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RESEARCH ARTICLE



Tracing transgenerational plasticity through ova fatty acid biomarkers in Giant Kōkopu (*Galaxias argenteus*)

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ABSTRACT

Broodstock management has traditionally focused on heritable traits and diet-induced gamete quality. However, the potential of transgenerational plasticity (TP) underscores the importance of understanding adaptive trait transfer in aquaculture, where the sustainability of production can be hampered by mortality arising from disease and stress. Notably, the role of fatty acids (FAs) as important biomarkers is an untapped area of TP research with implications for the management of new aquaculture species. We assessed TP in Giant Kōkopu (*Galaxias argenteus*) by comparing the ova FA profiles of broodstock across five captive generations; wild-caught, F1, and three successive F2s. Our study revealed intergenerational shifts in ova FA profiles. We encountered a significant decline in C18:1 (n-9) (Oleic Acid, OLA) and C20:4 (n-6) (Arachidonic acid, ARA), and an increase in C16:0 (Palmitic acid, PAL) from wild-caught to captive-bred broodstock. These shifts reveal that latter generations of captive-bred *G. argenteus* are reducing the provision of FAs associated with stress (OLA) and lower reproductive success (ARA), while increasing those linked to improved immune response (PAL), signalling a significant adaptation to captivity. Our findings help elucidate the theoretical understanding of TP through the use of FAs as biomarkers, providing practical insight for broodstock management.

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Introduction

The productivity and efficiency of successful aquaculture are underpinned by the quality and genetic robustness of broodstock—the individuals used for breeding (Mylonas et al. 2011; Duncan et al. 2013). Although conventional broodstock selection has historically prioritised heritable traits such as rapid growth and resistance to disease, more studies are now shifting their focus towards the critical role of gamete quality as a pivotal performance indicator (Izquierdo et al. 2001; Migaud et al. 2013; Valdebenito et al. 2015). Specifically, ova lipidomic profiles are being used as quantitative proxies of egg quality

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and predictive reproductive success in aquaculture (Hansen et al. 2022; Malzahn et al. 2022). More recently, the concept of transgenerational plasticity (TP) has also gained prominence in innovative broodstock management strategies (Roy et al. 2023). TP allows for the direct transfer of adaptive traits from parent to offspring based on the environmental experiences of the parent (Heckwolf et al. 2018). Mechanistically, TP can manifest through pathways like epigenetic modifications and hormonal signalling (Mousseau and Fox 1998; Spitzer 2004; Gavery and Roberts 2017). The link between TP and maternal provisioning of endogenous caloric reserves in broodstock eggs remains relatively unexplored (Hoang et al. 2021). This research gap is noteworthy given the roles lipids play in gamete quality (Valdebenito et al. 2015; Reading et al. 2018), making them excellent biomarkers for tracking TP through generations of broodstock.

Lipids, which are hydrocarbon-chained organic biomolecules synthesised mainly in the liver, serve both as vital energy reserves in egg yolk and as substrates for biochemical vectors like vitellogenin and lipoproteins (Wiegand 1996; Johnson 2009; Hiramatsu et al. 2015; Bruslé and i Anadon 2017). For example, lipids such as essential fatty acids (EFAs) C22:6 (n-3) (Docosahexaenoic acid, DHA) and C20:5 (n-3) (Eicosapentaenoic acid, EPA) are known to provide energy, structural integrity to cell membranes, serve as precursors to key signalling molecules, and aid in vital larval physiological processes (Bell et al. 2003; Tocher 2003, 2010; Salze et al. 2005; Yanes-Roca et al. 2009; Grote et al. 2011; Hauville et al. 2016). Uniquely, fishes can synthesise EFAs *de novo* (Tocher 2010) adding nuance to the untapped potential of tracking TP through EFA provisioning.

The roles of lipids have also been explored as identifiers of environmental stress (Yan et al. 2012) and mediators of immune response (Arts and Kohler 2009). For example, research indicates that C18:1 (n-9) (Oleic acid, OLA) can be used as a proxy to identify acute and chronic stress in aquacultured fish (Izquierdo 1996; Montero et al. 2001), while C16:0 (Palmitic acid, PAL) has been identified as having immunostimulant and antiviral properties (Nakamura et al. 1996; Librán-Pérez et al. 2019). Both OLA and PAL are non-EFAs. By extending the research focus beyond EFAs to include non-EFAs, we can develop strategies to enhance stress resilience and disease resistance in aquaculture, ultimately driving both productivity and sustainability.

Introducing wild species into aquaculture presents unique opportunities for sustainable food production and conservation (Anderson et al. 2017; Overton et al. 2024). However, the challenge for newly aquacultured species sourced from the wild is that they may require several generations to adapt to captivity. Giant Kōkopu (*Galaxias argenteus*) is one of five amphidromous galaxiid fish species supporting the New Zealand customary, commercial and recreational 'whitebait' fishery (McDowall 1988; McDowall and Kelly 1999). This is a wild-caught fishery where the post-larvae of primarily galaxiid, and to much lesser extent non-galaxiids (e.g. Anguillidae), are caught as they begin their upstream freshwater migration, having hatched in saltwater earlier (McDowall and Eldon 1980). *G. argenteus* has been listed by the IUCN-Redlist as 'vulnerable', and a large-scale spatio-temporal study of whitebait species composition around New Zealand has highlighted the importance of land-use changes and habitat loss to their historic decline (Yungnickel et al. 2020). Therefore, all indications are that this heavily wild-focused fishery is unsustainable, which underscores the importance of successful domestication to management and conservation of wild stocks. Aquaculture settings are ideal

controlled and stable environments in which the effects of broodstock management can be studied and improved (Duncan et al. 2013). However, the whitebait aquaculture industry is at infancy in New Zealand, with Manāki Whitebait the only company attempting to bring large-scale captive-bred whitebait to the market. Hence, innovative research to expedite the domestication process is needed, offering a potential lifeline for both a struggling fishery and the vulnerable species it relies upon.

In this study, we scrutinise the FA profiles of ova from five different captive broodstock generations of giant kōkopu; namely, wild-caught, F1, and F2 cohorts from 2015, 2016, and 2017. This unique opportunity allows us to probe the TP of captive giant kōkopu by examining maternal provisioning of FAs across multiple generations from the same genetic lineage, on the same diet, living in identical controlled conditions. Specifically, we identified PAL, OLA, and C20:4 (n-6) (Arachidonic acid, ARA) as three highly valuable biomarkers for broodstock quality, potentially identifying valuable proxies for studying TP in this species. Our study's aim was to track changes in the relative concentrations of FAs across generations raised in a stable aquaculture environment, thereby offering insight into the mechanisms of TP. The findings have significant implications not only for our theoretical understanding of FA allocation across generations, but also for optimising gamete quality for sustainable aquaculture practices.

Material and methods

Broodstock history

In 2007, Manāki Whitebait began to house approximately 30 wild-caught giant Kōkopu at their Warkworth aquaculture facility, experimenting with the most effective captive conditions to enhance acclimation. From that initial wild-caught batch, a few thousand individuals were successfully hatched in 2012, forming an F1 generation. From that F1 broodstock, random male and female pairs were used in 2015, 2016, and 2017, to breed three successive F2 generations—F2-2015, F2-2016, F2-2017. This nomenclature is employed in this study to emphasise their status as distinct, independently born F2-generations in separate years. In total, Manāki Whitebait now holds nearly 3000 F1 fish, and just over 30,000 F2 fish.

Broodstock management

All giant Kōkopu at Manāki Whitebait were kept in recirculating aquaculture systems (RAS) in order to regulate temperatures, simulating natural seasonal variations. Fish were sustained within each 'seasonal' condition for about three months before being prepared for stripping during the 'winter' months when water temperatures ranged between 10 and 12°C. The photoperiod emulated the typical daylight duration of each respective season, and the fish were fed manually twice weekly in the form of a commercial diet comprising of pellets (*Otohime*) and mealworms (*Tenebrio molitor*). The quantity of pellets to be fed was determined by the equation:

$$M_p = n \times \overline{M}_F \times 0.008 \quad (1)$$

where M_p is the weight of the pellets, n is the number of fish, and the mean weight of all

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

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

fish in the tank (M_F) multiplied by 0.008. The quantity of mealworms provided was set at half the pellet mass. We analysed the FA content of both the pellets and the mealworms, providing this information in Table 1.

Ova collection and fatty acid analysis

For each generation, eggs from multiple random female *G. argenteus* were stripped by trained Manāki Whitebait staff on following days; F2-2017 stripped on 25th of June 2020 ($n = 6$), F2-2016 stripped on 2nd of July 2020 ($n = 5$), F2-2015 stripped on 31st of July 2020 ($n = 6$), F1 stripped on 11th of November 2020 ($n = 10$), Wild-caught stripped on 4th of December 2021 ($n = 4$). Based on these collection dates, the F1 generation was 8-years old at the time of sampling, and the F2 generations were 5-years (F2-2015), 4-years (F2-2016), and 3-years (F2-2017) old, respectively. The age of the wild-caught samples are unknown, but since they were caught in 2007 they would be older than 14-years old at the time of sampling. From the stripped eggs roughly 100-150 g were packaged into plastic containers, placed on ice, transported to Auckland University of Technology's (AUT) School of Science laboratory. Once there, the eggs were sorted and underwent processing. Specifically, deionised water was used to cleanse potential excrement and gelatinous coatings enveloping each egg cluster. The eggs were then subjected to drying before being delicately placed into Eppendorf tubes, destined for preservation within a freezer set at -80°C .

Gas Chromatography-Mass Spectrometry (GC-MS) was used for identification and quantitation of ova FAs. The method of extraction and derivatisation of samples was based on Zhou et al. (2014). Samples were snap frozen with liquid nitrogen, then freeze-dried overnight, and homogenised using a ceramic mortar and pestle.

Approximately 20 mg of the homogenised sample from the eggs of each fish was then weighed into screw-cap glass culture tubes (Kimax). To each sample, surrogate solution (20 μL of (400 $\mu\text{g}/\text{mL}$) 20 mg of tridecanoic acid in 50 mL of toluene) and extraction solution (2 mL of ISTD A 128 μM (9.55 mg Nonadecanoic acid in 250 mL of toluene)) in 50 mL of methanol was added. Each sample then received 200 μL of acetyl chloride drop wisely over 1 min, then vortexed and incubated at 100°C for one hour. Once cooled, 4 mL of 6% potassium carbonate was added to each sample, vortexed and centrifuged at 2500 rpm for 5 min. For each sample the top organic phase was extracted 2 times with sodium sulphate. Samples were then transferred to 2 mL amber vial. For each sample, a further 10x dilution was prepared, with 100 μL of the sample extract, 800 μL of toluene and 100 μL of ISTD B 200 $\mu\text{g}/\text{mL}$ (10 mg biphenyl in 50 mL toluene) prior to GC-MS analysis.

The analytical instrument parameters were established following the methodology outlined by Kramer et al. (2008). The Agilent 7890A GC coupled with a 5975C mass spectrometer and equipped with a split/splitless inlet was employed as the analytical instrument. In this investigation, a sample volume of one microliter was introduced via a CTC PAL autosampler into a 4 mm ID glass inlet liner packed with deactivated glass wool (Restek Sky®). The inlet was set to a temperature of 250°C and operated in splitless mode, with a column flow rate of 1 mL/min and a column head pressure of 9 psi, resulting in an average linear velocity of 19 cm/s. A purge flow of 50 mL/min was initiated one-minute post-injection. The column choice followed the guidelines of (Mossoba and Kramer 2010) and entailed the use of a fused silica Rtx-2330 column with dimensions 100 m x 0.25 mm (length x internal diameter) and a highly polar stationary phase of 0.2 μm (90% biscyanopropyl, 10% cyanopropylphenyl polysiloxane, Shimadzu). The carrier gas employed was helium of instrument-grade quality (99.99%, BOC).

Temperature programming in the GC oven commenced with an isothermal hold at 45°C for 2 min, followed by a ramp of 10°C/min to 215°C, which was maintained for 35 min, and subsequently increased by 40°C/min to reach 250°C, where it was held for 10 min. The transfer line to the mass spectrometric detector (MSD) retained a temperature of 250°C, while the MSD source and quadrupole temperatures were set at 230°C and 150°C, respectively. Detection was initiated at 14.5 min into the run. The detector was operated in a positive-ion, electron-impact ionisation mode, utilising an electron energy of 70 eV. The electron multiplier was maintained at the autotune value without additional voltage. Data acquisition occurred in scan mode over the range of 41–420 atomic mass units, acquiring at a rate of 1463 amu/s, and a detection threshold of 100 ion counts was implemented. Quantitative analysis necessitated the implementation of various dilutions for the 37-mix calibration curves (Nu-Chek-Prep). These meticulously plotted curves enabled the subsequent computation of fatty acid sample concentrations. Employing the linear equation derived from the 37-mix calibration, fatty acid concentrations within the samples were ascertained.

Statistical analysis

For comparative analysis, we treated the broodstock generation as a fixed effect, as it represents distinct, predefined categories (Wild-caught, F1, F2-2015, F2-2016, F2-2017). One outlier was visually identified (F2-2016, 3) and subsequently removed.

The majority of the FA analysed met the assumptions of normal distribution (Shapiro–Wilk test) and homogenous variances (Fligner-Killeen test). We employed one-way Analysis of Variance (ANOVA) and Tukey-HSD post-hoc tests to determine intergenerational differences in FA profiles. Principal Component Analysis (PCA) was conducted on all FAs, sums, and FA ratios, identifying variations across generations based on significant loadings ($>|0.5|$). FAs exhibiting loadings above this threshold on the first two principal components were deemed key biomarkers. A refined PCA was then carried out, focusing solely on these selected biomarkers. All analyses were conducted in SAS JMP (16.0.0). A p -value of <0.05 was considered statistically significant.

Results

PAL, OLA, and DHA were found to contribute towards the majority (Mean 72%) of total ova FA across all five generations. ANOVA revealed significant differences in FA profile (denoted by * in Table 2) for Σ SAFA, C6:0, C14:0, PAL, C18:0, C23:0, C16:1 (n-7), OLA, Σ PUFA, C18:2 (n-6), ARA, Σ (n-3)/ Σ (n-6) and DHA/EPA ratios.

Specifically, the increase in Σ SAFA concentration was primarily driven by PAL, which was significantly higher in F2-2017 compared to Wild-caught generation. Although the decline in Σ MUFA concentration was marginally nonsignificant ($P = 0.0542$), there was significantly lower OLA content in F2-2017 compared to Wild-caught, F1, and F2-2015 generations. There was a significant decline in Σ PUFA content, primarily driven by lower concentration of ARA in F2-2017 generation compared with wild-caught, and lower concentrations of C18:2 (n-6) in F2-2017 compared with all four other generations.

Table 2. Mean (\pm SD) relative fatty acid content (%) in ova of Wild-caught, F1, F2(2015), F2(2016), F2 (2017) Giant Kōkopu (*Galaxias argenteus*).

Fatty Acid	Wild-caught (n = 4)	F1 (n = 10)	F2-2015 (n = 6)	F2-2016 (n = 5)	F2-2017 (n = 6)	p
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

Notes: 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.

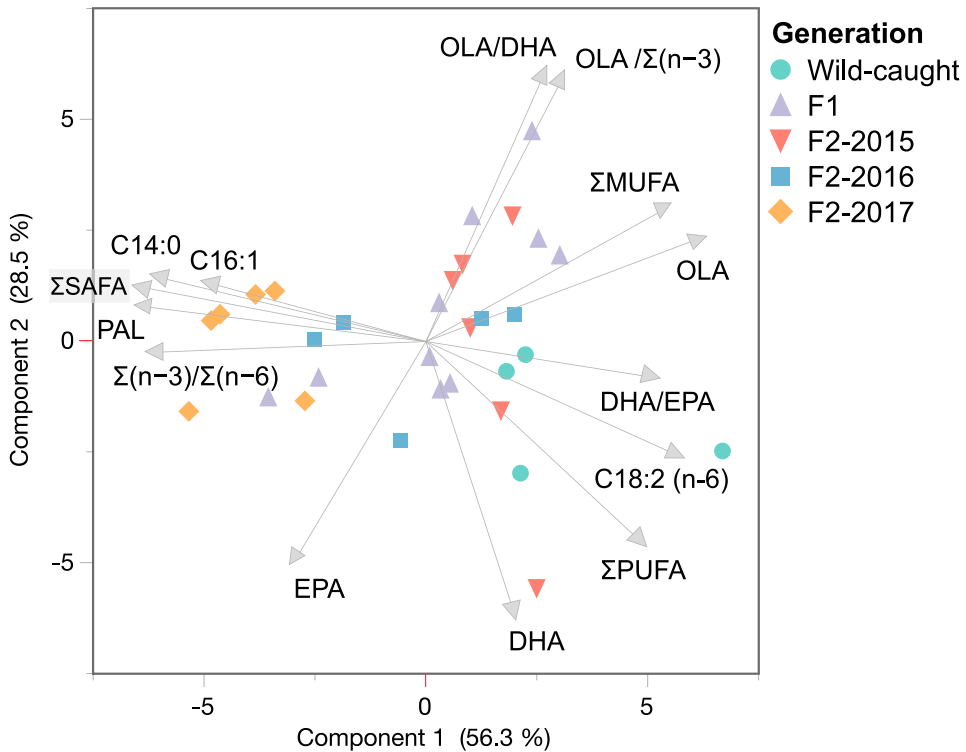


Figure 1. Biplot of the first two principal components of ova fatty acid profiles across different broodstock generations of Giant Kōkopu (*Galaxias argenteus*), highlighting the key fatty acids and ratios that contribute to the overall variance. DHA: C22:6 (n-3), Docosahexaenoic acid; EPA: C20:5 (n-3),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

Additionally, the ratio of $\Sigma(n-3)/\Sigma(n-6)$ and DHA/EPA was significantly higher in F2-2017 compared with the wild-caught generation.

In the PCA, the initial two principal components significantly captured the variations in ova FA profiles across different broodstock generations. The first principal component was heavily influenced by Σ SAFA (loading: -0.33847) and OLA (loading: 0.32655), indicating their pivotal roles in the variance. The second principal component was markedly driven by the ratios OLA/ $\Sigma(n-3)$ (loading: 0.43841) and OLA/DHA (loading: 0.44715). Specifically, eggs from the F2-2017 generation were marked by elevated levels of C14:0, C16:1, Σ SAFA, and $\Sigma(n-3)/\Sigma(n-6)$, whereas eggs from the Wild-caught generation were characterised by higher DHA/EPA and C18:2 (n-6) levels, along with Σ PUFA. Eggs from the F1, F2-2015, and F2-2016 generations exhibited intermediate profiles, notably influenced by OLA/ $\Sigma(n-3)$ and OLA/DHA (see Figure 1).

Discussion

Successful aquaculture is primarily underpinned by high quality broodstock, reproductive success and fish health in captivity. Although specific FAs have been identified for their roles in various aspects of these parameters, they have seldom been used as biomarkers to study broodstock TP. To our knowledge, this is the first study to conduct a

comprehensive assessment of TP across a multi-generational ‘wild-to-captivity’ lineage in an aquacultured fish species. We discovered that with each generation, captive-bred fish are depositing fewer FAs associated with stress and reduced reproductive success into their eggs, while simultaneously increasing the levels of FAs correlated with enhanced immune response. Our findings shed light on the broodstock’s capacity to adapt to captive conditions, suggesting implications for enhancing aquaculture productivity through targeted rearing strategies.

In profiling FAs across five generations of giant kōkopu broodstock, we established that PAL, OLA, and DHA are the most dominant FAs. Moreover, we uncovered significant dynamic trends for PAL, OLA, and ARA, and changes in $\Sigma(n-3)/\Sigma(n-6)$ and DHA/EPA ratios from the wild-caught to F2-2017 generations. These insights advance our understanding of TP as it pertains to the ability of broodstock to provision different amounts of specific FAs into their eggs. The decline in OLA concentration across successive generations seen in this study echoes insights from previous research by Izquierdo (1996) and Montero et al. (2001). These investigations identified high OLA content as a potential correlative biomarker of acute and chronic stress in aquacultured fish. Similarly, the decreasing trend in ARA and increasing trend in PAL from wild-caught to F2-2017 broodstock generations are not without precedence. For example, high levels of ARA have been linked to lower levels of fertilisation success, egg, and larval quality (Furuita et al. 2003, 2006; Nguyen et al. 2010), while higher levels of PAL have been noted for increased immunostimulant and antiviral properties, making it a crucial agent in bolstering mortality and morbidity-resistance in captive fish (Nakamura et al. 1996; Librán-Pérez et al. 2019).

This study confirms the findings of Hoang et al. (2021), which also showed a decreasing trend in OLA from F1 to F2-2015. However, by repeating the same analysis, and expanding our focus from two to five generations, we advanced our understanding of TP in this species through multi-generational tracing of key biomarkers that may have remained obscured in a binary comparison between only two generations. These findings have far-reaching implications, opening avenues for further research and practical applications in aquaculture. In particular, FA profiles can be used as indicators for maternal adaptability and health, thereby facilitating informed decision-making for broodstock selection and management of newly aquacultured species.

This study has one inherent bias and one limitation. The inherent challenge of experimental design bias is difficult to overcome in a study seeking to assess biomarkers across successively bred lineage, resulting in broodstock generations of progressively younger ages. In this study, the fourth generation bred in captivity, and thus the youngest (F2-2017), was the most adapted to reproductive success, disease and stress resistance. Recognising this bias is crucial, particularly because the influence of age cannot be decoupled from successive generations bred in captivity. Progeny are always going to be younger than their parents. However, the effects of age on maternal resource provisioning appear to be complicated. For example, the BOFFF hypothesis (big old fat fecund female fish) argues that older and bigger mothers provision more lipids into their larger eggs, which lead to robust larvae that can withstand starvation (Hixon et al. 2014). However, the notion that offspring quality improves with maternal age is a counter intuitive idea, for which little empirical evidence exists in animals (Marshall et al. 2010; Marasco et al. 2019). In fact, when fish studies have moved beyond simplistic

qualitative surrogates of egg quality (e.g. egg size or larval oil globule volume) and conducted analytical investigations of tissue lipid constituents, they have found no correlation between maternal age and lipid content (Carter et al. 2015; Allen et al. 2018). In terms of limitation, our analysis stops at the F2-2017 generation, leaving unanswered questions about whether subsequent generations bred in captivity would continue to exhibit improved FA profiles. Given that this study reaffirms the findings of Hoang et al. (2021), there may be more insight to be gained from repeated monitoring of FA biomarkers which could elucidate the effects of extended time (i.e. age) and targeted nutritional intervention in captivity.

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Disclosure statement

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