ALEXANDER GOIKOETXEA

Where's the Switch?[1]

Sex change occurs as a usual part of the life cycle for many ray-finned fish,[2] often following specific social cues. It has been shown that environmental factors can interact with, and sometimes override, genetic factors to control sexual development. More dramatically, in many marine fish, individuals can change sex as an adaptive response to environmental changes even during adulthood.[3] Such sensitivity to environmental stimuli may explain why teleost or bony fishes display such highly diverse sex determination and developmental systems, which make them good models for understanding vertebrate sexual development.[4]

However, the exact mechanism behind the transduction of the environmental signals into the molecular cascade that underlies this singular transformation remains largely unknown. Cortisol is the main glucocorticoid in fish and the hormone most directly associated with stress. In recent years, cortisol has been proposed as a key factor linking external environmental stimuli with internal physiological responses during sexual development, whereby environmental stress may initiate a steroidogenic shift at the onset of sex change.[5] However, the exact role of cortisol or stress in transducing the external signals to elicit physiological responses during sexual development and sex change remains a mystery.

Teleost fishes display a diversity of sex determination and developmental systems found in no other vertebrate lineage. They may exhibit gonochorism (fixed separate sexes), simultaneous (or synchronous) or sequential hermaphroditism.[6] With the exception of some species of frogs, teleost fish species are the only known vertebrates with sequentially hermaphroditic representatives,[7] a reproductive strategy that has multiple evolutionary origins in fishes, having been reported in 27 taxonomic families spread across nine orders.[8] In such species, reproductive value is typically strongly size-dependent, and sex change enables an individual to maximise lifetime reproductive fitness by reproducing as one sex while small, and the other sex when older and larger (the size advantage model or SAM[9]). Three strategies are observed: protogynous (female-to-male, common amongst coral reef fishes such as groupers, parrotfishes and wrasses); protandrous (male-to-female, as in anemone fishes); and sequentially bidirectional sex change (sex change in either direction, common in gobies). All three approaches entail modifications to behavior, gonadal restructuring and alterations in external morphology.[10] Sex change in teleost fish offers a dramatic example of environmentally cued phenotypic plasticity unprecedented in any other group of vertebrates.[11]

Many commercially valuable fish species naturally change sex (for example, grouper). In aquaculture settings, artificial control of sex ratios using sex-change technology has become essential for the accomplishment of successful operations.[12] For example, artificial achievement of monosex populations can be valuable for increasing growth rates, or to ensure availability of broodstock of a particular sex.[13] A molecular-level understanding of sex change, and especially the upstream factors that initiate this process, would enable development of efficient, low-cost genetic tools for controlling sex ratios, thereby avoiding the use of steroid treatments that pose significant environmental contamination risk. I anticipate that understanding the role of cortisol in the initial stages of sex change will further improve our understanding of sex determination and differentiation across vertebrates and may lead to new tools to control fish sex ratios in aquaculture.

In my research, I aim to elucidate the role of cortisol in mediating sex change in a protogynous (female-to-male) hermaphrodite, the endemic New Zealand spotty wrasse *(Notolabrus celidotus)* (Figure 1)

Figure 1. Specimens of female New Zealand spotty (A) and male New Zealand spotty wrasse (B). Photographs: Dr Malcom Francis.

Previous research revealed that protogynous sex change in spotties follows social cues: the removal of the dominant male from a social group leads to the sex change of the dominant female in the group (unpublished). To fulfill my aim, I designed the following pilot experiment: I wanted to test if, by implanting cortisol pellets (Figure 2) into female spotties, this could induce sex change under non-permissive conditions (the presence of at least one male).

During the first stages of my project, I aimed to address the following hypotheses: i) treatment with cortisol promotes sex change under inhibitory social conditions; ii) a correlation exists between the time series of all histological samples extracted during the process of sex change and the hormonal profile assays, and clear differences between control and treatments are observed; and iii) a genetic approach shows downregulation in the expression of female-pathway genes and upregulation of the male-promoting gene network across the stages of sex change.

Figure 2. Cortisol pellets with different concentrations (0, 50, 500 and 5000 μg of cortisol per pellet).

To address these objectives, high concentration cortisol pellets were implanted into female spotty wrasses to promote sex change under inhibitory conditions. Each female was monitored daily for behavioral and morphological signs of sex change. To track the interrenal hormonal changes across the process of sex change, blood plasma samples were collected on eight time-points across a 71-day period. During those samplings, brain, pituitary, liver, head kidney and gonadal tissue were also obtained to create a histological time series and to conduct transcriptome-wide expression analysis (Figure 3).

Figure 3. Alexander Goikoetxea extracting blood from a New Zealand spotty wrasse specimen (A) and experimental tank set-up (B).

Cortisol did not induce sex change in the female spotties under socially inhibitory conditions as we had anticipated. Hormone measurement of cortisol and sex steroids in blood plasma from this experiment and expression analysis of key male and female genes are underway, which should shed some light on why the experiment did not work as expected. Further experimental designs are planned for the future using a higher number of replicates, and we are exploring the possibility of making corticotropin-releasing hormone (CRH) inhibitors, a different kind of pellets that would allow us to deepen our knowledge about the role of the stress axis in fish sex change from a different perspective. We are also very interested in culturing New Zealand spotty gonads *in vitro*, as the technique of organ culture would open a whole range of possibilities by accurately modelling the functions of spotty ovaries and testes in various states and conditions.

Alexander Goikoetxea Pérez de Mendiola is a PhD candidate in the Department of Anatomy, University of Otago. His research area is fish genetics.

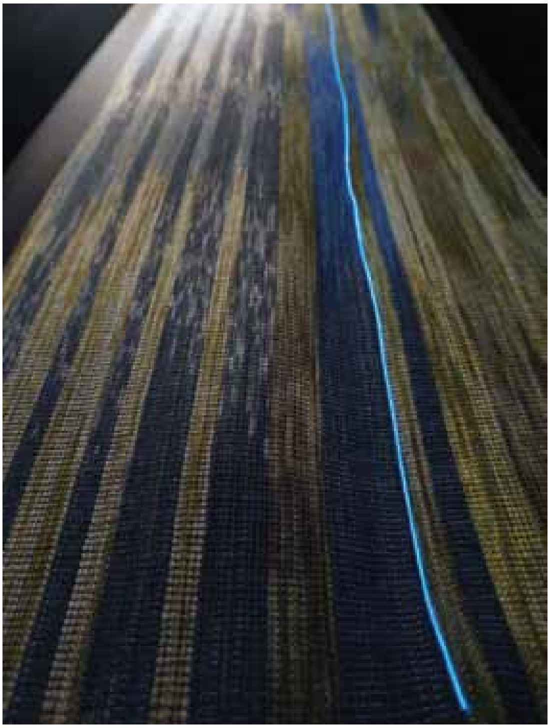
1. Text adapted from A Goikoetxea, EV Todd and NJ Gemmell, "Stress and Sex: Does Cortisol Mediate Sex Change in Fish?" *Reproduction*, 154:6 (2017), R149-R160.
2. DR Robertson and JH Choat, "Protogynous Hermaphroditism and Social Systems in Labrid Fish," in *Proceedings of the Second International Coral Reef Symposium*, 1, eds AM Cameron et al. (Brisbane: The Great Barrier Reef Committee, 1974), 217-26.
3. Y Kobayashi, Y Nagahama and M Nakamura, "Diversity and Plasticity of Sex Determination and Differentiation in Fishes," *Sexual Development*, 7:1-3 (2013), 115-25.
4. Reviewed by D Wilhelm and P Koopman, "The Makings Of Maleness: Towards an Integrated View of Male Sexual Development," *Nat Rev Genet*, 7:8 (2006), 620-31; CK Matson and D Zarkower, "Sex and the Singular DM Domain: Insights into Sexual Regulation, Evolution and Plasticity," *Nature Reviews Genetics*, 13:3 (2012), 163-74; A Herpin and M Schartl, "Plasticity of Gene-Regulatory Networks Controlling Sex Determination: Of Masters, Slaves, Usual Suspects, Newcomers, And Usurpators," *Embo Reports*, 16:10 (2015), 1260-74.
5. TK Solomon-Lane, EJ Crespi and MS Grober, "Stress and Serial Adult Metamorphosis: Multiple Roles for the Stress Axis in Socially Regulated Sex Change," *Front Neurosci*, 7 (2013), 210; R Nozu and M Nakamura, "Cortisol Administration Induces Sex Change from Ovary to Testis in the Protogynous Wrasse, Halichoeres trimaculatus," *Sex Dev*, 9:2 (2015), 118-24.
6. RH Devlin and Y Nagahama, "Sex Determination and Sex Differentiation in Fish: An Overview of Genetic, Physiological, and Environmental Influences," *Aquaculture*, 208:3-4 (2002), 191-364.
7. Robertson and Choat, "Protogynous Hermaphroditism;" DY Shapiro, "Social Behavior, Group Structure, and the Control of Sex Reversal in Hermaphroditic Fish," *Advances in the Study of Behavior*, 10 (1979), 43-102.
8. PL Munday, PM Buston and RR Warner, "Diversity and Flexibility of Sex-Change Strategies in Animals," *Trends in Ecology & Evolution*, 21:2 (2006), 89-95; JC Avise and JE Mank, "Evolutionary Perspectives on Hermaphroditism in Fishes," *Sexual Development*, 3:2-3 (2009), 152-63; J Godwin, "Social Determination of Sex in Reef Fishes," *Semin Cell Dev Biol*, 20:3 (2009), 264-70.
9. RR Warner, "Sex Change and the Size-advantage Model," *Trends in Ecology & Evolution*, 3:6 (1988), 133-36.
10. RR Warner, "Mating Behavior and Hermaphroditism in Coral-Reef Fishes," *American Scientist*, 72:2 (1984), 128-36; M Nakamura and Y Kobayashi, "Sex Change in Coral Reef Fish," *Fish Physiology and Biochemistry*, 31:2-3 (2005), 117-22; Godwin, "Social Determination of Sex in Reef Fishes."
11. A Frisch, "Sex-change and Gonadal Steroids in Sequentially-hermaphroditic Teleost Fish," *Reviews in Fish Biology and Fisheries*, 14:4 (2004), 481-99; AP Moczek, "Developmental Plasticity and Evolution – Quo Vadis?" *Heredity*, 115:4 (2015), 302-05.
12. A Frisch, "Sex-change and Gonadal Steroids;" A Budd, Q Banh, J Domingos and D Jerry, "Sex Control in Fish: Approaches, Challenges and Opportunities for Aquaculture," *Journal of Marine Science and Engineering*, 3:2 (2015), 329-55.
13. Frisch, "Sex-change and Gonadal Steroids."

PAM MCKINLAY

Where's the Switch?

Alex Goikoetxea's research focuses on the potential role of stress in the process of sex change in the New Zealand spotty wrasse, through histological analysis of the fish gonads (testes and ovaries) from groups of fish manipulated in captivity, and the correlation of the observed changes with the expression of particular genes. Male and female spotties are easily distinguished by colour and size. The females have an inky spot mid-flank from which the species takes their name. Males have light 'electric-blue' wavy patterns on their cheeks.[1]

Figure 1. Dyed warp and workbook used to develop dye palette. Figure 2. Weaving in progress. Figure 3. Skeins ready for first tie-offs. Figure 4 Dyeing in progress, with last tie-offs.



Figures 4 and 5. Pam McKinlay, switch 000I⏻, 2018, hand-dyed wool (multiple ikat dye baths), woven on 8-shaft table loom, EL wire. Installlation and detail with EL wire turned on.

This artwork focused its attention on the story behind the genetic assay and imagined the gene encoding positions in the hormone feedback loop for sex maintenance. In a recent genetics and art project, I learned that we have millions of cells in our bodies and each contain long lengths of DNA. Rather than the image of a rigid twisted ladder, the DNA inside of a cell looks more like a bowl of noodle soup. According to genetics researcher Julia Horsfeld, cells solve the 'packing problem' of squeezing all the DNA into a body by looping and binding. The conceptual model used to explain this involves the chromatin strand forming loops held in place by a molecule called cohesin, which acts like a climbing carabiner.[2] It guides how the chromosome folds into a compact space, but still has the ability to freely move when needed. When the 'switch' is flicked in the dominant female spotty, the genes controlling sex determination are revealed, the loops 'shift' and the genes for maleness are 'turned on.'

Warp yarns were produced using a multiple ikat dye process to tie in the 'gene positions' in the skeins of yarns. Ikat is a technique by which skeins of yarn are bound or tied and dyed before weaving. [3] It was an elaborate process to create the markings on the warps. The warp went through multiple dye baths, was dyed in sections, then rebound to either retain an under-colour or to overdye an initial white section and then dip-dyed to unify the overall schema. The colour palette was drawn from diveNZ photos of reef environments where New Zealand spotty fish are endemic. The end result of the weaving project produced a pattern very similar to DNA gel electrophoresis patterns. The textile had soft shifting colours reminiscent of light under the sea, where the colours are ever-changing, and the ikat 'blur' suggested the constant motion at a cellular level until conditions are favourable to 'flick the switch' for change.

Each pair of (vertical) warps tell the story of the genetic potential for individual fish to be both female and male at birth, with the gene positions for sex clearly marked. Reading from the left, the first two warps are 'female,' with typically male-like expression genes turned 'OFF.' The 'male' warp(s) have the same information, but is wider (to represent its dominant position in the social hierarchy of the group) and is reversed, because it has flicked 'ON' its maleness genes and undergone its complete functional gonad restructuring and the change from female to male in external appearance. To signify the transformation to male was complete, I added a strand of electroluminescent (EL) wire to the 'male' warp, running its length in 'electric blue.' He still retained the female element, but his transformation was highlighted by the electric blue line of EL wire that now pulsed down the fabric. At the right end of the piece were the 'transitional' or 'indeterminate' sex warps. These contained the same basic genetic information, but the gene positions were now less well ordered and they appeared in flux, as they are in nature with the 'initial phase' males.

Pam McKinlay has a Bachelor Arts and Diploma of Home Science (clothing/design and textile science) from the University of Otago. She works at the Dunedin School of Art and Research Office at Otago Polytechnic. Her art practice is in the sphere of Sci Art.

Photographs: Pam McKinlay.

---

1. "Notolabrus celidotus, Spotty Fish," *Marine Life Database*, http://www.marinelife.ac.nz/species/997.
2. P McKinlay, Christine Keller with Julia Horsfield, "It's Written in your DNA – Or is it?" *Junctures: The Journal for Thematic Dialogue*, 18 (2017), 82.
3. Ikat is derived from the Malayan word *mengikat*, which means "to bind, knot or wind round." Ikat is produced in many traditional textile centres around the world, from India to Central Asia, Southeast Asia, Japan (where it is called *kasuri*), Africa and Latin America.