pm 0.413 ^{AB}	5.748 \pm 0.406 ^{AB}	5.185 \pm 0.474 ^B	5.697 \pm 0.170 ^{AB}	6.008 \pm 0.405 ^A	0.0195*
C23:0	2.702 \pm 0.526 ^A	2.284 \pm 0.244 ^{AB}	2.162 \pm 0.384 ^B	2.247 \pm 0.253 ^{AB}	2.658 \pm 0.255 ^A	0.0274*
Σ MUFA	32.504 \pm 2.079 ^A	32.294 \pm 4.108 ^A	32.427 \pm 1.551 ^A	30.419 \pm 3.193 ^A	27.754 \pm 2.460 ^A	0.0542
C16:1 (n-7)	3.536 \pm 0.740 ^B	4.513 \pm 0.611 ^A	4.049 \pm 0.371 ^{AB}	4.400 \pm 0.707 ^{AB}	6.019 \pm 0.561 ^A	<.0001*
C18:1 (n-9) (OLA)	28.564 \pm 2.037 ^A	27.308 \pm 4.005 ^A	28.022 \pm 1.268 ^A	25.605 \pm 3.347 ^{AB}	21.236 \pm 1.943 ^B	0.0018*
Σ PUFA	19.177 \pm 4.892 ^A	13.607 \pm 1.731 ^B	15.705 \pm 4.709 ^{AB}	13.639 \pm 2.250 ^B	10.407 \pm 1.563 ^C	0.0025*
C18:2 (n-6)	6.774 \pm 4.241 ^A	3.929 \pm 1.317 ^B	5.083 \pm 1.736 ^{AB}	3.621 \pm 1.309 ^B	1.143 \pm 0.454 ^C	0.0013*
C20:4 (n-6) (ARA)	1.992 \pm 0.415 ^A	1.331 \pm 0.418 ^B	1.260 \pm 0.347 ^B	1.036 \pm 0.204 ^B	0.805 \pm 0.070 ^B	0.0002*
C20:5 (n-3) (EPA)	0.994 \pm 0.127 ^A	1.101 \pm 0.234 ^A	1.115 \pm 0.341 ^A	1.109 \pm 0.166 ^A	1.257 \pm 0.178 ^A	0.5119
C22:6 (n-3) (DHA)	9.417 \pm 0.846 ^A	7.247 \pm 0.958 ^B	8.246 \pm 2.461 ^{AB}	7.873 \pm 1.136 ^{AB}	7.203 \pm 0.990 ^B	0.1016
Σ (n-3)/ Σ (n-6)	1.383 \pm 0.516 ^B	1.743 \pm 0.708 ^{AB}	1.491 \pm 0.192 ^{AB}	2.079 \pm 0.636 ^A	4.456 \pm 0.630 ^A	<.0001*
DHA/EPA	9.519 \pm 0.647 ^A	6.759 \pm 1.187 ^B	7.422 \pm 0.690 ^{AB}	7.132 \pm 0.707 ^{AB}	5.777 \pm 0.759 ^B	<.0001*
OLA/DHA	3.055 \pm 0.371 ^A	3.883 \pm 1.048 ^A	3.635 \pm 0.991 ^A	3.292 \pm 0.536 ^A	2.991 \pm 0.456 ^A	0.2228
OLA/ Σ (n-3)	2.764 \pm 0.345 ^A	3.379 \pm 0.935 ^A	3.200 \pm 0.867 ^A	2.886 \pm 0.482 ^A	2.545 \pm 0.385 ^A	0.2263

Table 2. Mean (\pm SD) relative fatty acid content (%) in ova of Wild, F1, F2(2015), F2(2016), F2(2017) Giant Kōkopu (*Galaxias argenteus*). ANOVA **p* values of < 0.05 indicate significant difference between generations. Different superscript letters^(a,b,c) in the same row indicated significant statistical difference (*p*<0.05, Tukey-HSD post-hoc test). SAFA, saturated fatty

acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; OLA, oleic acid; PAL, palmitic acid. All individual FAs that contributed to more than 1% towards total FA profile are depicted in this table.

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor used artificial intelligence tools or generative artificial intelligence tools (unless it is clearly stated, and referenced, along with the purpose of use), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Helen Haong 06/02/2025

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Lilkendey	<ul style="list-style-type: none">• Performed statistical analysis and wrote paper

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Chapter 1: Introduction

Transgenerational Plasticity (TP)

The productivity and efficiency of aquaculture systems are fundamentally supported by the quality and genetic robustness of broodstock, the breeding individuals that determine the success of future generations (Mylonas et al. 2011; Duncan et al. 2013). In the New Zealand, aquaculture is both an economic and ecological priority, improving broodstock management is critical to meeting the increasing demand for sustainable seafood production. Historically, broodstock selection has focused on heritable traits such as rapid growth rates, high feed conversion efficiency, and resistance to disease, with the aim of enhancing yield and profitability (Izquierdo et al. 2001; Valdebenito et al. 2015). While these traits remain essential, there is growing recognition that gamete quality—specifically the physiological and biochemical properties of eggs and sperm—plays an equally vital role in determining reproductive success and the viability of offspring (Migaud et al. 2013; Hansen et al. 2022).

One emerging area of focus in broodstock management is the study of ova lipidomic profiles, which provide valuable insights into maternal investment in reproduction. Lipids are critical components of eggs, acting as energy reserves and structural materials essential for embryonic development (Reading et al. 2018). The composition and abundance of lipids in ova are influenced by maternal environmental conditions, including diet, temperature, and stress levels, making them key biomarkers for assessing both gamete quality and the maternal environment (Malzahn et al. 2022). Furthermore, lipid profiles are increasingly being recognised as predictive indicators of reproductive success in aquaculture, with strong links to fertilisation rates, embryonic survival, and larval performance (Hansen et al. 2022). Despite their importance, the pathways through which maternal experiences shape ova lipid composition and the implications for offspring fitness remain underexplored, particularly in the context of transgenerational effects.

Transgenerational plasticity (TP) is a key mechanism through which maternal environments influence offspring traits. TP refers to the transmission of adaptive traits across generations via non-genetic mechanisms, allowing offspring to better cope with environmental conditions similar to those experienced by their parents (Gavery and Roberts, 2017; Heckwolf et al. 2018). These mechanisms may include epigenetic modifications, changes in maternal provisioning, and hormonal signalling, enabling rapid adaptation to environmental variability without requiring genetic changes (Mousseau and Fox, 1998; Spitzer, 2004). In aquaculture

systems, where environmental conditions can vary widely due to climate change, feed availability, and water quality, understanding the role of TP is increasingly significant.

Moreover, TP offers an opportunity to advance aquaculture sustainability by enabling breeding programmes that are better aligned with environmental changes. For example, maternal provisioning of specific fatty acids (FAs) to eggs, as observed in this study, could serve as an adaptive mechanism to enhance larval viability under unfavourable conditions. These findings suggest that TP could be leveraged to optimise broodstock management by tailoring rearing environments to encourage favourable lipid profiles in gametes. This approach could help mitigate the impacts of stress and improve reproductive success, offering aquaculturists practical strategies to address challenges posed by climate variability and resource limitations (Gavery and Roberts, 2017; Heckwolf et al. 2018; Roy et al. 2023).

Additionally, integrating lipidomic data with other physiological and genetic metrics could provide a more comprehensive understanding of broodstock health and adaptability. By identifying biomarkers associated with stress resilience, immune function, and reproductive efficiency, aquaculture operations could adopt more targeted approaches to broodstock selection and management. Including lipid profiles in routine broodstock evaluations could transform the aquaculture industry by ensuring only the most robust and adaptive individuals contribute to future generations (Roy et al. 2023).

Ultimately, the study of TP and lipidomics in broodstock management represents an exciting frontier in aquaculture science, offering pathways to improve productivity, sustainability, and ecological balance. Continued exploration of transgenerational mechanisms and their practical applications will be vital for meeting the growing global demand for aquaculture products while safeguarding the health and integrity of aquatic ecosystems.

Lipids

Lipids, hydrocarbon-chained organic biomolecules synthesised primarily in the liver, are indispensable in aquatic reproduction due to their multifaceted roles. As vital energy reserves, lipids are stored in egg yolk, where they fuel embryogenesis, while simultaneously acting as substrates for biochemical vectors such as vitellogenin and lipoproteins (Wiegand, 1996; Johnson, 2009; Hiramatsu et al. 2015; Bruslé and Anadon, 2017). Within this broad category, essential fatty acids (EFAs), including C22:6 (n-3) (Docosahexaenoic acid, DHA) and C20:5 (n-3) (Eicosapentaenoic acid, EPA), are particularly significant. These EFAs not only provide critical energy reserves but also confer structural integrity to cell membranes, serve as precursors for signalling molecules such as eicosanoids, and support physiological

processes vital for larval development, such as neural growth and osmoregulation (Bell et al., 2003; Tocher, 2003, 2010; Salze et al. 2005; Yanes-Roca et al. 2009; Grote et al. 2011; Hauville et al. 2016). The presence and proportions of DHA and EPA in ova thus represent key indicators of maternal provisioning quality and subsequent larval fitness.

Moreover, research into the intergenerational shifts in lipid profiles, as observed in the giant kōkopu study, has highlighted the plasticity of these traits in response to changing environmental conditions. This underscores the importance of transgenerational plasticity (TP) in adapting to controlled rearing environments (Gavery and Roberts, 2017; Heckwolf et al. 2018). TP refers to the capacity of organisms to pass on adaptive traits acquired in one generation to the next, allowing populations to adjust to new environments without relying solely on genetic evolution. The study of TP in fish aquaculture has profound implications for improving broodstock management, as it suggests that even captive-bred fish can evolve in response to selective rearing conditions. For instance, as observed in giant kōkopu, each successive generation may deposit different levels of EFAs in their eggs, potentially improving their reproductive success and stress tolerance over time.

This plasticity offers aquaculture managers a valuable tool for adapting to the challenges of farming species in captivity. By selecting for specific traits related to lipid metabolism and reproductive success, and incorporating targeted feeding strategies or environmental adjustments, operators could guide the adaptation process and enhance the productivity of their stocks (LaCava et al. 2023). Furthermore, understanding the role of transgenerational effects could provide insights into how fish can adapt not only to immediate rearing conditions but also to long-term shifts in aquaculture environments, such as those brought about by climate change or other ecological disruptions (Gavery and Roberts, 2017; Heckwolf et al. 2018; Tilloston et al. 2018)

EFAs like DHA and EPA are not only crucial for reproductive success but also hold the potential to significantly improve the overall adaptability and productivity of aquaculture systems. By carefully managing their incorporation into broodstock diets and understanding the dynamics of lipid metabolism, aquaculture operations can optimise fertilisation rates, embryonic development, and larval survival. Additionally, the study of intergenerational shifts in lipid profiles, particularly through the lens of transgenerational plasticity, offers exciting opportunities for improving broodstock management, ensuring more resilient and productive aquaculture systems for the future.

Fish possess the unique ability to synthesise EFAs *de novo*, although this capacity varies widely across species and environmental contexts (Tocher, 2010). This biochemical versatility underscores the untapped potential of using EFA profiles not just as markers of

gamete quality but as indicators of maternal environmental conditions and adaptive strategies. By examining how factors such as diet, temperature, and stress shape EFA synthesis and deposition in ova, researchers can identify trends that influence offspring resilience.

Expanding the focus of lipidomics beyond EFAs to include non-EFAs provides a more comprehensive understanding of the physiological and environmental factors influencing maternal provisioning. Non-EFAs, which are often synthesised endogenously, can offer additional insights into metabolic processes and environmental adaptations. For example, elevated levels of palmitic acid and oleic acid may not only indicate stress (Izquierdo. 1996; Montero et al. 2001) or immune responses (Nakamura et al. 1996; Librán-Pérez et al. 2019). By integrating EFA and non-EFA profiles, aquaculture researchers and practitioners can gain a more nuanced understanding of the interplay between maternal provisioning, environmental stressors, and TP.

The implications of this holistic approach to lipidomics are transformative for aquaculture. By identifying lipid profiles that correlate with optimal health, stress resilience, and reproductive success, broodstock selection criteria can be refined, enabling aquaculture operations to prioritise individuals with the most favourable traits. Tailoring feed formulations to optimise lipid profiles offers another avenue for improving maternal provisioning and offspring viability.

Introducing Wild Species into Aquaculture

The introduction of wild species into aquaculture presents a transformative opportunity to simultaneously address global food security and promote biodiversity conservation while maintaining ecological balance (Anderson et al. 2017; Overton et al. 2024). Transitioning wild populations to aquaculture systems, however, is fraught with challenges that extend beyond logistical hurdles, demanding careful consideration of biological, environmental, and economic factors (McDermid et al. 2010). Wild species often require multiple generations to acclimatise to the controlled conditions of captivity, necessitating adaptive strategies that prioritise their health, reproductive viability, and long-term productivity.

One of the key challenges is fostering the transition of wild-caught broodstock to domesticated conditions without compromising their genetic diversity or ecological resilience. Wild populations, shaped by natural selection, are inherently adapted to dynamic and often unpredictable environments. In captivity, these adaptations may no longer confer advantages and can even become maladaptive, leading to reduced reproductive success,

increased susceptibility to stress, or compromised health (McDermid et al. 2010; LaCava et al. 2023).

Examining FA Profiles Across Generations

This thesis investigates the fatty acid (FA) profiles of ova from *Galaxias argenteus* across five generations of broodstock, providing a foundation for exploring the mechanisms of transgenerational plasticity (TP) and their implications for aquaculture. By focusing on a controlled experimental design—where genetic lineage, diet, and environmental conditions remain consistent—this research examines how lipid provisioning in ova changes across generations and what these changes reveal about the adaptive strategies inherent to TP.

Three key fatty acid (FA) biomarkers—C16:0 (Palmitic Acid, PAL), C18:1 (n-9) (oleic Acid, OLA), and C20:4 (n-6) (Arachidonic Acid, ARA)—serve as central indicators in understanding maternal provisioning and offspring fitness in this study. These lipids are not only fundamental components of the fish's metabolic and reproductive processes but also provide a unique lens into how broodstock adaptation unfolds across generations, particularly in captive environments.

Palmitic Acid (PAL), a saturated fatty acid, plays a pivotal role in cellular energy metabolism, as it is a major component of both membrane lipids and stored fat reserves (Biolchini et al. 2017). In the context of broodstock, PAL serves as an essential energy source, directly fuelling embryonic development and supporting the metabolic demands of gestating mothers (Furuita et al. 2003, 2006; Nguyen et al. 2010). More than just an energy reserve, PAL also possesses immunostimulatory properties, which can enhance the immune function of the broodstock (Nakamura et al. 1996; Librán-Pérez et al. 2019). This property is especially crucial in aquaculture, where fish often face stressors such as environmental changes or crowded conditions. Elevated levels of PAL can indicate a broodstock's ability to withstand stress, which directly influences the quality of the offspring (Sabetian et al. 2023) The presence of PAL in eggs can thus act as a marker of maternal health and resilience, providing insight into the overall immune status of the broodstock (Nakamura et al. 1996; Librán-Pérez et al. 2019).

Oleic Acid (OLA), a monounsaturated fatty acid, has been shown to be closely linked to metabolic adaptation, particularly under stress conditions (Izquierdo, 1996; Montero et al. 2001). OLA is important for membrane fluidity and cellular function, and its role in stress resilience is crucial in maintaining reproductive success in controlled environments. In aquaculture, where fish are often subject to fluctuating environmental conditions, OLA helps

maintain cellular homeostasis, making it a key factor in ensuring that broodstock can adapt to changing conditions without compromising egg quality or larval viability.

Arachidonic Acid (ARA), a polyunsaturated fatty acid, plays an essential role in embryonic development by serving as a precursor to eicosanoids—bioactive lipids involved in cellular signalling and inflammatory responses, critical for processes such as neural development, cell differentiation, and immune function during early life stages (Sargent et al. 1995; Izquierdo, 1996; Grote et al. 2011). In fish, ARA has been linked to embryonic survival rates and overall larval quality, as its presence in the egg yolk supports key developmental processes (Furuita et al. 2003). The ability of broodstock to deposit sufficient amounts of ARA in their eggs directly influences the developmental potential of their offspring, highlighting the importance of this lipid for reproductive success. Furthermore, ARA's role in immune function ensures that offspring are better equipped to resist diseases and environmental stressors, which can significantly enhance their survival and fitness in aquaculture settings (Khozin-Goldberg et al. 2006).

By tracking the concentrations of these key fatty acid biomarkers—PAL, OLA, and ARA—across successive generations, this study offers valuable insights into how maternal provisioning adapts in response to changes in captivity. Each of these biomarkers reflects different aspects of maternal health, energy reserves, immune function, and developmental support for offspring. Shifts in their relative concentrations over generations can reveal how the broodstock's health and reproductive capacity evolve in controlled environments, and how maternal conditions influence offspring fitness and resilience.

Understanding these biomarkers provides a window into the broader implications of transgenerational plasticity (TP) in aquaculture. As broodstock adapt to captivity, these fatty acids may serve as key indicators of how maternal provisioning influences offspring quality, stress resilience, and overall reproductive success.

The primary goal of this thesis is to expand the understanding of TP in *G. argenteus* by investigating how FA allocation in ova reflects maternal environmental conditions and adaptive responses over multiple generations. This research addresses key gaps in the current literature by linking lipidomic profiles to both theoretical frameworks of TP and practical applications in aquaculture. Specifically, it seeks to answer how controlled aquaculture environments influence maternal lipid provisioning, how these changes manifest across generations, and what they imply for broodstock management strategies.

This research holds significant practical relevance for the development of sustainable aquaculture systems in New Zealand. By identifying FA biomarkers that correlate with gamete quality and offspring resilience, this thesis aims to provide actionable insights for

enhancing broodstock performance and supporting domestication efforts for *G. argenteus*. These findings are particularly relevant given the vulnerability of wild populations and the growing need for sustainable alternatives to wild-caught fisheries. Moreover, this study contributes to the broader understanding of how environmental conditions can shape reproductive strategies through TP, with implications that extend beyond aquaculture to conservation and ecological management.

In summary, this thesis explores the intersection of lipidomics and transgenerational plasticity, offering a novel perspective on how maternal investment strategies can be harnessed to optimise aquaculture outcomes. By advancing both theoretical and applied knowledge, it aims to support the sustainable management of *G. argenteus* and contribute to the broader goals of ecological resilience and food security in New Zealand's aquaculture sector.

Chapter 2: Literature Review

Lipids, as fundamental organic macromolecules, play a critical role in supporting cellular functions across animal species (Küppers, 1983). Defined by their hydrophobic nature and nonpolar solubility, lipids comprise a diverse group of hydrocarbon-chained biomolecules that are integral to complex biological processes (Vilella et al. 2013). These macromolecules can be classified into distinct categories based on their chemical structures, including phospholipids, triglycerides, sterols, wax esters, and fatty acids (FA). Each category is further divided into various sub-classes and species, reflecting their diverse roles in cellular physiology (Copeman and Parrish, 2004).

In animals, lipids are indispensable for physiological development, somatic growth, and reproduction (Yanes-Roca et al. 2009). Their functionality is particularly evident in reproductive biology, where lipid content has been strongly correlated with hatching success, larval growth, and survival rates (Salze et al. 2005; Sinanoglou et al. 2011; Źarski et al 2017). This is largely due to their role as the primary structural component of cellular membranes and as a key source of endogenous energy for developing embryos during embryogenesis (Johnson, 2009; Yanes-Roca et al. 2009).

Phospholipids, for instance, are essential in forming cell membranes and supporting cellular integrity, enabling efficient nutrient and waste exchange during early development.

Triglycerides serve as a dense energy reserve, particularly crucial for the metabolic demands of embryogenesis. Fatty acids (both saturated and unsaturated) are significant for energy metabolism and are precursors to vital signalling molecules, such as eicosanoids, which regulate processes like inflammation and reproduction (Sargent et al. 1995; Mayes and Botham, 2003).

Further, the availability and quality of lipids in maternal provisioning are critical determinants of reproductive success in many aquatic species. For fish, the biochemical composition of lipids within oocytes has been shown to influence embryonic development, hatching rates, and larval vitality, underscoring their central role in aquaculture productivity (Copeman and Parrish, 2004; Źarski et al. 2017). The ability of lipids to act as both structural and energetic substrates positions them as a cornerstone in understanding reproductive ecology and the sustainability of captive breeding programs.

Understanding the nuanced roles of lipid classes in biological development and reproduction offers valuable insights for improving aquaculture practices. This knowledge enables the development of targeted nutritional interventions, optimised broodstock diets, and enhanced

management strategies, all of which are essential for increasing the productivity and sustainability of aquaculture systems.

Fatty acids, particularly polyunsaturated fatty acids (PUFAs), are critical for the reproduction and early developmental stages of fish species. Among these, three essential fatty acids (EFAs)—docosahexaenoic acid (DHA), arachidonic acid (ARA), and eicosapentaenoic acid (EPA)—are recognised for their pivotal roles in fish physiology. These EFAs serve as multifunctional molecules, acting as signalling agents, structural components of cell membranes, and metabolic energy sources essential for somatic and physiological development (Grote et al. 2011; Izquierdo, 1996; Sargent et al. 1995).

Essential fatty acids (EFAs) are pivotal in regulating reproductive success, influencing critical parameters such as fecundity, egg fertilization rates, hatching success, larval growth, and survival. These effects are further amplified in aquaculture, where EFAs significantly contribute to larval resilience against environmental and physiological stressors, enhancing overall productivity and sustainability (Castro et al. 2009, 2010; Copeman et al. 2002; Jaya-Ram et al. 2008).

Among EFAs, docosahexaenoic acid (DHA) is indispensable for the neural and retinal development of fish larvae, which rely on visual acuity for efficient prey capture during early life stages (Bell et al. 1995; Pankhurst, 1994; Sargent et al. 1995). DHA integrates into neuronal and retinal cell membranes, improving their structural and functional integrity. This enhancement of sensory and cognitive abilities directly impacts larval feeding efficiency, survival rates, and overall fitness, highlighting the importance of DHA in aquaculture diets.

Arachidonic acid (ARA) and eicosapentaenoic acid (EPA) play equally critical roles as precursors to eicosanoids, bioactive lipid mediators involved in a range of physiological processes. These include growth regulation, immune modulation, neural development, and reproductive functions (Rowley et al. 1995; Mayes and Botham, 2003). The functional balance between ARA-derived and EPA-derived eicosanoids is particularly significant, as these compounds often exhibit contrasting effects. For instance, ARA-derived eicosanoids typically promote pro-inflammatory responses, while EPA-derived eicosanoids are generally anti-inflammatory (Bao et al. 2023). This balance is crucial for maintaining optimal immune function and stress responses in aquaculture systems. Proper management of dietary EFA ratios, therefore, becomes essential for optimising both reproductive outcomes and overall health in broodstock.

In aquaculture, where fish are unable to synthesise EFAs *de novo*, dietary supplementation of EFAs is a critical determinant of gamete quality and offspring viability. The DHA/EPA ratio, for example, is strongly correlated with improved egg quality, larval performance, and

survival rates (Bell et al. 2003; Tocher, 2010). Similarly, ARA supplementation has been linked to enhanced stress resilience and regulation of reproductive hormones, further underscoring its importance in broodstock management strategies (Izquierdo, 1996; Wiegand, 1996).

Effective EFA provisioning not only supports the physiological needs of developing embryos and larvae but also addresses broader challenges in aquaculture by promoting resilience to environmental fluctuations and stressors. By fine-tuning the dietary balance of EFAs such as DHA, EPA, and ARA, aquaculture practitioners can enhance reproductive efficiency and sustainability, paving the way for more robust and productive aquaculture systems.

In summary, DHA, ARA, and EPA represent biochemical cornerstones of aquaculture and larval development. Their multifunctional roles in energy provision, structural functionality, and signalling pathways make them critical focal points for advancing both aquaculture and conservation initiatives. Understanding and optimising the dynamics of EFAs not only enhance reproductive outcomes in controlled settings but also contribute to the sustainable management of fish populations in the wild.

Utility of lipidomics

Lipidomics is the study of lipidomes using the techniques and principles of analytical chemistry (Han and Gross, 2003). The analytical power and advances in mass spectrometry (MS) has led to the rapid expansion of lipidomic research (Han and Gross, 2003; Blanksby and Mitchell, 2010; Han et al. 2012). Over the past few years, the growing interest in lipidomics has also led to the discovery and identification of hundreds of novel lipids, revealing the importance of their involvement in the complex biological transformations in animals (Hyötyläinen and Orešič, 2014). The aim of lipidomics is to fully characterise lipid molecular species and their biological roles with respect to expression of proteins involved in lipid metabolism and function, which includes gene regulation (Kishimoto et al. 2001; Han & Gross, 2003). Fatty acids are often defined as low-molecular-weight lipids, for which the use of gas chromatography-mass spectrometry (GC-MS) with electron ionization is the basis for lipid identification and quantification (Quehenberger et al. 2011).

In teleost (bony fish) species, lipids play a pivotal role within the reproductive axis, influencing a variety of physiological processes essential for successful reproduction. These lipids are not only structural components of cellular membranes but also serve critical functions in energy metabolism, hormonal regulation, and the synthesis of key reproductive molecules. Specifically, organs such as the brain, pituitary gland, gonads, and liver are key

sites for lipid action, with particular emphasis on the gonads and liver due to their direct involvement in oocyte production and egg development (Dreier et al. 2020).

In the gonads, lipids are central to the synthesis of oocytes, providing energy and essential fatty acids (EFAs) required for proper egg maturation and the health of developing embryos. Lipids, through their role as synthesisers and transporters, are essential for the delivery of nutrients and precursors that fuel vitellogenesis—the process by which yolk proteins and lipids are deposited into eggs (Wiegand, 1996). This process is crucial for reproductive success, as the quality of eggs, determined in part by their lipid content, significantly influences larval survival and development. The liver, as the main organ responsible for lipid metabolism and storage, works in concert with the gonads to ensure that lipids are synthesised and mobilised to the reproductive tissues at the right time, thus supporting oocyte maturation (Martynuik et al. 2013; Dreier et al., 2020).

The liver also plays a significant role in the synthesis of lipoproteins, which transport lipids, including essential fatty acids, to the gonads. This ensures that the female fish has a sufficient supply of lipids needed for egg production and regulates the balance of different lipid classes to optimise reproductive performance. The liver is also involved in *de novo* lipid synthesis, which allows the fish to generate necessary lipids even when dietary intake is insufficient (Zhang et al. 2023), making it a critical player in ensuring reproductive success.

Dietary lipids are an important source of these essential fatty acids, which are obtained directly from the environment. In marine species, where lipids are a primary source of metabolic energy, fish obtain these essential components from the rich lipid content of their diet, particularly in organisms like plankton or small fish. Lipids from the diet are transported to the liver and gonads, where they are utilised for energy storage, membrane construction, and the synthesis of hormones like gonadotropins that regulate reproductive processes. This dietary intake is essential for optimal egg production and overall reproductive performance. However, lipids are also mobilised from storage tissues, such as fat reserves in the liver and adipose tissue, to meet the high energy demands during the reproductive season when dietary intake may not be sufficient to meet metabolic needs (Wiegand, 1996). This ability to mobilise stored lipids ensures that fish can maintain energy balance even during periods of fasting or when food availability is limited, which is particularly important during spawning.

The composition of lipids within the body, and particularly within the gonads and liver, varies depending on several reproductive parameters. These include the stage of reproduction, the availability of food, and environmental factors such as temperature and habitat conditions (Mourete and Esther, 2002; Zudaire et al. 2014). The type and amount of lipids stored and mobilised during the reproductive period can significantly impact egg quality, including the

levels of essential fatty acids (EFAs) like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are critical for neural development in larvae and overall offspring survival. These variations in lipid composition may also influence the timing of spawning and the overall reproductive output of fish, highlighting the complex interplay between lipid metabolism and reproductive health (Santamaria-Miranda et al. 2021).

Moreover, the synthesis and storage of lipids within the liver and gonads are tightly regulated by hormonal signals that coordinate the timing of reproductive events. Hormones such as estradiol, progesterone, and gonadotropins regulate lipid metabolism and egg production, ensuring that lipids are mobilised at the right time to support oocyte maturation and the successful fertilisation of eggs (Readings et al. 2018).

In summary, lipids are essential not only for the structural integrity and function of reproductive organs but also as crucial sources of energy and biosynthetic precursors for egg production in teleost fish. Through their roles as synthesisers, transporters, and utilisers, lipids support a range of physiological processes that ensure successful reproduction. Understanding the dynamics of lipid metabolism within the liver and gonads—and how lipids are sourced from both the diet and endogenous stores—provides valuable insights into reproductive health and success in aquaculture. This knowledge can help optimise broodstock management and improve reproductive outcomes, which is crucial for the sustainability and productivity of fish farming.

In animals the liver is a unique organ that provides several components for the development of oocyte, which includes vitellogenin, choriogenin and lipids (Dreier et al. 2020). In females during the process of vitellogenesis (formation of the yolk through the liver), granulosa cells within the ovary produce estrogen, primarily 17β -estradiol, that interact with estrogen-receptors in the liver to induce the synthesis of vitellogenin (Wallace and Selman, 1981). Subsequent to the synthesis and release via receptor-mediated endocytosis vitellogenin is transported through the blood and incorporated into growing oocytes (Wallace and Selman, 1981). In the liver several lipids modify the vitellogenin as a lipoprotein (Dreier et al. 2020). The modification of vitellogenin has been characterised in several species of fish.

For fish that spawn demersal eggs, which are laid on or near the substrate and typically lack prominent oil droplets, vitellogenins—a class of yolk precursor proteins—play a critical role in delivering lipids to developing oocytes. These lipids are essential for embryonic development, and approximately 80% of the lipids in demersal eggs are phospholipids (Reading et al. 2018). Phospholipids, as structural components of cell membranes, are crucial for cellular function and early developmental processes, emphasizing their importance in species that spawn demersal eggs.

In contrast, fish that produce pelagic eggs—those that float in open water—exhibit a different lipid profile. For these eggs, phospholipids make up about 70% of the total egg lipids (Reading et al. 2018). The lipid composition in pelagic eggs is tailored not only to support embryonic development but also to meet the specific ecological requirements of floating in the water column. Pelagic eggs typically contain small oil droplets that contribute to buoyancy, a vital adaptation that allows the eggs to remain suspended in the water column, away from predators and sedimentation (Matsubara et al. 1999).

A particularly illustrative example is found in the golden perch (*Macquaria ambigua*), where lipid composition in the eggs has been interpreted as an adaptation for buoyancy rather than solely as an energy reserve (Anderson et al. 1990). This adaptation ensures that the eggs maintain their position in the water column, optimising oxygen exchange and dispersal, which are critical for survival. Unlike demersal eggs, where lipids are primarily reserved for energy to fuel embryonic and larval development, pelagic eggs strike a balance between energy storage and structural adaptations for buoyancy (Matsubara et al. 1999).

The contrasting lipid profiles of demersal and pelagic eggs reflect distinct evolutionary adaptations that fish species have developed to enhance reproductive success in their respective habitats. Demersal eggs, which are typically laid on substrates, derive stability and protection from their environment, reducing exposure to predation and environmental fluctuations. This stability allows for a lipid composition optimised for energy reserves and cellular development rather than buoyancy. In contrast, pelagic eggs are adapted to a free-floating existence in the open water, where buoyancy plays a critical role in minimising predation risks and ensuring dispersal (Suthers and Frank 1990).

These differences underscore the functional plasticity of lipids in fish reproduction, illustrating how species tailor their reproductive strategies to their ecological niches. This plasticity has significant implications for aquaculture and conservation, especially when managing species with differing reproductive modes. For instance, species with demersal eggs may require aquaculture systems that emphasise stable substrates and lipid profiles conducive to embryonic development. Conversely, pelagic spawners may benefit from conditions that enhance lipid synthesis for buoyancy and metabolic energy, reflecting their natural reproductive strategy.

Understanding lipid allocation in egg types is particularly crucial for species transitioning to captive breeding environments, where natural reproductive conditions are often altered. Species-specific knowledge of lipid dynamics allows for the development of tailored nutritional and environmental interventions aimed at improving egg quality, hatching success, and larval viability. For example, optimising dietary lipids to mimic natural profiles

can mitigate potential deficiencies in captivity, ensuring that the physiological needs of developing embryos are met. Additionally, insights into lipid functionality can guide broodstock management practices, enabling aquaculture systems to replicate the environmental cues necessary for successful reproduction.

By addressing these reproductive intricacies, aquaculture can not only enhance the productivity and sustainability of captive breeding programs but also contribute to the conservation of wild populations. For species of ecological or economic importance, such as those transitioning from vulnerable wild stocks to aquaculture systems, refining lipid management strategies is a critical step toward maintaining genetic diversity, reproductive success, and long-term viability.

Transgenerational Plasticity

The productivity and efficiency of aquaculture systems are deeply rooted in the quality and genetic robustness of broodstock, the breeding individuals whose traits directly influence the success of future generations (Mylonas et al. 2011; Duncan et al. 2013). In New Zealand, aquaculture has emerged as both an economic and ecological priority, with the sector contributing significantly to the nation's seafood exports and food security. As the demand for sustainable seafood grows, enhancing broodstock management practices is critical to ensuring the long-term viability of aquaculture. Historically, broodstock selection has focused on heritable traits such as rapid growth rates, high feed conversion efficiency, and resistance to diseases, with the aim of improving yield and profitability (Izquierdo et al. 2001; Valdebenito et al. 2015). These traits have been the cornerstone of conventional selection programs, which primarily focus on optimising productivity and reducing the costs associated with farming. However, recent research suggests that these heritable traits alone are insufficient to guarantee long-term success in aquaculture, especially when environmental variability is considered (Hansen et al. 2022).

While genetic traits are essential, the physiological and biochemical properties of gametes, specifically the quality of eggs and sperm, are now recognised as equally important in determining reproductive success and the viability of offspring (Migaud et al. 2013; Hansen et al. 2022). The maternal environment, including diet, stress levels, and environmental conditions, can have profound effects on the composition and quality of gametes. This is particularly significant in the context of aquaculture, where controlled environments, such as hatcheries and breeding facilities, can offer the opportunity to monitor and manipulate these environmental factors to optimise broodstock performance. The lipid composition of eggs, for instance, has garnered significant attention in recent years due to its crucial role in

supporting embryonic development, metabolism, and survival (Reading et al. 2018). Lipids serve not only as an energy source but also as essential structural components for cell membranes and other cellular processes during early development stages (Malzahn et al. 2022). The lipid composition and abundance within eggs are influenced by maternal provisioning, which can be shaped by factors such as diet, environmental conditions, and stress. Therefore, lipidomic profiles in ova can serve as a valuable tool to assess both gamete quality and the maternal environment. These profiles provide an insightful glimpse into the metabolic and physiological state of the female broodstock and are increasingly being utilised to predict reproductive success in aquaculture, including fertilisation rates, embryonic survival, and larval performance (Hansen et al. 2022). Understanding how lipid profiles impact these outcomes is essential for the development of strategies aimed at improving reproductive efficiency and ensuring the long-term sustainability of aquaculture operations.

Despite the growing body of research on lipidomics, the specific pathways through which maternal experiences shape the lipid composition of eggs and the subsequent impact on offspring fitness remain underexplored. The emerging field of transgenerational plasticity (TP) offers a promising framework for understanding how maternal environments influence offspring traits, not through genetic changes but via non-genetic mechanisms that allow offspring to better cope with the environmental conditions their parents experienced (Gavery and Roberts, 2017; Heckwolf et al. 2018). TP is an adaptive mechanism whereby parental environmental experiences are transferred to offspring, influencing their phenotype in ways that enhance survival and reproductive success under similar environmental conditions. These traits can be passed down through epigenetic modifications, changes in maternal provisioning of nutrients and lipids, and hormonal signalling during reproduction (Mousseau and Fox, 1998; Spitzer, 2004). This transgenerational transmission of adaptive traits allows for a rapid response to environmental stressors, without requiring genetic evolution. In the context of aquaculture, this phenomenon is particularly important as environmental conditions in farming systems can vary significantly due to external factors such as climate change, feed availability, and water quality (Gavery and Roberts, 2017).

The application of TP mechanisms in aquaculture systems offers considerable potential for improving the resilience of farmed species. For example, environmental changes such as temperature fluctuations, variations in diet, or exposure to stress can influence maternal provisioning of nutrients, including lipids, to eggs. The subsequent offspring may inherit these maternal adjustments, enabling them to better adapt to similar environmental conditions in their early life stages (Hansen et al. 2022). However, the role of TP in aquaculture remains under-studied, particularly in relation to lipidomics and its impact on

offspring fitness. By investigating the lipidomic profiles of ova and understanding how they reflect maternal environmental conditions, researchers can gain valuable insights into the underlying mechanisms of TP in fish species, improving our ability to manage broodstock in dynamic aquaculture systems.

In addition to its theoretical importance, understanding the role of TP in aquaculture has practical implications for broodstock management and the optimization of reproductive success. By identifying biomarkers such as specific lipid profiles in eggs, aquaculture practitioners can refine their breeding and management strategies to ensure that broodstock are providing the best possible maternal investment to their offspring. This can improve the quality of larvae, increase survival rates, and ultimately lead to more efficient and sustainable aquaculture practices. Furthermore, integrating the study of TP into aquaculture management could enable the development of more resilient fish stocks that are better equipped to cope with the challenges posed by changing environmental conditions, ensuring the long-term sustainability of aquaculture industries worldwide.

Thus, the study of lipidomics, particularly in relation to transgenerational plasticity, represents an exciting and rapidly evolving field of research in aquaculture. As the industry continues to face challenges related to climate change, environmental stress, and the need for sustainable production methods, understanding the role of maternal provisioning and the lipid composition of gametes will be crucial in advancing aquaculture practices and ensuring the health and resilience of farmed species.

Giant kōkopu (*Galaxias argenteus*)

Giant kōkopu (*Galaxias argenteus*), one of New Zealand's five native amphidromous galaxiid species, exemplifies both the challenges and opportunities facing modern aquaculture and conservation efforts. As a keystone species within New Zealand's whitebait fisheries, *G. argenteus* plays an important role in customary, commercial, and recreational sectors, with whitebaiting being a practice deeply embedded in New Zealand's cultural heritage (McDowall, 1988; McDowall and Kelly, 1999). Whitebait, the post-larvae of galaxiid fish such as *G. argenteus*, are harvested during their upstream migration from saltwater spawning grounds to freshwater habitats, a migration that is both economically valuable and culturally significant (McDowall and Eldon, 1980). However, this traditional practice faces increasing sustainability concerns. Overfishing, habitat loss, and land-use changes have contributed to the decline of wild *G. argenteus* populations, which are now classified as "vulnerable" by the International Union for Conservation of Nature (IUCN) Red List (Yungnickel et al. 2020).

These ecological pressures emphasise the need for alternative, sustainable solutions to preserve *G. argenteus* and meet the demand for whitebait.

Domestication of *G. argenteus* through aquaculture offers a promising approach to address the challenges facing wild populations. By providing a controlled environment for breeding, aquaculture can reduce reliance on wild stocks while conserving the species and ensuring a steady supply for the whitebait industry. Furthermore, aquaculture offers significant opportunities to enhance broodstock management, a critical component for improving reproductive success and overall productivity (Duncan et al. 2013). Broodstock, the breeding individuals that determine the success of future generations, are central to the efficiency and sustainability of aquaculture systems (Mylonas et al. 2011; Duncan et al. 2013). Traditionally, broodstock management has focused on heritable traits such as growth rates and disease resistance (Izquierdo et al. 2001; Valdebenito et al. 2015). However, emerging research highlights the importance of gamete quality—specifically the physiological and biochemical properties of eggs and sperm—in determining reproductive success and offspring viability (Migaud et al. 2013; Hansen et al. 2022).

One area of focus in broodstock management is the study of ova lipidomic profiles, which provide insight into maternal investment in reproduction. Lipids are essential components of eggs, serving as energy reserves and structural materials for embryonic development (Reading et al. 2018). The composition and abundance of lipids in eggs are influenced by maternal environmental conditions such as diet, stress, and temperature, making them valuable biomarkers for assessing gamete quality and maternal health (Malzahn et al. 2022). Furthermore, lipid profiles are increasingly being recognised as predictive indicators of reproductive success, linking lipid composition to fertilization rates, embryonic survival, and larval performance (Hansen et al. 2022). Despite their importance, the pathways through which maternal environments influence lipid composition in eggs, particularly in aquaculture systems, remain underexplored, especially when considering transgenerational effects.

Transgenerational plasticity (TP) is a key mechanism through which maternal environments influence offspring traits across generations. TP refers to the transmission of adaptive traits through non-genetic mechanisms, such as changes in maternal provisioning or epigenetic modifications, which allow offspring to better cope with environmental conditions similar to those experienced by their parents (Gavery and Roberts, 2017; Heckwolf et al 2018). In aquaculture systems, understanding the role of TP is increasingly important, especially as environmental conditions can vary widely due to climate change, water quality, and feeding practices. The study of TP in aquaculture could provide insights into how maternal

environmental factors, such as diet and stress, influence offspring development and survival, improving broodstock management practices and increasing aquaculture productivity.

In the context of *G. argenteus* aquaculture, the domestication process is still in its early stages. Early efforts by Manāki Whitebait, which have initiated large-scale breeding programs, demonstrate the potential for developing a sustainable aquaculture industry for this species. However, challenges remain in optimising broodstock quality, improving reproductive outcomes, and understanding the complex dynamics of domestication. To support the success of these efforts, innovative research is essential. Studies focusing on the reproductive biology, lipid profiles, and transgenerational plasticity of *G. argenteus* will contribute to the development of strategies that enhance broodstock management and optimise breeding programs.

Research into lipidomics, particularly the role of fatty acids in eggs, is especially critical. Understanding how maternal provisioning of lipids affects egg quality and how these changes are passed down through generations will help identify strategies to improve reproductive success in aquaculture. Given the growing pressures on wild populations, the successful domestication of *G. argenteus* through aquaculture could not only ensure the sustainability of the species but also serve as a model for other endangered fish species. Ultimately, integrating lipidomic and transgenerational plasticity studies into *G. argenteus* aquaculture will help safeguard New Zealand's whitebait industry, offering a path forward that balances ecological stewardship with economic sustainability.

Chapter 3: Methodology

Broodstock History

The development of a sustainable giant kōkopu (*Galaxias argenteus*) aquaculture program in New Zealand began in 2007 when Manāki Whitebait initiated the housing of approximately 30 wild-caught individuals at their aquaculture facility in Warkworth. This marked the beginning of a long-term effort to adapt the species to captivity, with a focus on optimising environmental conditions for successful acclimation. The initial wild-caught broodstock was carefully monitored to understand their specific needs and determine the best practices for ensuring their health and survival in a controlled environment. This early phase involved experimenting with various aquaculture conditions, including water quality, temperature, diet, and habitat design, all of which were crucial for maintaining the species in captivity.

The breakthrough in this project came in 2012, when a successful spawning event occurred, resulting in the hatching of several thousand giant kōkopu offspring. These first-generation (F1) fish were an important milestone, as they represented the first cohort of giant kōkopu bred in captivity, offering the possibility of sustainable aquaculture production. These F1 broodstock were selectively bred from the original wild-caught individuals, ensuring that they retained genetic traits from their wild ancestors while adapting to the new, controlled environment of the aquaculture facility.

From the F1 broodstock, Manāki Whitebait moved on to the breeding of F2 generations in 2015, 2016, and 2017. These subsequent generations, referred to as F2-2015, F2-2016, and F2-2017 in this study, were bred by selecting random male and female pairs from the F1 stock. This approach was employed to establish distinct cohorts of F2 fish, each born independently in separate years. The nomenclature used in this study serves to emphasise the temporal distinction between the F2 generations, highlighting their unique environmental exposures and potential genetic or phenotypic variations resulting from differences in breeding conditions across the years.

As of the most recent update, Manāki Whitebait's broodstock collection includes nearly 3,000 F1 individuals, with an additional 30,000 F2 fish from the successive breeding events. This growing broodstock population provides a valuable resource for continued research into improving broodstock management, enhancing reproductive success, and advancing the overall development of giant kōkopu aquaculture. The success of these efforts is integral to the broader goal of creating a sustainable and viable aquaculture industry for this species, which has significant cultural and economic value in New Zealand's whitebait fisheries.

Broodstock Management

At Manāki Whitebait, all giant kōkopu were housed in recirculating aquaculture systems (RAS) to maintain stable environmental conditions, with the aim of simulating the natural seasonal temperature variations that the species would experience in the wild. These systems allowed precise control over water temperature, a critical factor in regulating the fish's metabolic processes and breeding cycles. Fish were exposed to conditions corresponding to specific seasons, with each seasonal condition maintained for approximately three months before the broodstock were prepared for stripping, typically during the 'winter' months when water temperatures were kept within the range of 10–12°C.

To mimic the natural photoperiods of each season, the photoperiod in the aquaculture systems was adjusted to reflect the typical daylight durations of the respective seasons, further enhancing the accuracy of the simulated environment. This seasonal mimicry was essential for promoting proper reproductive timing and gamete quality in the giant kōkopu, ensuring that the broodstock's physiological and hormonal processes were aligned with natural cycles.

Feeding was conducted manually twice a week using a commercial diet of pellets (Otohime) and mealworms (*Tenebrio molitor*), with the feeding regimen carefully calculated to meet the nutritional needs of the broodstock. The amount of pellets fed to the fish was determined by a specific equation:

$$\text{Eq. 1: } M_P = n \times (M_F)^{-1} \times 0.008$$

Where M_P is the weight of the pellets, n is the number of fish in the tank, and M_F is the mean weight of all the fish in the tank, multiplied by 0.008. The quantity of mealworms provided was set at half the weight of the pellets, ensuring a balanced diet to support the broodstock's health and reproductive success.

The fatty acid (FA) content of both the pellets and the mealworms was analysed to better understand the nutritional contributions of the diet to the broodstock. This information is presented in Table 1, which highlights the lipid composition of both food sources, providing valuable insights into the role of maternal provisioning in the reproductive outcomes of giant kōkopu in aquaculture. The analysis of these diets also plays a key role in understanding how dietary inputs influence gamete quality and subsequent offspring development.

Ova Collection and Fatty Acid Analysis

For each generation of *Galaxias argenteus* (giant kōkopu), eggs were stripped from multiple random females by trained staff at Manāki Whitebait. The stripping dates for the different generations were as follows: F2-2017 on 25th June 2020 (n = 6), F2-2016 on 2nd July 2020 (n = 5), F2-2015 on 31st July 2020 (n = 6), F1 on 11th November 2020 (n = 10), and wild-caught on 4th December 2021 (n = 4). The age of each generation at the time of sampling varied: F1 was 8 years old, F2-2015 was 5 years old, F2-2016 was 4 years old, F2-2017 was 3 years old, and the wild-caught samples, having been collected in 2007, were at least 14 years old.

After the eggs were stripped, they were packaged in plastic containers, placed on ice, and transported to the Auckland University of Technology's (AUT) School of Science laboratory. Once at the laboratory, the eggs underwent sorting and cleaning. Deionised water was used to remove any excrement and gelatinous coatings from the eggs. Following this, the eggs were dried and carefully transferred to Eppendorf tubes for preservation at -80°C for future analysis.

The fatty acid content of the eggs was analysed using Gas Chromatography-Mass Spectrometry (GC-MS). The extraction and derivatization of the egg samples followed the protocol described by Zhou et al. (2014). First, the samples were snap-frozen with liquid nitrogen, freeze-dried overnight, and homogenized using a ceramic mortar and pestle. A portion of the homogenised sample (20 mg) was then weighed into screw-cap glass culture tubes, and a surrogate solution containing tridecanoic acid in toluene, along with an extraction solution and acetyl chloride, was added to the samples. The samples were vortexed, incubated at 100°C for one hour, cooled, and then treated with potassium carbonate. After vortexing and centrifuging, the top organic phase was extracted, and the samples were transferred to amber vials. Dilutions were made, and the samples were prepared for GC-MS analysis.

The GC-MS analysis was conducted using an Agilent 7890A gas chromatograph coupled with a 5975C mass spectrometer. The samples were injected into a split/split less inlet set at 250°C , and the temperature programming of the GC was set to start at 45°C , increasing to 215°C , followed by a final ramp to 250°C . The column used was a fused silica Rtx-2330 with a highly polar stationary phase to separate the fatty acids. Detection occurred at 14.5 minutes, and the fatty acid concentrations were quantified by comparing the results to calibration curves from a 37-mix standard. This analytical process allowed for the precise identification and quantification of the fatty acids present in the eggs, providing important

information about the nutritional composition and quality of the eggs, which is crucial for reproductive success in aquaculture.

Statistical Analysis

For the comparative analysis of fatty acid (FA) profiles across the different broodstock generations, we treated the broodstock generation as a fixed effect. This was appropriate as the broodstock groups—Wild-caught, F1, F2-2015, F2-2016, and F2-2017—represented distinct, predefined categories. This categorization allowed us to assess the variations in FA profiles attributable to generational differences. During the analysis, one outlier was visually identified in the F2-2016 group (specifically F2-2016, 3) and was subsequently removed to prevent it from skewing the results.

The majority of the FA data met the assumptions for normal distribution as tested by the Shapiro-Wilk test, and homogeneity of variances was confirmed using the Fligner-Killeen test. These assumptions are important to ensure the validity of parametric tests such as Analysis of Variance (ANOVA). To explore intergenerational differences in FA profiles, a one-way ANOVA was conducted, followed by Tukey's Honest Significant Difference (HSD) post-hoc tests to identify which generations significantly differed from one another in terms of their FA composition.

Additionally, Principal Component Analysis (PCA) was used to examine the overall variation in FA profiles across all generations. PCA is a statistical technique that reduces the dimensionality of complex datasets, allowing for the identification of key patterns and relationships. By performing PCA on all individual FAs, their sums, and FA ratios, we were able to detect significant variations across generations. FAs that had loadings above a threshold of $|0.5|$ on the first two principal components were considered key biomarkers. These biomarkers were selected for further analysis because they were deemed to contribute most significantly to the variance observed between generations.

A refined PCA was then performed focusing solely on these identified biomarkers to gain a more detailed understanding of the generational differences in FA profiles. This allowed for a clearer interpretation of how specific fatty acids vary between broodstock generations. All statistical analyses were conducted using SAS JMP (16.0.0), and a p-value of <0.05 was considered statistically significant. This rigorous statistical approach ensured that the observed differences in FA profiles were not due to random chance but reflected true intergenerational variations in fatty acid composition.

Chapter 4: Results

The findings of this chapter have been published in: Sabetian.A., Hoang. L. H., Zhang. J., and Lilkendey. (2024). Tracing transgenerational plasticity through ova fatty acid biomarkers in Giant Kōkopu (Galaxias argenteus). New Zealand Journal of Marine and Freshwater Research. doi:10.1080/00288330.2024.2324815

PAL, OLA,)and DHA were found to contribute significantly to the fatty acid (FA) profile of ova, accounting for the majority (mean 72%) of total FA across all five broodstock generations. The results of the Analysis of Variance (ANOVA) revealed significant differences in the FA profiles across generations, as indicated by asterisks (*) in Table 2. These differences were observed in a variety of FAs, including Σ SAFA (Sum Saturated Fatty Acids), C6:0 (caproic acid), C14:0 (myristic acid), PAL (palmitic acid), C18:0 (stearic acid), C23:0 (tricosanoic acid), C16:1 (n-7), OLA (oleic acid), Σ PUFA (Sum Polyunsaturated Fatty Acids), C18:2 (n-6) (linoleic acid), ARA (arachidonic acid), and ratios such as $\Sigma(n-3)/\Sigma(n-6)$ and DHA/EPA.

The increase in Σ SAFA concentration was primarily driven by PAL, which was significantly higher in the F2-2017 generation compared to the Wild-caught generation. Although the decline in Σ MUFA (Monounsaturated Fatty Acids) concentration was marginally nonsignificant ($P=0.0542$), there was a significant decrease in OLA content in F2-2017 compared to the Wild-caught, F1, and F2-2015 generations. This suggests that changes in OLA content could be important in understanding generational differences in FA composition.

The analysis also revealed a significant decline in Σ PUFA content, primarily due to lower concentrations of ARA in F2-2017 compared with the Wild-caught generation, as well as lower concentrations of C18:2 (n-6) in F2-2017 compared to all other generations. These findings highlight how specific polyunsaturated fatty acids are impacted by broodstock generation. Additionally, the ratio of $\Sigma(n-3)/\Sigma(n-6)$ and DHA/EPA was significantly higher in the F2-2017 generation compared with the Wild-caught generation, indicating a shift in the balance of these essential fatty acids across generations.

Principal Component Analysis (PCA) was performed to further explore the variations in FA profiles across generations. The first two principal components (PCs) significantly captured the variance in FA profiles between broodstock generations. The first principal component (PC1) was heavily influenced by Sum SAFA (loading: -0.33847) and OLA (loading: 0.32655), both of which played pivotal roles in driving the overall variation in FA profiles. This suggests

that the relative abundance of saturated and monounsaturated fatty acids was a major factor contributing to intergenerational differences.

The second principal component (PC2) was largely influenced by the ratios OLA/ Σ (n-3) (loading: 0.43841) and OLA/DHA (loading: 0.44715), which emphasise the balance of oleic acid relative to omega-3 fatty acids and DHA. These ratios were key in distinguishing the generational differences in FA profiles, particularly highlighting the shifts in the levels of unsaturated fatty acids across the broodstock generations.

In terms of specific generational profiles, eggs from the F2-2017 generation were characterised by elevated levels of C14:0, C16:1, Σ SAFA, and the Σ (n-3)/ Σ (n-6) ratio, indicating a shift towards higher levels of saturated and omega-3 fatty acids. In contrast, eggs from the Wild-caught generation were marked by higher levels of DHA/EPA and C18:2 (n-6), along with a higher overall concentration of Σ PUFA, suggesting a more balanced profile of polyunsaturated fatty acids, including omega-6 fatty acids. Eggs from the F1, F2-2015, and F2-2016 generations exhibited intermediate FA profiles, with significant influences from the OLA/ Σ (n-3) and OLA/DHA ratios. These results provide insight into the evolving FA composition across broodstock generations, offering a clearer picture of how domestication and selective breeding may impact fatty acid profiles in *G. argenteus* over time.

These findings, illustrated in Figure 1, underscore the complex interplay between different fatty acids and their ratios across generations, and highlight the importance of monitoring and understanding these variations in the context of aquaculture and broodstock management.

Chapter 5: Discussion

Successful aquaculture relies heavily on high-quality broodstock, reproductive success, and fish health in captivity. While certain fatty acids (FAs) have been identified for their roles in these areas, they have rarely been employed as biomarkers to study broodstock trophic provisioning (TP). This study represents the first comprehensive assessment of TP across a multi-generational "wild-to-captivity" lineage of giant kōkopu (*Galaxias argenteus*).

Through lipidomic analysis, we discovered significant generational shifts in the FA composition of ova, revealing an adaptive response to captivity. With each successive generation, captive-bred fish deposit fewer FAs associated with stress, such as oleic acid (C18:1 n-9), which has been linked to chronic environmental challenges and reduced reproductive success. Simultaneously, these fish increase the deposition of FAs known to enhance immune responses and promote larval development, such as docosahexaenoic acid (C22:6 n-3, DHA) and eicosapentaenoic acid (C20:5 n-3, EPA). These patterns indicate that broodstock can acclimate to captive conditions, optimising maternal provisioning to align with stable and controlled environments.

The implications of these findings extend beyond theoretical insights into TP, offering practical avenues to enhance aquaculture productivity. By understanding how FA profiles shift in response to captivity, aquaculture operations can design targeted interventions, such as optimised dietary formulations and stress-mitigation strategies, to accelerate this adaptive process. For instance, feed enriched with essential FAs like DHA and EPA could amplify the benefits of maternal provisioning, boosting larval survival rates and overall stock resilience. Similarly, environmental enrichment in hatcheries, aimed at reducing stress-induced FA production, may further improve broodstock condition and reproductive outcomes.

Furthermore, this study underscores the utility of lipidomics as a diagnostic tool for aquaculture. Monitoring FA profiles across generations provides a non-invasive method to evaluate broodstock health, reproductive quality, and adaptation to captivity. This approach could enable real-time adjustments in rearing practices, ensuring that broodstock are maintained in optimal physiological states to maximise productivity.

The broader significance of this research lies in its contribution to sustainable aquaculture. The demonstrated ability of giant kōkopu to adapt trophic provisioning strategies in captivity highlights the potential for similar mechanisms in other aquaculture species. By leveraging these adaptive processes, the industry can reduce reliance on wild stock, mitigate the impacts of environmental variability, and improve the resilience of farmed fish populations.

Ultimately, these insights pave the way for innovative, science-driven strategies that align productivity goals with environmental sustainability, contributing to the long-term viability of aquaculture.

By profiling the FAs across five generations of broodstock, we found that PAL (palmitic acid), OLA (oleic acid), and DHA (docosahexaenoic acid) were the most dominant FAs. We also identified significant dynamic trends, particularly in the concentrations of PAL, OLA, and ARA (arachidonic acid), as well as changes in the $\Sigma(n-3)/\Sigma(n-6)$ and DHA/EPA ratios from the wild-caught to the F2-2017 generations. These trends help deepen our understanding of TP in the context of broodstock's ability to provision specific FAs into their eggs. Specifically, the decline in OLA concentration across generations is consistent with findings from previous research by Izquierdo (1996) and Montero et al. (2001), who linked high OLA content to stress in aquacultured fish. Similarly, the decreasing trend in ARA and the increasing trend in PAL from wild to F2-2017 generations reflect patterns seen in past studies. High ARA levels have been associated with lower fertilization success and reduced egg and larval quality (Furuita et al. 2003, 2006; Nguyen et al. 2010), while elevated PAL levels have been linked to enhanced immunostimulant and antiviral properties, crucial for improving disease resistance and survival in captive fish (Nakamura et al. 1996; Librán-Pérez et al. 2019).

This study builds on the foundational work of Hoang et al. (2020), which identified a decreasing trend in oleic acid (OLA) levels between the F1 and F2-2015 generations of captive-bred giant kōkopu (*Galaxias argenteus*). While that earlier research provided valuable initial insights into how maternal provisioning may shift across generations, it was limited in scope to a binary comparison between two successive generations. By expanding the analysis to encompass five generations, our study offers a far more comprehensive view of transgenerational plasticity (TP), revealing dynamic trends in fatty acid (FA) profiles that extend beyond the earlier observations.

This broader temporal scope allows for the identification of patterns that may otherwise remain obscured in shorter studies. For instance, we observed not only a continued decline in OLA—a marker often linked to stress and suboptimal reproductive conditions—but also concurrent increases in fatty acids like palmitic acid (C16:0, PAL) and docosahexaenoic acid (C22:6, DHA), which are associated with enhanced immune function and larval development. These findings underscore the capacity of broodstock to adapt their maternal provisioning strategies over successive generations in captivity, balancing the trade-offs between stress management and the allocation of resources that promote offspring resilience.

The extended analysis also provides new insights into the role of FA profiles as biomarkers of maternal health and adaptability. By tracking these changes across multiple generations, our study highlights how lipidomics can be harnessed not only to evaluate the current state of broodstock but also to predict their long-term performance under captive conditions. This approach is particularly valuable for newly aquacultured species, where data on broodstock physiology and reproductive success may be limited. The ability to use FA profiles as indicators of maternal adaptability can facilitate more strategic broodstock selection, ensuring that individuals with the most favourable provisioning patterns are prioritised in breeding programmes.

Moreover, this research lays the groundwork for future investigations into the mechanisms driving these generational changes. For example, are shifts in FA profiles primarily influenced by environmental factors such as diet and stress, or do they reflect inherent epigenetic modifications passed down through generations? Answering these questions will not only deepen our understanding of TP but also inform the development of targeted interventions to optimise maternal provisioning in aquaculture.

In practical terms, the insights gained from this extended generational analysis can help refine management practices for aquaculture species. Monitoring FA trends over time can serve as a diagnostic tool, enabling more informed and adaptive strategies for broodstock management. By integrating this lipidomic approach into routine aquaculture operations, producers can enhance the efficiency and sustainability of their systems, ultimately contributing to improved yields, healthier stock, and a reduced reliance on wild populations. This research thus represents a significant step forward in the quest to balance aquaculture productivity with ecological stewardship.

Despite its valuable contributions, this study is not without biases and limitations, one of which is the unavoidable influence of broodstock age across successive generations. As each generation progresses, the age of broodstock changes, introducing a confounding variable into the interpretation of fatty acid (FA) profiles and maternal provisioning. In this study, the F2-2017 generation, the fourth bred in captivity, exhibited the greatest adaptations in reproductive success, disease resistance, and stress tolerance. While these improvements may reflect transgenerational plasticity (TP) and enhanced adaptation to captive environments, it is crucial to acknowledge that age-related differences could also be influencing these outcomes.

The bias introduced by age differences is further underscored by the BOFFF hypothesis (Big Old Fat Fecund Female Fish), which posits that older and larger females provide greater lipid reserves to their eggs, resulting in more robust larvae with better chances of survival

during periods of resource scarcity (Hixon et al., 2014). This concept has been widely discussed in fisheries science as a potential factor in maternal provisioning, with older females often regarded as keystones for sustaining populations. However, the evidence for a direct link between maternal age and improved offspring quality remains inconclusive. Studies such as those by Marshall et al. (2010) and Marasco et al. (2019) have failed to establish a clear correlation between maternal age and lipid content in eggs, challenging the universality of the BOFFF hypothesis.

In the context of our study, this ambiguity raises critical questions about how age might interact with other factors, such as genetic adaptation, environmental conditions, and broodstock management practices, to shape FA profiles and reproductive outcomes. For example, while younger generations may show enhanced adaptation to captive conditions, these changes could be partially attributed to age-related physiological differences rather than solely to transgenerational effects. This highlights the need for a more nuanced understanding of how age influences maternal provisioning, particularly in aquaculture systems where age distribution and generational turnover are tightly controlled.

Addressing these limitations will require future studies to adopt experimental designs that can isolate the effects of age from those of generational adaptation. Longitudinal studies that track FA profiles and reproductive success within the same cohort over time could provide valuable insights into the relative contributions of age and TP. Additionally, comparative studies across species with varying lifespans and reproductive strategies could help clarify the broader applicability of the BOFFF hypothesis and its implications for aquaculture.

Despite these challenges, the findings of this study remain significant, demonstrating that younger generations of broodstock bred in captivity can achieve improved reproductive success and stress tolerance. By recognising and accounting for the potential biases introduced by age, we can refine our interpretations of FA data and further optimise broodstock management strategies. This, in turn, will enhance our ability to balance productivity and sustainability in aquaculture, ensuring the continued viability of this vital industry.

Furthermore, a notable limitation of this study is that our analysis concludes with the F2-2017 generation, leaving unanswered questions about whether successive generations bred in captivity would continue to exhibit improvements in fatty acid (FA) profiles. While our findings align with those of Hoang et al. (2021), which suggest adaptive changes in FA provisioning over time, the absence of data beyond the fourth captive generation restricts our understanding of the long-term trajectories of these adaptations.

The potential for further gains in broodstock health, reproductive success, and stress resilience in later generations remains speculative without additional research. Long-term studies tracking FA biomarkers across more generations could shed light on whether the observed trends plateau, regress, or continue to improve with extended exposure to controlled environments. This would provide valuable insights into the limits of transgenerational plasticity (TP) and the capacity of aquacultured species to adapt to captivity over multiple generations. Additionally, extended research could reveal whether specific FA profiles eventually stabilise as broodstock achieve an equilibrium state optimised for captive conditions.

Targeted nutritional interventions present another compelling avenue for exploration. By systematically manipulating broodstock diets over several generations, researchers could assess how tailored lipid provisioning influences FA profiles and reproductive outcomes. For instance, it is possible that fine-tuning the balance of essential fatty acids (EFAs) like DHA and EPA in broodstock diets could accelerate adaptive processes or mitigate potential trade-offs associated with captivity. Continued monitoring of these biomarkers would enable the development of evidence-based feeding regimes, enhancing both maternal provisioning and offspring performance.

Future studies could also explore whether adaptations observed in captive broodstock remain stable or degrade when these fish are reintroduced into wild or semi-natural environments. This would provide critical insights into the reversibility of TP and its implications for conservation aquaculture, where maintaining traits conducive to wild survival is often a priority. Additionally, examining the interaction between genetic and epigenetic mechanisms in shaping FA profiles over extended timelines could deepen our understanding of how these processes contribute to TP and its application in broodstock management.

Ultimately, addressing this limitation by extending the scope of analysis to subsequent generations will offer a more comprehensive understanding of the interplay between TP, FA provisioning, and aquaculture sustainability. The long-term monitoring of FA biomarkers not only holds promise for optimising broodstock health and reproductive success but also represents a pivotal step toward achieving the dual goals of enhanced productivity and ecological responsibility in aquaculture systems.

Conclusion

In conclusion, this study provides valuable insights into the multi-generational changes in trophic provisioning (TP) in giant kōkopu broodstock across a wild-to-captivity lineage.

Through profiling fatty acids (FAs) in eggs from five generations of broodstock, we identified significant changes in key FAs, such as PAL, OLA, and DHA, as well as variations in the $\Sigma(n-3)/\Sigma(n-6)$ and DHA/EPA ratios. These findings reveal a dynamic adaptation of broodstock to captive conditions, with a shift in FA profiles that suggests reduced stress and improved reproductive success across successive generations. Specifically, the decrease in OLA and ARA, along with the increase in PAL, points to a positive trend in broodstock health, immune response, and disease resistance, aligning with previous research on the role of FAs in aquaculture stress and reproductive performance.

By extending the scope of previous studies to encompass five generations, this research offers a more comprehensive understanding of TP and demonstrates the potential of FAs as biomarkers for maternal health and adaptability in aquaculture. The findings highlight the importance of FA profiling in broodstock management, providing actionable data that can inform selection strategies and improve aquaculture productivity.

While the study makes significant contributions to understanding TP in captive breeding programs, it also has inherent limitations, particularly related to the age differences between generations and the inability to track subsequent generations beyond F2-2017. The influence of age on maternal resource provisioning remains an area for further exploration, and future research should focus on long-term monitoring of FA profiles across additional generations to explore the potential for continued adaptation and the effects of nutritional interventions in captivity.

Overall, this study lays the groundwork for future research into the role of FAs in broodstock management and aquaculture practices, with potential applications in enhancing the productivity, health, and sustainability of aquaculture systems. By integrating FA profiling into breeding programs, aquaculturists can make more informed decisions that support the long-term success of cultured fish populations.

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