

Research Report

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## Genomic and Developmental Approaches to Enhance Reproductive Success and Growth in Eel (*Anguilla* spp.)

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**Abstract** Eels (*Anguilla* spp.) exhibit complex life cycles and face challenges such as habitat loss and overexploitation, necessitating innovative approaches to enhance their reproductive success and growth. The primary objective of this study is to explore genomic and developmental methods to improve eel reproduction and growth, with a focus on understanding the underlying biological mechanisms and identifying strategies for aquaculture improvements. Recent research has highlighted several key findings. In Japanese eel (*Anguilla japonica*), androgen regulation has been shown to significantly influence ovarian growth and follicle development. In European eel (*Anguilla anguilla*), recombinant gonadotropins can effectively induce spermatogenesis and sperm release, improving reproductive outcomes. Additionally, studies have revealed complex relationships between growth rates and environmental factors such as temperature, which affect eel development over time. The expression of gonadotropin subunits and receptors during different stages of oogenesis has also been characterized, providing insights into the hormonal regulation of reproduction. These findings underscore the potential of genomic and developmental approaches to enhance eel reproductive success and growth. By leveraging hormonal regulation, recombinant gonadotropins, and understanding environmental influences, significant advancements can be made in eel aquaculture. These strategies not only improve reproductive efficiency but also contribute to the conservation and sustainable management of eel populations.

**Keywords** *Anguilla* spp.; Reproductive success; Androgenic modulation; Gonadotropins; Environmental factors; Maternal transfer; Aquaculture

### 1 Introduction

The genus *Anguilla*, commonly known as eels, encompasses species with complex life cycles and significant ecological and economic importance. Eels are renowned for their intricate life cycles, which include long-distance migrations and unique reproductive strategies, and they face numerous challenges in their natural habitats (Hakim et al., 2019). Major challenges include habitat loss, environmental changes, and population decline due to overexploitation. To address these issues, considerable research efforts have been devoted to understanding the reproduction and growth dynamics of eels.

Eels exhibit a remarkable catadromous life cycle, with adult individuals migrating thousands of kilometers to the Sargasso Sea in the Atlantic Ocean for spawning. After hatching, the juvenile eels begin a long journey back to freshwater and estuarine habitats, where they grow and mature over several years (Reismann and Frankowski, 2021). The complexity of eel reproduction, from the mysterious spawning grounds to the development and recruitment of juveniles, poses significant challenges for conservation biologists and fisheries managers. The reproductive biology of the Japanese eel (*Anguilla japonica*), in particular, has become a focal point of research due to the population decline of this species and the urgent need for effective aquaculture practices (Lee and Lou, 2019). The primary ovarian growth stages of female eels are crucial for successful reproduction, and understanding the hormonal regulation of these stages is essential for enhancing reproductive success (Churcher et al., 2015; Jéhannet et al., 2019).

Enhancing eel reproductive success is crucial for maintaining viable populations and ecosystem health. Firstly, the populations of many eel species, including the Japanese eel, have been severely dwindling due to overfishing,

habitat loss, and other anthropogenic factors (Lee and Lou, 2019). Effective aquaculture practices are necessary to meet the demand for eel products and to reduce pressure on wild populations. Secondly, the unique life cycle of eels, which involves long migrations and complex developmental stages, poses significant challenges for conservation and aquaculture efforts (Hanzen et al., 2020). By improving our understanding of the reproductive mechanisms and growth processes in eels, researchers can develop strategies to enhance their reproductive success and ensure sustainable populations.

This study aims to explore genomic and developmental approaches to enhance the reproductive success and growth of eel species. Specifically, it includes researching the hormonal regulation of key ovarian growth stages and their impact on reproductive success, evaluating the practicality of DNA barcoding in species identification, and its implications for conservation and aquaculture. Based on the findings from genomic and developmental research, the study will propose strategies to improve eel aquaculture practices. By addressing these objectives, we hope to contribute to the knowledge base of eel reproduction and growth, providing insights that can support conservation and aquaculture practices.

## 2 Genomic Approaches

### 2.1 Genomic sequencing and analysis

Genomic sequencing and analysis are fundamental tools in understanding the genetic makeup of eels, which can significantly contribute to enhancing their reproductive success and growth. By sequencing the genome of eels, researchers can identify genetic variations and understand the genetic basis of traits related to growth and reproduction (Burgerhout et al., 2018). For instance, comprehensive expression analysis using quantitative real-time PCR and RNA sequencing has been employed to study the effects of phytoestrogens on sex differentiation in Japanese eels (*Anguilla japonica*). This approach revealed that genistein, a soy isoflavone, induces feminization by altering the expression of sex-specific genes, thereby providing insights into the molecular mechanisms of sex differentiation in eels (Inaba et al., 2022).

### 2.2 Identification of key genes

Identifying key genes involved in reproductive processes and growth is crucial for developing strategies to enhance these traits in eels. The study on Japanese eels demonstrated that genistein up-regulates female-specific genes and down-regulates male-specific genes in the gonads, suggesting that these genes play a significant role in sex differentiation. Such findings can be leveraged to manipulate the sex ratio in eel populations, favoring the production of females, which have higher commercial value due to their greater body weight (Inaba et al., 2022). Additionally, the use of recombinant gonadotropins in European eels (*Anguilla anguilla*) has shown that specific hormones can induce spermatogenesis and spermiation, highlighting the importance of hormonal regulation in reproductive success (Peñaranda et al., 2018; Kottmann et al., 2021).

### 2.3 Genomic selection techniques

Genomic selection techniques involve using genetic information to select individuals with desirable traits for breeding. This approach can accelerate the improvement of growth and reproductive traits in eel populations. By integrating genomic data with traditional breeding methods, it is possible to enhance the efficiency and accuracy of selection. For example, the identification of sex-specific genes and their expression patterns in Japanese eels can be used to develop genomic selection strategies aimed at increasing the proportion of females in aquaculture settings (Inaba et al., 2022). Similarly, the application of recombinant gonadotropins in European eels demonstrates the potential of combining genomic and hormonal approaches to optimize reproductive outcomes (Peñaranda et al., 2018; Kottmann et al., 2020).

## 3 Developmental Approaches

### 3.1 Hormonal regulation of reproduction

Hormonal regulation plays a pivotal role in orchestrating the reproductive cycle of eels, from gonadal maturation to spawning. Key hormones, such as gonadotropins and sex steroids, govern critical stages of reproductive development (Burgerhout et al., 2018; Peñaranda et al., 2018). One significant approach involves the use of

specific recombinant gonadotropins to induce spermatogenesis and spermiation in European eels (*Anguilla anguilla*). In a study, recombinant European eel follicle-stimulating hormone (aarFsh) and luteinizing hormone (aarLh) were used to stimulate androgen synthesis and induce spermiation. The best results were achieved with a combination of constant doses of aarFsh and increasing doses of aarLh, leading to high-quality sperm production with over 50% motile cells (Peñaranda et al., 2018). Additionally, the use of phytoestrogens such as genistein has been shown to induce feminization in Japanese eels (*Anguilla japonica*) by altering the expression of sex-specific genes, thereby enhancing the female ratio which is commercially desirable (Inaba et al., 2022).

### 3.2 Embryonic development and growth

Embryonic development and growth in eels can be significantly influenced by hormonal treatments. The administration of recombinant gonadotropins not only induces spermatogenesis but also ensures the production of high-quality sperm, which is essential for successful fertilization and subsequent embryonic development (Peñaranda et al., 2018; Kottmann et al., 2021). Furthermore, the feminization of eels through dietary phytoestrogens like genistein can lead to a higher proportion of females, which are known to achieve greater body weight and better growth rates compared to males (Inaba et al., 2022). This approach can be strategically used to enhance the overall growth performance in eel aquaculture.

### 3.3 Environmental influences on development

Environmental factors such as diet and hormonal treatments play a pivotal role in the developmental processes of eels. The study on Japanese eels demonstrated that dietary inclusion of soy isoflavones like genistein can significantly influence sex differentiation and growth by modulating the expression of sex-specific genes (Inaba et al., 2022). Similarly, controlled administration of recombinant gonadotropins in European eels under aquaculture conditions has shown to effectively induce reproductive maturation and improve sperm quality, which are critical for successful breeding and development (Peñaranda et al., 2018; Palstra et al., 2023). These findings highlight the importance of optimizing environmental conditions to enhance reproductive success and growth in eel aquaculture.

## 4 Enhancing Reproductive Success

### 4.1 Breeding programs and techniques

Breeding programs for eels, particularly the Japanese eel (*Anguilla japonica*) and European eel (*Anguilla anguilla*), have focused on overcoming the challenges of inducing maturation and successful reproduction in captivity. Techniques such as hormone injections have been employed to stimulate ovarian growth and spermatogenesis. For instance, the use of salmon pituitary homogenate (SPH) combined with methyltestosterone (MT) has shown to enhance synchronous follicle development in Japanese eels (Lee and Lou, 2019). Similarly, recombinant gonadotropins have been used to induce spermatogenesis and spermiation in European eels, with varying doses of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) yielding significant results (Peñaranda et al., 2018).

### 4.2 Genetic improvement strategies

Genetic improvement strategies have been pivotal in enhancing reproductive success and growth in eels. The high-quality genome assembly of the Japanese eel has provided insights into gene duplication and chromosomal reorganization, which are crucial for understanding the genetic basis of traits related to reproduction and growth (Wang et al., 2022). Additionally, the study of sex differentiation at the molecular level has identified key genes involved in gonadal development, which can be targeted for genetic improvement (Horiuchi et al., 2022). The transfer of maternal steroids and mRNA transcripts to eggs has also been shown to influence embryonic development, suggesting that genetic selection for favorable traits could enhance reproductive outcomes (Kottmann et al., 2021).

### 4.3 Case studies of successful reproduction

Several case studies have demonstrated successful reproduction in eels through various interventions. For example, Lee and Lou (2019) studied the regulatory role of androgens in the primary ovarian growth of the Japanese eel

(*Anguilla japonica*). The study found that injecting salmon pituitary homogenate (SPH) and 17 $\alpha$ -methyltestosterone (MT) significantly promoted synchronous follicle development. Experimental results showed that the ovarian growth index (GSI) and follicle-stimulating hormone receptor (fshr) mRNA expression levels in the SPH + MT group were higher than those in the SPH-only group (Figure 1). Additionally, it was found that using pituitary extract and maturation-inducing hormone to induce follicle maturation in European eels resulted in successful vitellogenesis and enhanced embryonic development capabilities (Kottmann et al., 2021).

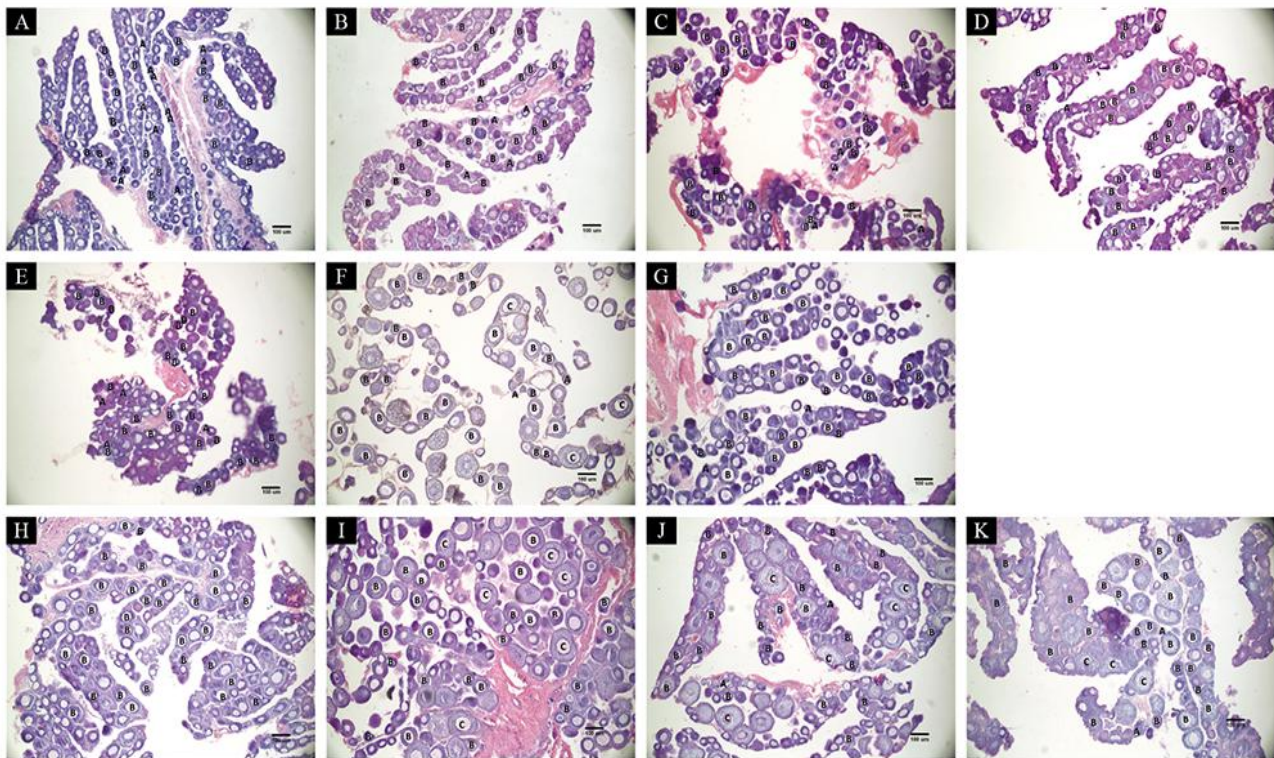


Figure 1 Histological comparison of ovarian development among the control and three weekly SPH- and SPH + MT- injected groups (Adopted from Lee and Lou, 2019)

Image caption: The figure shows that primary follicle growth in the control group (A-D) was slow (A, GSI = 0.42%; B, GSI = 0.46%; C, GSI = 0.5%; D, GSI = 0.75%), while the group injected with salmon pituitary homogenate (SPH) (E-G) showed greater follicle development (E, GSI = 0.63%; F, GSI = 1.02%; G, GSI = 1.07%). The group injected with both SPH and 17 $\alpha$ -methyltestosterone (MT) (H-K) (H, GSI = 0.93%; I, GSI = 1.34%; J, GSI = 1.38%; K, GSI = 1.41%) exhibited the most significant follicle development, with a notable increase in the number of primary follicles. The results indicate that the combined action of androgens and SPH promotes synchronized ovarian development (Adapted from Lee and Lou, 2019)

Another study explored the impact of cold seawater treatment on the early sexual development stages of European eel. Rozenfeld et al. (2019) found that cold seawater (10°C) treatment significantly promoted the activity of the male reproductive gland axis (BPG axis) in European eel. Specifically, the cold seawater treatment increased the proliferation and differentiation of reproductive cells, especially at the type A spermatogonia stage, and elevated plasma testosterone and 11-ketotestosterone levels (Figure 2). Gene expression analysis showed significant differences in gene expression profiles between the treatment group and the control group, particularly in aspects such as circadian rhythm, histone modification, and meiotic nuclear division. The results suggest that cold seawater treatment may influence temperature perception through epigenetic mechanisms, thereby promoting the early sexual development stages of eel. Furthermore, the cryopreservation of eel sperm has been optimized, with recent protocols using methanol as a cryoprotectant showing improved motility and fertilization rates, which is crucial for large-scale breeding programs (Herranz-Jusdado et al., 2019).

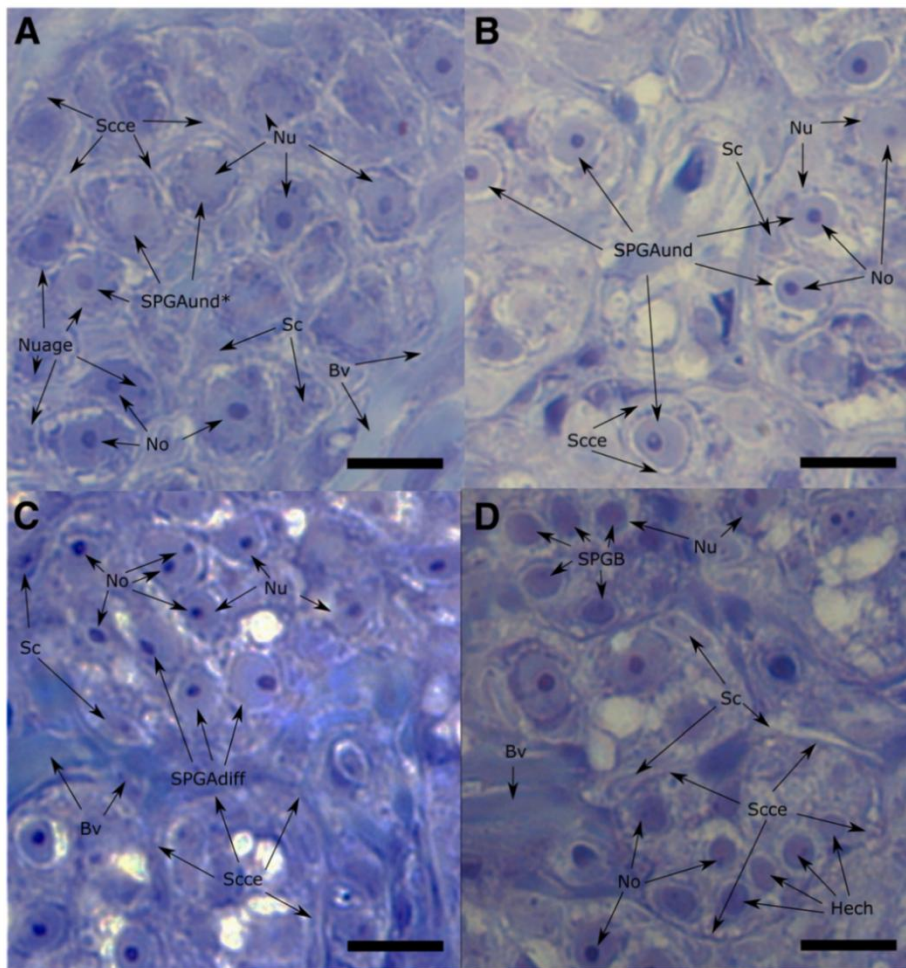


Figure 2 Identified spermatogonia types (Adopted from Rozenfeld et al., 2019)

Image caption: Forty times magnification fields of selected histological sections representing the spermatogonia stages: the most undifferentiated spermatogonia type A (SPGAund\*; panel a), the second most undifferentiated spermatogonia type A (SPGAund; panel b), differentiated spermatogonia type A cells (SPGAdiff; panel c), and early spermatogonia type B cells (SPGB; panel d). These identifiable characteristics are further labeled with arrows: blood vessels (Bv), nucleus (Nu), nucleoli (No), Sertoli cells (Sc), Sertoli cell cytoplasmic extensions (Scce), heterochromatin (Hech), and nuage (Nuage). The results show that the proportion of SPGAdiff cells in the cold seawater treatment group (T10) is significantly higher than in other groups, while the proportion of undifferentiated SPGAund cells is lower, indicating that cold seawater treatment promotes the proliferation and differentiation of spermatogonia (Adapted from Rozenfeld et al., 2019)

## 5 Promoting Growth

### 5.1 Nutritional interventions

Nutritional interventions play a crucial role in enhancing the growth performance of eels. Various studies have demonstrated the positive effects of dietary supplements on growth metrics. For instance, the inclusion of Chinese herbal medicines mixture (CHMM) in the diet of European eels (*Anguilla anguilla*) significantly improved weight gain and specific growth rate (SGR) when administered at concentrations of 5-12.5 g/kg (Huang et al., 2020). Similarly, the supplementation of *Macleaya cordata* extract (MCE) in the diet of juvenile American eels (*Anguilla rostrata*) at levels of 50 mg/kg or 100 mg/kg resulted in enhanced growth performance and improved intestinal health (Chen et al., 2022). Additionally, the enrichment of eel diets with essential fatty acids from fish oil and corn oil has been shown to significantly increase the growth rate and feed efficiency in *Anguilla bicolor* (Sari et al., 2019; Ismayanti et al., 2019).

### 5.2 Growth hormones and supplements

Growth hormones and other supplements have been investigated for their potential to enhance growth in eels. The administration of salmon or carp pituitary extract (PE) as an exogenous source of gonadotropins has been used to

induce vitellogenesis in female European eels (*Anguilla anguilla*), which is crucial for reproductive success and subsequent growth (Kottmann et al., 2021). Furthermore, the use of *Bacillus amyloliquefaciens* (GB-9) and *Yarrowia lipolytica* lipase2 (YLL2) as dietary supplements has been shown to significantly improve growth performance, digestive enzyme activity, and innate immunity in Japanese eels (*Anguilla japonica*) (Zheng et al., 2019). These findings suggest that hormonal and supplementary interventions can be effective strategies for promoting growth in eel aquaculture.

### 5.3 Genetic factors influencing growth

Genetic factors also play a significant role in the growth and development of eels. The expression of specific genes related to growth and development can influence the overall growth performance of eels. For example, the mRNA transcript profiles of growth-related genes such as growth hormone (gh), insulin-like growth factors (igf1, igf2a, igf2b), and thyroid hormone receptors (thr $\alpha$ b, thr $\beta$ a, thr $\beta$ b) have been shown to vary during embryogenesis in European eels (*Anguilla anguilla*) (Kottmann et al., 2021). Additionally, the expression of androgen receptors (ara, ar $\beta$ ) and follicle-stimulating hormone receptor (fshr) has been linked to the modulation of primary ovarian growth and vitellogenic development in Japanese eels (*Anguilla japonica*) (Lee and Lou, 2019). Understanding these genetic factors can provide insights into the mechanisms underlying growth and development in eels, thereby informing breeding and management practices to enhance growth performance.

A combination of nutritional interventions, growth hormones and supplements, and genetic factors can significantly enhance the growth performance of eels in aquaculture. By leveraging these approaches, it is possible to improve the efficiency and sustainability of eel farming practices.

## 6 Integration of Genomic and Developmental Approaches

### 6.1 Combined strategies for improvement

The integration of genomic and developmental approaches has shown significant promise in enhancing reproductive success and growth in eel species. One effective strategy involves the use of phytoestrogens, such as soy isoflavones, to manipulate sex differentiation. In Japanese eels (*Anguilla japonica*), genistein has been found to induce feminization more efficiently than daidzein, leading to a higher ratio of females, which are commercially more valuable due to their higher body weight (Inaba et al., 2022). This approach leverages the molecular pathways responsible for sex differentiation, as evidenced by the up-regulation of female-specific genes and down-regulation of male-specific genes in the gonads.

### 6.2 Technological advances

Technological advancements have played a crucial role in the application of genomic and developmental approaches to eel aquaculture. The development of specific recombinant gonadotropins, such as European eel (*Anguilla anguilla*) recombinant gonadotropins (aarGths), has enabled precise control over the reproductive processes (Kottmann et al., 2021). These recombinant hormones, produced in Chinese hamster ovarian cells, have been used to induce spermatogenesis and spermiation in captive male eels. The use of a combination of recombinant follicle-stimulating hormone (aarFsh) and luteinizing hormone (aarLh) has been particularly effective, with the best results achieved through a constant dose of aarFsh and an increasing dose of aarLh (Peñaranda et al., 2018). This technological innovation has significantly improved sperm quality and reproductive success in European eels.

### 6.3 Case studies of integrated approaches

Several case studies highlight the successful integration of genomic and developmental approaches in eel aquaculture. In one study, Japanese eels were fed a diet treated with estradiol-17 $\beta$  or genistein for 45 days (Inaba et al., 2022). The study found that adding soy isoflavones to the feed can significantly increase the proportion of female eels, with the effect being more pronounced when genistein is added (Inaba et al., 2022). The experiments demonstrated that genistein could induce feminization by upregulating female-specific genes (such as *foxl2a* and *cyp19a1*) and downregulating male-specific genes (such as *amh* and *gsdf*) (Figure 3). Additionally, the study showed that genistein exhibits estrogen-like effects similar to estradiol in eels. This discovery provides a potential

method for sex control in eel aquaculture without using synthetic hormones, thereby enhancing the commercial value of eels (Inaba et al., 2022). Another study demonstrated the efficacy of recombinant gonadotropins in inducing spermatogenesis and spermiation in European eels. The best sperm quality, with over 50% motile cells, was observed in males treated with the highest dose of aarFsh, showcasing the potential of these recombinant hormones to enhance reproductive outcomes (Peñaranda et al., 2018).

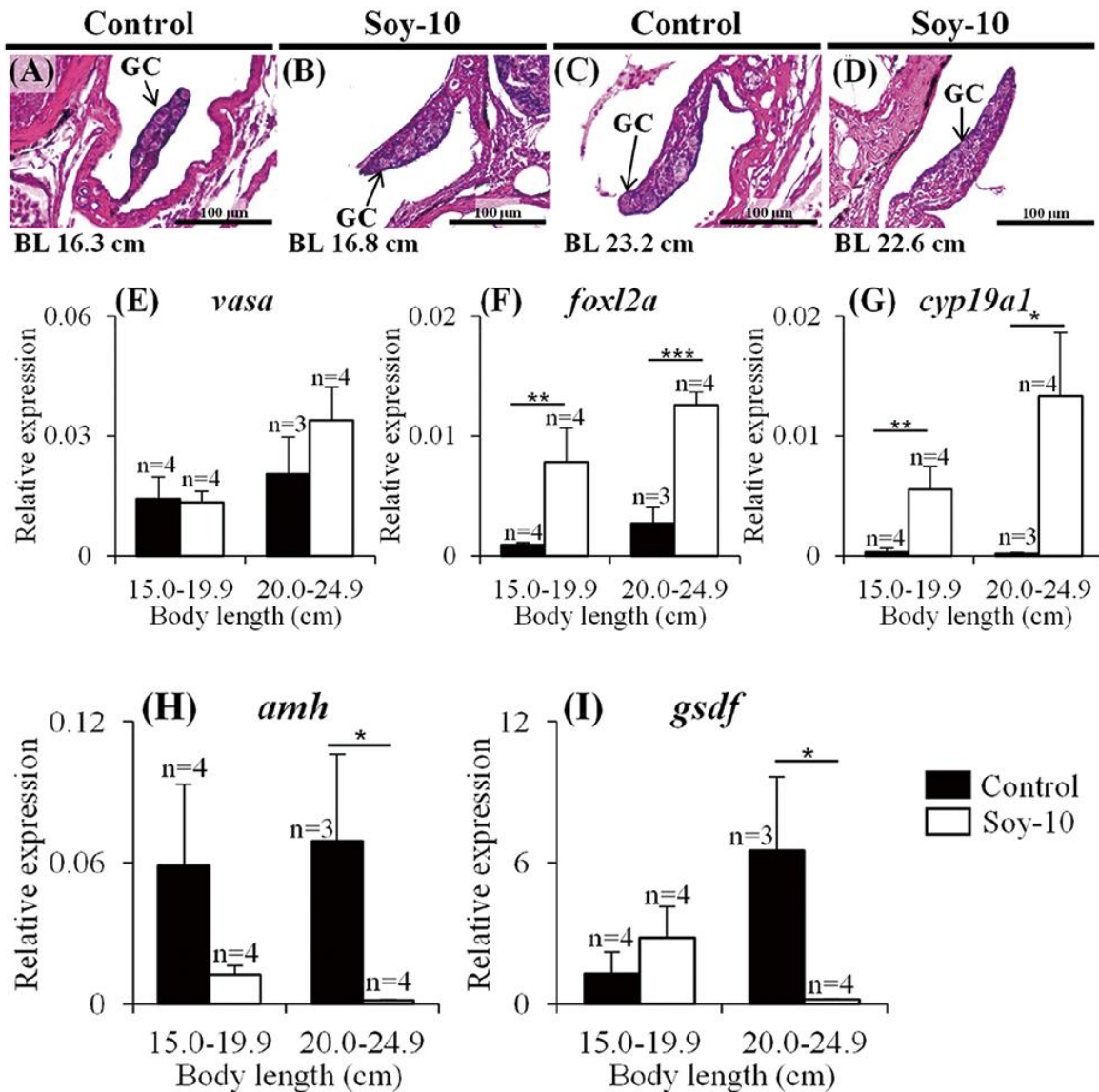


Figure 3 Histological analysis of germ cells and quantitative real-time PCR (qPCR) analysis of sex-specific genes in the gonads of Japanese eels raised to 45 days (Adopted from Inaba et al., 2022)

Image caption: The figure details the impact of soy isoflavones on the expression of sex-specific genes in Japanese eels. Panels (A – D) show histological sections of undifferentiated gonads after hematoxylin and eosin staining in body-length groups medium (A, B) and large (C, D), revealing the gonadal morphology of eels after 45 days in different treatment groups, with all germ cells remaining undifferentiated. Panels (E – I) present the expression levels of five sex-specific genes (*vasa* (E), *foxl2a* (F), *cyp19a1* (G), *amh* (H), *gsdf* (I)) analyzed by quantitative PCR. The results indicate that the expression of female-specific genes (*foxl2a* and *cyp19a1*) is significantly higher in the isoflavone-treated group compared to the control group, while the expression of male-specific genes (*amh* and *gsdf*) is significantly lower in the isoflavone-treated group. These findings confirm that soy isoflavones induce feminization in Japanese eels by regulating the expression of sex-specific genes (Adapted from Inaba et al., 2022)

By combining genomic insights with developmental interventions, these integrated approaches offer a robust framework for improving the reproductive success and growth of eel species in aquaculture settings.

## 7 Applications and Implications

### 7.1 Aquaculture practices

The application of genomic and developmental approaches in eel aquaculture has shown significant promise in enhancing reproductive success and growth. For instance, the use of soy isoflavones, such as genistein, has been demonstrated to induce feminization in Japanese eels (*Anguilla japonica*), which is beneficial since females gain higher body weight and have better commercial value than males. This feminization is achieved by altering the molecular pathways responsible for sex differentiation, as evidenced by the up-regulation of female-specific genes and down-regulation of male-specific genes in the gonads (Inaba et al., 2022). Additionally, the use of specific recombinant gonadotropins, such as European eel follicle-stimulating hormone (aarFsh) and luteinizing hormone (aarLh), has been effective in inducing spermatogenesis and spermiation in European eels (*Anguilla anguilla*). These treatments have led to improved sperm quality and successful maturation of captive male eels, which is crucial for breeding programs (Peñaranda et al., 2018; Guarniero et al., 2020).

### 7.2 Conservation efforts

Genomic and developmental techniques also play a vital role in the conservation of eel species (Nomura et al., 2018; Takeuchi et al., 2019; Hanzen et al., 2020). By understanding and manipulating the sex differentiation pathways, it is possible to manage the population structure more effectively, ensuring a balanced sex ratio that supports sustainable breeding practices. The ability to induce spermatogenesis and spermiation using recombinant gonadotropins can aid in the captive breeding of endangered eel species, thereby contributing to their conservation (Peñaranda et al., 2018). These approaches can help maintain genetic diversity and prevent the decline of eel populations in the wild.

### 7.3 Economic and ecological impact

The economic implications of these advancements are substantial. By increasing the proportion of female eels in aquaculture, producers can achieve higher yields and better market prices due to the larger size and higher commercial value of females (Inaba et al., 2022). Moreover, the ability to induce reproductive processes in captive eels reduces the reliance on wild populations, thereby decreasing the pressure on natural stocks and contributing to the sustainability of eel fisheries. Ecologically, these practices help maintain the balance of eel populations in their natural habitats, supporting the overall health of aquatic ecosystems. The integration of genomic and developmental approaches in eel aquaculture and conservation not only enhances productivity but also promotes ecological sustainability and economic viability (Peñaranda et al., 2018; Inaba et al., 2022).

## 8 Challenges and Future Directions

Despite significant advancements, the integration of genomic and developmental approaches in enhancing reproductive success and growth in eels faces several challenges and offers promising avenues for future research and innovation.

### 8.1 Technical and methodological challenges

Technical challenges persist in genomic and developmental research in eels, including the complexity of genome assembly due to repetitive sequences and genome size variation among species (Tian et al., 2022; Wang et al., 2022). Methodological limitations in studying early developmental stages and larval physiology hinder our understanding of critical factors influencing larval survival and growth (Parmeggiani et al., 2020). Additionally, standardizing protocols for hormonal manipulation and optimizing environmental conditions in captive breeding programs remain challenging, requiring interdisciplinary collaboration and the development of specialized techniques tailored to eel biology.

### 8.2 Research gaps and opportunities

Several research gaps present opportunities for advancing knowledge and application in eel biology. Understanding the epigenetic mechanisms regulating gene expression during critical developmental stages could reveal novel strategies for enhancing reproductive outcomes and growth performance. Further exploration of environmental cues and their influence on eel migration patterns and spawning behaviors is essential for



predicting and mitigating the impacts of climate change on eel population (Gaillard et al., 2018). Integrating multi-omics approaches, such as proteomics and metabolomics, with genomic analyses could provide comprehensive insights into physiological responses and adaptive mechanisms in eels facing environmental stressors (Kho et al., 2023).

### 8.3 Future trends and innovations

Future trends in genomic and developmental research are poised to revolutionize eel management and conservation practices. Advancements in genome editing technologies, such as CRISPR-Cas9, hold promise for targeted genetic modifications to enhance desirable traits in eel populations. Continued innovation in bioinformatics and computational tools will facilitate the interpretation of large-scale genomic data and accelerate the discovery of genetic markers associated with reproductive success and growth. Embracing interdisciplinary collaborations and fostering international partnerships will promote knowledge exchange and harmonize conservation efforts across eel range countries, ensuring sustainable management and resilience of eel populations in a rapidly changing world.

## 9 Concluding Remarks

The research on enhancing reproductive success and growth in eels (*Anguilla* spp.) has yielded significant insights through genomic and developmental approaches. One study demonstrated that soy isoflavones, particularly genistein, effectively induce feminization in Japanese eels (*Anguilla japonica*) by altering the expression of sex-specific genes, leading to a higher female ratio which is commercially desirable. Another study highlighted the use of specific recombinant gonadotropins to induce spermatogenesis and spermiation in European eels (*Anguilla anguilla*), showing that a combination of recombinant follicle-stimulating hormone (aarFsh) and luteinizing hormone (aarLh) can significantly enhance sperm quality and motility.

Genomic and developmental approaches are crucial for the aquaculture of eels due to their complex life cycles and reproductive challenges. The ability to manipulate sex differentiation through dietary supplements like genistein not only improves the yield of commercially valuable females but also provides a deeper understanding of the molecular mechanisms governing sex differentiation. Similarly, the application of recombinant gonadotropins to induce and enhance spermatogenesis and spermiation addresses the bottleneck of male maturation in captivity, thereby facilitating controlled breeding programs and improving overall reproductive success.

Future research should focus on optimizing the dosage and administration protocols of phytoestrogens and recombinant hormones to maximize their efficacy and minimize any potential adverse effects. Additionally, exploring the genetic basis of sex differentiation and reproductive maturation through advanced genomic techniques such as CRISPR/Cas9 could provide new avenues for targeted interventions. Long-term studies on the ecological and physiological impacts of these interventions on eel populations are also recommended to ensure sustainable aquaculture practices. Expanding these approaches to other eel species and integrating them with environmental and nutritional management strategies could further enhance the productivity and sustainability of eel aquaculture.

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### Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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