Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Neurobiology

Timing reproduction in teleost fish: cues and mechanisms

Scott A Juntti and Russell D Fernald

Fish comprise half of extant vertebrate species and use a rich variety of reproductive strategies that have yielded insights into the basic mechanisms that evolved for sex. To maximize the chances of fertilization and survival of offspring, fish species time reproduction to occur at optimal times. For years, ethologists have performed painstaking experiments to identify sensory inputs and behavioral outputs of the brain during mating. Here we review known mechanisms that generate sexual behavior, focusing on the factors that govern the timing of these displays. The development of new technologies, including high-throughput sequencing and genome engineering, has the potential to provide novel insights into how the vertebrate brain consummates mating at the appropriate time.

Address
Department of Biology, Stanford University, Stanford, CA 94305, USA

Corresponding authors: Juntti, Scott A (sjuntti@stanford.edu) and Fernald, Russell D (rfernald@stanford.edu)

Current Opinion in Neurobiology 2016, **38**:57–62

This review comes from a themed issue on **Neurobiology of sex**

Edited by **Barry Dickson** and **Catherine Dulac**

http://dx.doi.org/10.1016/j.conb.2016.02.006

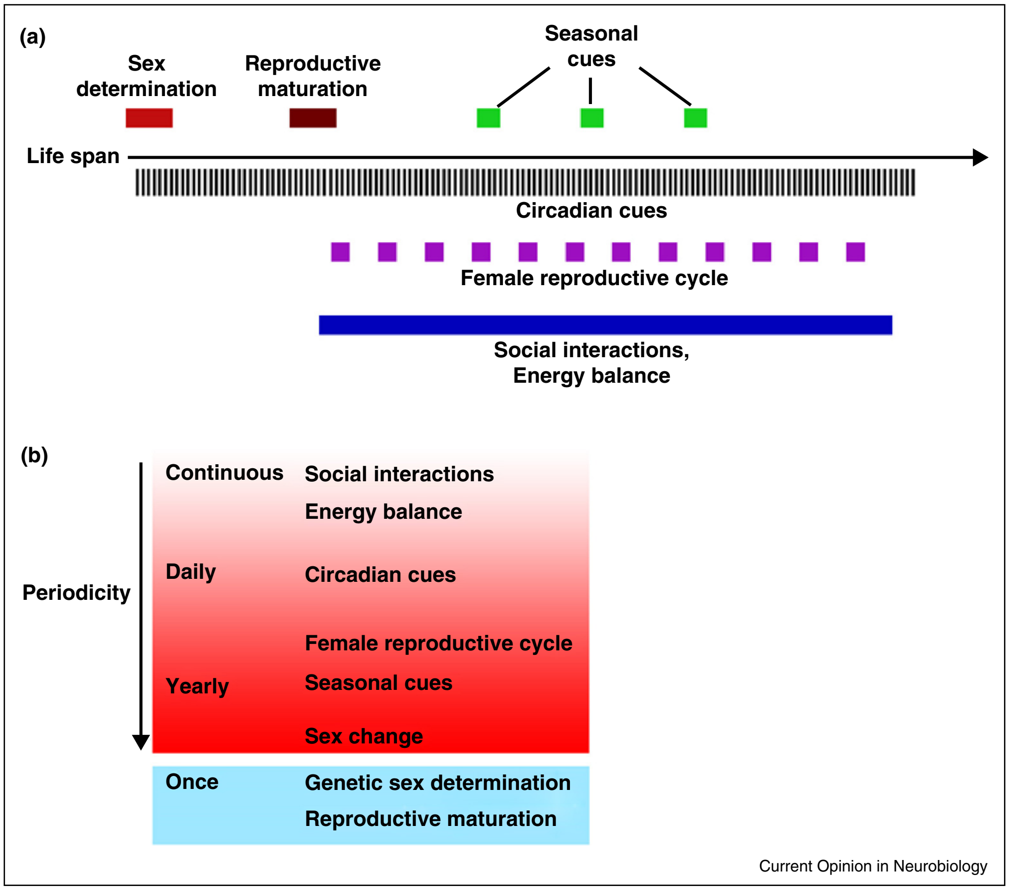
All animals face the critical challenge of deciding when to mate, to maximize their lifetime reproductive success. The factors that regulate the timing of mating can be divided into two groups, which we will call 'chronological' and 'continuous' (Figure 1). Chronological factors promote reproduction in specific, restricted time windows, while continuous factors may affect reproduction at any time. Among chronological factors are processes such as sexual differentiation and puberty, as well as environmental influences like seasonal and circadian cues. Continuous challenges that animals face include finding food, avoiding danger, and struggling for social dominance. Negative outcomes of these challenges may result in hunger, stress, or social subordination, which can prevent reproduction. In this review, we highlight some of the factors that induce reproductive behavior in the fish, and what is known about the physiological mechanisms responsible.

Chronological factors that control reproduction

Sex determination in fish occurs early in embryogenesis, and may be initiated by either chromosomes or environmental influences [1]. Unlike most mammals, which carry a single gene, *Sry*, that determines sex, fish species may utilize multiple different genes. In some species, a single gene appears sufficient to specify sex [2], while other species carry multiple alleles that interact to determine sex [3]. Despite the wide range of signals that may initiate male or female differentiation, these pathways ultimately converge on a core network of genes that is conserved across vertebrates, and some of the sex-determining genes are themselves known components of the core in other animals [2]. As a result of sex determination, the gonads differentiate into testes or ovaries, and the hormones they produce (i.e. androgens, estrogens, and progestins) shape the neural circuits that control sexual behavior in parallel with effects throughout the body. These effects may in fact be more extensive than those in mammals, since treating some species of fish with gonadal hormones results in a fully sex-reversed — and fertile — adult [4]. Many of these effects are likely mediated through sex steroid receptors that are ligand-activated transcription factors. The neural changes effected by these hormones are not well understood in fish, but may include changes in cell number, connectivity, gene expression, and neural activity patterns. Unlike mammals, fish sex is not always fixed for life. Some species of fish also change sex, a process regulated by the social environment discussed in more detail below.

Factors that influence fish reproduction act on many timescales, from daily to once in a lifetime (Figure 1). Although the sex of most fish species is set early in development, there is a period of sexual quiescence as juveniles grow. Like puberty in mammals, fish begin to exhibit sexual behavior when they have reached a size sufficient to fend off rivals and attract a mate, and/or care for young. Timing of sexual maturity can be advanced or delayed by factors including energy balance and the composition of the social environment. One of the most dramatic examples of the timing of reproduction occurs in salmon (*Salmo salmo*). These fish hatch in rivers, then move to the ocean for years while they grow to reproductive maturity. Salmon spawn once in their lives, and will navigate vast distances to return to their spawning site.

Figure 1



Control of reproduction acts at multiple timescales. **(a)** During the lifespan of a typical fish, sex is determined early in life, and after reproductive maturation, cues with a variety of timescales may affect reproduction. For a given species, one or more of these factors may regulate the onset of sexual behavior. **(b)** Categorization of classes of regulators of reproduction by the periodicity at which they act.

There is good evidence that an inherited magnetic map guides their return from the ocean to their natal stream [5]. Upon finding a stream that matches the olfactory signature of their natal stream, salmon initiate a search for a spawning site [6,7]. Once they release their gametes, the majority of salmon die.

In contrast to the life history of salmon, other fishes exhibit asynchronous egg development, in which only a portion of eggs are laid at a given time and others remain in an immature state. This strategy occurs in fish such as medaka (*Oryzias latipes*), which can lay eggs daily. In medaka, a long light:dark ratio and warm temperatures initiate reproduction [8,9]. The light phase is also important for fish including medaka and zebrafish to determine the timing of spawning within the reproductive season [10]. In these species, ovulation occurs in the dark phase, and spawning occurs shortly after light onset (i.e. dawn). In geographical regions where environmental conditions remain relatively constant, such as tropical zones, mating may occur year-round, depending on food availability. Females of these species also tend to exhibit asynchronous egg production, but spawning in these species is still regulated by environmental cues such as solar or lunar cycles [11]. In the California grunion (*Leuresthes tenuis*), spawning occurs at high tide, which is tied to the lunar cycle [12]. Eggs are buried and fertilized in the sand and at the next high tide young are washed out to sea. The tides, controlled by the moon, also open up new territories that species such as the grass pufferfish (*Takifugu niphobles*) use for spawning twice per month [13]. During mass spawnings of this species, hundreds of fish crowd the shorelines.

Continuing factors

Fish sense and interpret specific environmental cues to reproduce at a time that has been selected to maximize the survival of offspring. Correspondingly, in some species, social interactions reinforce hierarchical status that can be a crucial factor in reproduction. In an African cichlid fish, *Astatotilapia burtoni*, males live as one of two reversible, socially controlled phenotypes: dominant (D) and non-dominant (ND) males. D males are brightly colored, aggressively defend territories and are very reproductively active [14•]. In striking contrast, ND males have a dull coloration, mimic female behavior, school with females and other ND males except when fleeing from an attacking D male. ND males attend closely to the unfolding social scene, assessing when they might be able to gain a territory by defeating a resident male. When this happens, there is typically a dramatic fight during which males engage in mouth-to-mouth biting, hitting each other with their bodies and nipping at each other's fins. If the ND male successfully displaces the resident, he rapidly turns on his bright body colors and will begin performing behaviors characteristic of dominant males [14•,15]. Over a few days, the reproductive system of the ascending male is remodeled, enhancing the reproductive capacity of the male at several levels along the hypothalamic–pituitary–gonadal (HPG) axis [16]. These changes include gonad maturation, androgen level rises, and an 8-fold increase in GnRH1 cell volume [17,18] that changes electrophysiological properties [19•]. However, as for mammals, a neural engram that registers social status is elusive, as are its inputs to the reproductive axis.

In some species where a dominant male monopolizes breeding opportunities, alternative mating tactics have evolved in which male coloration mimics that of females or non-reproductive males. These 'alternate' males will then 'sneak' fertilization opportunities by entering a spawning site with a breeding pair and release sperm during egg laying. In some such species, the choice between becoming a 'traditional' male and a 'sneaker' male appears to be influenced by the social environment [20]. In the peacock blenny (*Salaria pavo*), the outcomes of agonistic interactions among juvenile males are predictive of the decision to become a territory-holding or sneaker male [21•].

A more extreme version of alternative reproductive tactics can be found in fish that change sex, a feature that has independently evolved $\geq$7 times in fish [22]. As males and females have different reproductive potentials across life stages and environments, there is an advantage to change to the sex most advantageous for a given stage of life [23]. The social environment is the main determinant of whether a fish will change sex. For example, when a large breeding male bluehead wrasse (*Thalassoma bifasciatum*) is removed from the reef, a large female may change sex and take his place as the breeding male [24]. In other fish species, such as the anemone fish (genus *Amphiprion*), the reverse switch will occur when a breeding female is removed [25]. Thus, fish have a keen sense of their size relative to the social hierarchy and are sensitive to changes within the community.

Mechanisms that link timing cues to reproductive behaviors

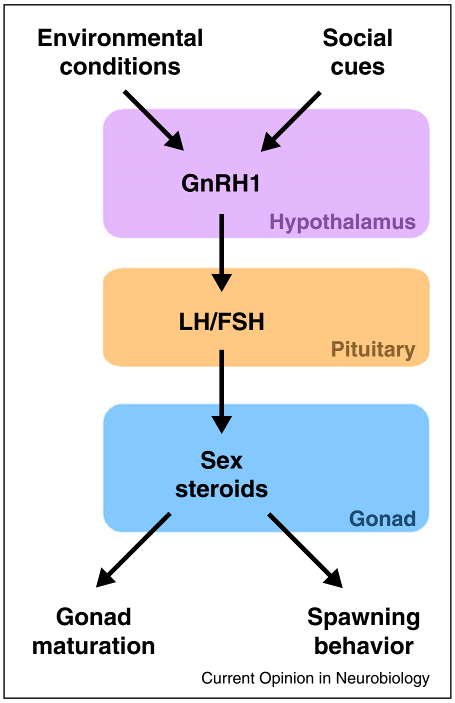
At present, there are numerous open questions about the regulation of reproduction in fish. What is the signaling pathway from social or environmental inputs to reproductive control? The gonadal hormones that sexually differentiate the brain between sexes are also commonly implicated in receiving environmental signals that promote reproductive behavior. During the breeding season, levels of androgens rise in males, and levels of estrogens and/or progestins rise in females. These hormones are under the control of the hypothalamic–pituitary–gonadal axis: at the apex, a small population of neurons in the hypothalamus expressing the decapeptide gonadotropin-releasing hormone (GnRH1) cause the pituitary to deliver the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) into the bloodstream (Figure 2). LH and FSH, in turn, stimulate the gonads to produce sex steroids, which have diverse effects on tissues throughout the body with the cumulative effect of preparing diverse tissues for reproduction [26,27]. Whereas the inputs to the GnRH1 neurons have been described in mammals [28,29•,30], these are not known in fish. Thus it is not known how the brain communicates social status to the GnRH1 neurons. It is also unclear how steroids exert their effects; they bind receptors that regulate gene expression, but it is not known for any vertebrate which genes they bind in the brain to drive reproduction.

How does the brain transduce seasonal signals to the reproductive axis? On the ventral side of the masu salmon (*Oncorhynchus masou masou*) brain, the saccus vasculosus contains cells that are light sensitive, and respond to changes in the light:dark cycle [31]. These cells initiate the thyroid hormone signaling pathway, which may result in increased GnRH1 signaling at the pituitary, as shown for birds [32].

Reproduction in the midshipman fish (*Porichthys notatus*), is also responsive to light:dark cycle. Levels of the hormone melatonin rise during the dark phase, when midshipman fish court females. In neurons that control courtship vocalizations, melatonin is important for setting the excitability of the circuit [33]. During the breeding season, androgen levels in the midshipman fish also rise in the male. These androgens and their metabolites (i.e. estrogens) also modulate the vocal circuit [34]. Gravid females respond to the courtship hum of the males, and their sensitivity to the frequency range of the hum increases during the summer when they lay eggs.

Across all vertebrates, GnRH1 neurons exhibit coordinated, pulsatile activity, which is postulated to overcome a signaling threshold in the pituitary [35•]. Gonadotropin-producing cells of the pituitary rapidly desensitize to tonic levels of GnRH1 [36••]. By firing synchronous

Figure 2



Known environmental and social cues that regulate timing of social behavior activate the hypothalamic–pituitary–gonadal axis. Through pathways that remain unclear, these cues promote the signaling of GnRH1 in the pituitary, which cause the release of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH), peptides that induce the gonads to produce steroid hormones including androgens, estrogens, and progestins. These hormones in turn act on receptors in target tissues including brain and gonads to promote pro-reproduction processes and provide negative feedback on the neurons that control their production.

action potentials at distributed intervals, GnRH1 neurons can avoid desensitization and drive the production of gonadal steroids. While this phenomenon had been first observed in mammals decades ago [36••], a mechanism for their synchronization had not been discovered. Recently, we found in cichlid fish that GnRH1 neurons are connected by electrical synapses [37], providing a mechanism by which these cells can coordinate their action potentials. Future work will determine whether gap junction among GnRH neurons is a solution for synchronization common to all vertebrates, or if cichlids have evolved a mechanism different from other vertebrate lineages.

Due to the whole-genome duplication that occurred in fish, after the split from the lineage leading to mammals, fish carry extra paralogs of many genes including three GnRH genes. In medaka, GnRH3 has been implicated in female mate choice [38]. Medaka females prefer to spawn with familiar males but ablation of GnRH3 neurons or mutation of the *Gnrh3* gene change the latency of females to spawn with an unfamiliar male. The role of GnRH2, a gene expressed in the midbrain of fish and mammals, is not established in any vertebrate. Although GnRH2 and GnRH3 could function as ligands for the GnRH receptors found in the pituitary [39], how they regulate reproduction remain unknown.

The reproductive and aggressive behaviors of male fish have been considerably better studied than that of females. While estrogen and progestin have been implicated in the control of female reproductive behavior, less is known about signaling systems in the brain. One important mediator in fish is prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which has been shown to potently activate female spawning behavior in several fish species [40,41]. Strikingly, systemic injection of $PGF_{2\alpha}$ appears to activate a pattern of behavior resembling spawning. This effect is evident even in females without eggs ready to be laid. $PGF_{2\alpha}$ is produced by the ovary around the time of ovulation [42], suggesting that this signal links the presence of fertile eggs with the neural circuits important for locating a suitable partner to fertilize them. A recent series of experiments identified a receptor for $PGF_{2\alpha}$ and used CRISPR/Cas9 (discussed below) gene editing to show that this signaling pathway is necessary for female sexual behavior in the cichlid *A. burtoni* [43]. The paper also identified four neural loci for $PGF_{2\alpha}$ signaling, indicating that these regions are central to the initiation of spawning behavior.

Since the discovery of environmental cues that lead fish to initiate reproduction, researchers have sought mechanisms through which these percepts change the brain. Despite excellent work in several model systems, fish have been traditionally neglected in research, which has led to a relative dearth of tools developed for these systems. Recent years, however, have witnessed a proliferation of approaches to identify mechanisms, including rapid and inexpensive determination of transcriptomic and genomic sequences. These approaches have the potential to enable discovery — in an unbiased manner — of gene expression changes that occur in response to environmental signals, and to identify the genomic targets to which steroid hormone receptors bind and alter gene expression. Importantly, techniques now exist to make targeted mutations and modifications in the genome, allowing a direct test of gene function. The most recent instantiation of such technologies, Crispr/Cas9, has proved inexpensive and highly efficient in numerous species, including several fishes [44••,45,46]. While previously some signaling pathways could be associated with the control of reproduction, direct tests of their roles have been difficult to perform in non-traditional model organisms.

Genes ultimately effect changes in the patterns of neural activity in discrete brain regions to drive reproductive behavior. The identity of these neurons remains shrouded, though candidates can be identified using activity responsive changes in the brain [47•,48]. Using genetic approaches, actuators of neural activity can be expressed in subsets of neurons, allowing the activation or inhibition of firing [49]. These actuators may activate or inhibit neurons using light (optogenetics) or chemicals (chemogenetics), and analysis of behavioral patterns that result allows the testing of hypotheses. Given the important role of gonadal hormones in controlling the onset of reproduction, one might hypothesize that their receptors are important for spawning. However, due to genomic duplications and the conversion of androgens to estrogens in the brain, the specific receptors that transduce the cues to reproduce are not known. Crispr/Cas9 can be used to directly test the function of these receptors to ask what subsets of neurons responsive to steroids may regulate different functions. By stimulating optogenetically, or mutating gene function in restricted subsets of cells, a more complete picture will emerge of how the brain integrates diverse cues and produces complex behaviors.

Conflict of interest statement

Nothing declared.

Acknowledgements

Supported by NIH 1F32HD071755 to S. Juntti and NIH-NS 034950 and NIGMS 101095 to RDF.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Baroiller JF, D'Cotta H, Saillant E: **Environmental effects on fish sex determination and differentiation**. *Sex Dev* 2009, **3**:118-135.
2. Kikuchi K, Hamaguchi S: **Novel sex-determining genes in fish and sex chromosome evolution**. *Dev Dyn* 2013, **242**:339-353.
3. Ser JR, Roberts RB, Kocher TD: **Multiple interacting loci control sex determination in lake Malawi cichlid fish**. *Evolution* 2010, **64**:486-501.
4. Hunter GA, Donaldson EM: **Hormonal sex control and its application to fish culture**. In *Fish Physiology.* Edited by Hoar WS, Randall DJ, Donaldson EM. Academic Press; 1983:223-303.
5. Putman NF, Scanlan MM, Billman EJ *et al.*: **An inherited magnetic map guides ocean navigation in juvenile Pacific salmon**. *Curr Biol* 2014, **24**:446-450.
6. Wisby WJ, Hasler AD: **Effect of olfactory occlusion on migrating silver salmon (*Oncorhynchus kisutch*)**. *J Fish Res Bd Can* 1954, **11**:472-478.
7. Hasler AD, Scholz AT, Goy RW: *Olfactory Imprinting and Homing in Salmon: Investigations into the Mechanism of the Imprinting Process*. Springer-Verlag; 1983.
8. Egami N: **Effect of artificial photoperiodicity on time of oviposition in the fish, *Oryzias latipes***. *Annot Zool Jpn* 1954, **27**:57-62.
9. Koger CS, Teh SJ, Hinton DE: **Variations of light and temperature regimes and resulting effects on reproductive parameters in medaka (*Oryzias latipes*)**. *Biol Reprod* 1999, **61**:1287-1293.
10. Blanco-Vives B, Sanchez-Vazquez FJ: **Synchronisation to light and feeding time of circadian rhythms of spawning and locomotor activity in zebrafish**. *Physiol Behav* 2009, **98**:268-275.
11. Taylor MH: **Lunar synchronization of fish reproduction**. *T Am Fish Soc* 1984, **113**:484-493.
12. Clark FN: **The life history of *Leuresthes tenuis*, an atherine fish with tide controlled spawning habits**. *Fish Bull* 1925, **10**:1-51.
13. Ando H, Shahjahan M, Hattori A: **Molecular neuroendocrine basis of lunar-related spawning in grass puffer**. *Gen Comp Endocrinol* 2013, **181**:211-214.
14. • Fernald RD, Hirata NR: **Field-study of *Haplochromis burtoni* — quantitative behavioral observations**. *Anim Behav* 1977, **25**:964-975.

This field study showed that male cichlid fish fall into two reversible types with differential mating opportunities. It was later shown that this system can be ported to the lab where the fish continue to show social distinctions and natural behaviors.

15. Burmeister SS, Jarvis ED, Fernald RD: **Rapid behavioral and genomic responses to social opportunity**. *PLoS Biol* 2005, **3**:e363.
16. Maruska KP: **Social regulation of reproduction in male cichlid fishes**. *Gen Comp Endocrinol* 2014, **207**:2-12.
17. Davis MR, Fernald RD: **Social control of neuronal soma size**. *J Neurobiol* 1990, **21**:1180-1188.
18. Francis RC, Soma K, Fernald RD: **Social regulation of the brain–pituitary–gonadal axis**. *Proc Natl Acad Sci U S A* 1993, **90**:7794-7798.
19. • Greenwood AK, Fernald RD: **Social regulation of the electrical properties of gonadotropin-releasing hormone neurons in a cichlid fish (*Astatotilapia burtoni*)**. *Biol Reprod* 2004, **71**:909-918.

The authors report the incredible finding that reef territories support a single breeding male wrasse, and upon his removal the largest female changes sex to take his place. This fish immediately displays male behaviors and gains fully functional testes in as little as 8 days.

20. Oliveira RF, Ros AF, Goncalves DM: **Intra-sexual variation in male reproduction in teleost fish: a comparative approach**. *Horm Behav* 2005, **48**:430-439.
21. • Fagundes T, Simoes MG, Goncalves D, Oliveira RF: **Social cues in the expression of sequential alternative reproductive tactics in young males of the peacock blenny, *Salaria pavo***. *Physiol Behav* 2012, **107**:283-291.

This study found that the social environment of juvenile blennies determines whether they will become territorial males or sneaker males.

22. de Mitcheson YS, Liu M: **Functional hermaphroditism in teleosts**. *Fish Fish* 2008, **9**:1-43.
23. Kuwamura T, Kadota T, Suzuki S: **Testing the low-density hypothesis for reversed sex change in polygynous fish: experiments in *Labroides dimidiatus***. *Sci Rep* 2014, **4**:4369.
24. Warner RR, Swearer SE: **Social-control of sex-change in the Bluehead Wrasse, *Thalassoma bifasciatum* (Pisces, Labridae)**. *Biol Bull-US* 1991, **181**:199-204.
25. Fricke H, Fricke S: **Monogamy and sex change by aggressive dominance in coral reef fish**. *Nature* 1977, **266**:830-832.
26. Forlano PM, Bass AH: **Neural and hormonal mechanisms of reproductive-related arousal in fishes**. *Horm Behav* 2011, **59**:616-629.
27. Goncalves DM, Oliveira RF: **Hormones and sexual behavior of teleost fishes**. In *Hormones and Reproduction of Vertebrates.* Edited by Norris DO., Lopez KH. Academic Press; 2011:119-147.
28. Yoon H, Enquist LW, Dulac C: **Olfactory inputs to hypothalamic neurons controlling reproduction and fertility**. *Cell* 2005, **123**:669-682.
29. • Boehm U, Zou Z, Buck LB: **Feedback loops link odor and pheromone signaling with reproduction**. *Cell* 2005, **123**:683-695.

The authors show that tissue deep below the brain contains the signal transduction components necessary to detect light. They develop an explant system that shows the system is responsive to light:dark cycle *ex vivo*.

30. Han SK, Gottsch ML, Lee KJ *et al.*: **Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty**. *J Neurosci* 2005, **25**:11349-11356.

31. Nakane Y, Ikegami K, Iigo M *et al.*: **The saccus vasculosus of fish is a sensor of seasonal changes in day length**. *Nat Commun* 2013, **4**:2108.

32. Yamamura T, Yasuo S, Hirunagi K *et al.*: **T(3) implantation mimics photoperiodically reduced encasement of nerve terminals by glial processes in the median eminence of Japanese quail**. *Cell Tissue Res* 2006, **324**:175-179.

33. Feng NY, Bass AH: **Melatonin action in a midbrain vocal–acoustic network**. *J Exp Biol* 2014, **217**:1046-1057.

34. Forlano PM, Sisneros JA, Rohmann KN, Bass AH: **Neuroendocrine control of seasonal plasticity in the auditory and vocal systems of fish**. *Front Neuroendocrinol* 2015, **37**: 129-145.

35. Moenter SM: **Identified GnRH neuron electrophysiology:**
• **a decade of study**. *Brain Res* 2010, **1364**:10-24.

This study shows that GnRH1 neurons are interconnected by electrical synapses, providing a possible mechanism for the synchronization of activity across these cells.

36. Belchetz PE, Plant TM, Nakai Y *et al.*: **Hypophysial responses to**
•• **continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone**. *Science* 1978, **202**:631-633.

37. Ma Y, Juntti SA, Hu CK *et al.*: **Electrical synapses connect a network of gonadotropin releasing hormone neurons in a cichlid fish**. *Proc Natl Acad Sci U S A* 2015, **112**:3805-3810.

The function of GnRH3 was previously not well characterized. The authors use several technologies including behavioral assays, mutagenesis, and cell ablation to suggest a role for this peptide in social behaviors.

38. Okuyama T, Yokoi S, Abe H *et al.*: **A neural mechanism underlying mating preferences for familiar individuals in medaka fish**. *Science* 2014, **343**:91-94.

39. Millar RP, Lu ZL, Pawson AJ *et al.*: **Gonadotropin-releasing hormone receptors**. *Endocrine Rev* 2004, **25**:235-275.

40. Kidd MR, Dijkstra PD, Alcott C *et al.*: **Prostaglandin F2 alpha facilitates female mating behavior based on male performance**. *Behav Ecol Sociobiol* 2013, **67**:1307-1315.

41. Stacey NE: **Effects of indomethacin and prostaglandins on the spawning behaviour of female goldfish**. *Prostaglandins* 1976, **12**:113-126.

42. Goetz FW, Cetta F: **Ovarian and plasma PGE and PGF levels in naturally ovulating brook trout (*Salvelinus fontinalis*) and the effects of indomethacin on prostaglandin levels**. *Prostaglandins* 1983, **26**:387-395.

43. Juntti SA, Hilliard AT, Kent K, Kumar A, Nguyen A, Jimenez MA, Loveland JL, Mourrain P, Fernald RD: **A neural basis for control of cichlid female reproductive behavior by prostaglandin F2α**. *Curr Biol* (in press) http://dx.doi.org/10.1016/j.cub.2016.01.067

44. Hwang WY, Fu YF, Reyon D *et al.*: **Efficient genome editing in**
•• **zebrafish using a CRISPR-Cas system**. *Nat Biotechnol* 2013, **31**:227-229.

This was the first study to report CRISPR/Cas9-mediated genome editing in a teleost fish species. This technology is highly efficient and inexpensive.

45. Li M, Yang H, Zhao J *et al.*: **Efficient and heritable gene targeting in tilapia by CRISPR/Cas9**. *Genetics* 2014, **197**:591-599.

46. Ansai S, Kinoshita M: **Targeted mutagenesis using CRISPR/Cas system in medaka**. *Biol Open* 2014, **3**:362-371.

47. Knight ZA, Tan K, Birsoy K *et al.*: **Molecular profiling of activated**
• **neurons by phosphorylated ribosome capture**. *Cell* 2012, **151**:1126-1137.

The authors identify a post-translational ribosome modification that results from neural activity. They show that immunoprecipitation of ribosomes tagged with these epitopes enables the transcriptional profiling of active neurons. This system can be used to discover genes enriched in behaviorally relevant cell populations.

48. Flavell SW, Greenberg ME: **Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system**. *Annu Rev Neurosci* 2008, **31**:563-590.

49. Rogan SC, Roth BL: **Remote control of neuronal signaling**. *Pharmacol Rev* 2011, **63**:291-315.