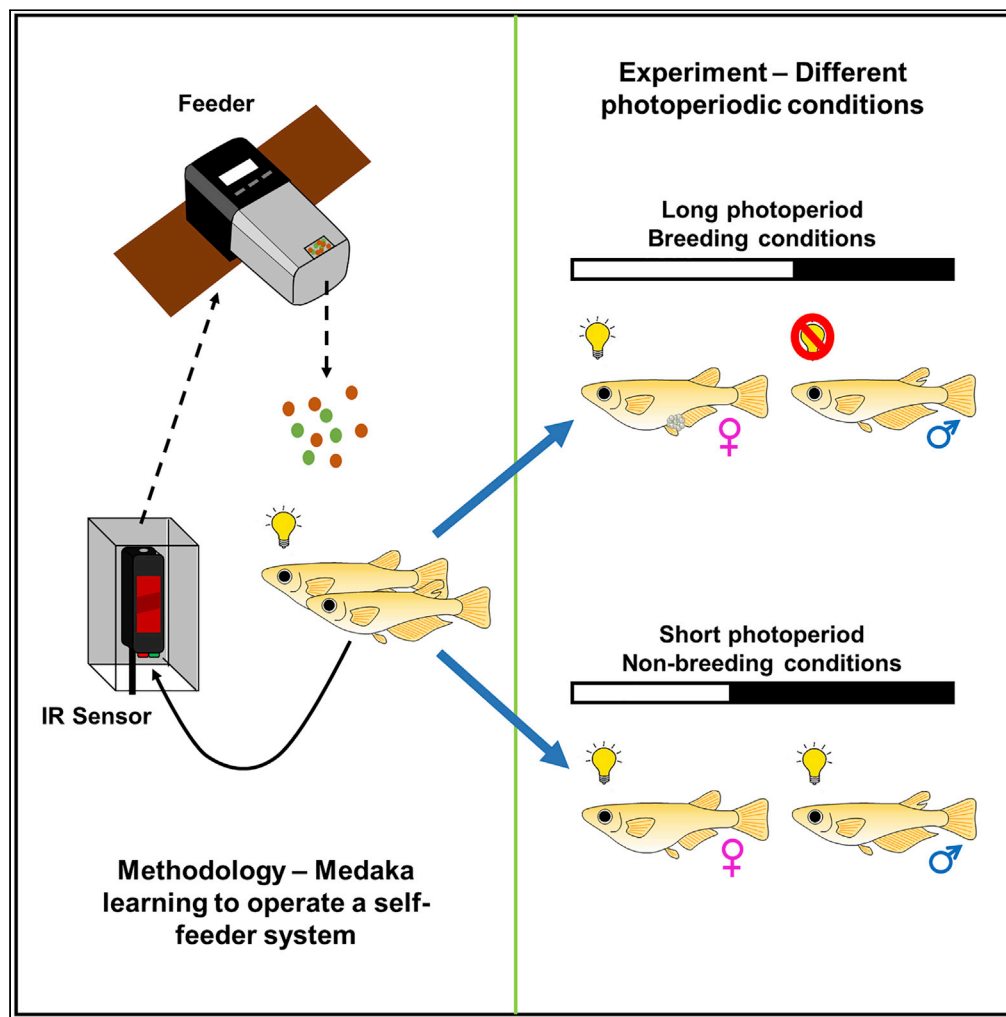


Article

Long photoperiod impairs learning in male but not female medaka



Jose Fernando López-Olmeda, Haiyu Zhao, Markus Reischl, Christian Pylatiuk, Tyrone Lucon-Xiccato, Felix Loosli, Nicholas S. Foulkes

felix.loosli@kit.edu (F.L.)
nicholas.foulkes@kit.edu (N.S.F.)

Highlights

Fish can learn by association using a self-feeder system and food reward

Medaka show sex-specific and seasonal differences in cognitive performance

On long days, sexually active male medaka are poor learners compared with females

Under short days, non-mating male medaka learn better

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Article

Long photoperiod impairs learning in male but not female medaka

Jose Fernando López-Olmeda,^{1,2,6} Haiyu Zhao,^{1,5,6} Markus Reischl,³ Christian Pylatiuk,³ Tyrone Lucon-Xiccato,⁴ Felix Loosli,^{1,*} and Nicholas S. Foulkes^{1,7,*}

SUMMARY

Day length in conjunction with seasonal cycles affects many aspects of animal biology. We have studied photoperiod-dependent alterations of complex behavior in the teleost, medaka (*Oryzias latipes*), a photoperiodic breeder, in a learning paradigm whereby fish have to activate a sensor to obtain a food reward. Medaka were tested under a long (14:10 LD) and short (10:14 LD) photoperiod in three different groups: mixed-sex, all-males, and all-females. Under long photoperiod, medaka mixed-sex groups learned rapidly with a stable response. Unexpectedly, males-only groups showed a strong learning deficit, whereas females-only groups performed efficiently. In mixed-sex groups, female individuals drove group learning, whereas males apparently prioritized mating over feeding behavior resulting in strongly reduced learning performance. Under short photoperiod, where medaka do not mate, male performance improved to a level similar to that of females. Thus, photoperiod has sex-specific effects on the learning performance of a seasonal vertebrate.

INTRODUCTION

Living organisms are faced with periodic, seasonal oscillations in their environments, which have a profound effect on many aspects of their biology. In particular, photoperiod (day length) often serves as a cue to synchronize various physiological and behavioral adaptations (Hazlerigg and Loudon, 2008; Wood and Loudon, 2014). These include changes in reproductive activity, hibernation, and migration (Chemineau et al., 2007; Dunlap et al., 2004), which represent key strategies to survive the annual seasonal cycle. Although the influence of photoperiod on endocrine and reproductive system function has received considerable attention, related alterations in complex behavior remain poorly understood. Flexibility in feeding behavior likely represents a key adaptation to survive seasonal changes in food availability. Indeed, for many species the abundance, type, and location of prey vary considerably according to the seasons (Djurhuus et al., 2020; Nunn et al., 2012). Learning allows animals to flexibly modulate feeding behavior and adapt to their current situation (Kieffer and Colgan, 1992). Animals may display seasonal differences in learning and cognitive abilities, which can also be influenced by the sex of the individuals (Carbia and Brown, 2020).

In the last decade, the importance of teleost fish for research on cognition and complex behaviors has grown substantially (Brown et al., 2011; Vila Pouca and Brown, 2017). Given their phylogenetic position, teleost fish can provide important insight into vertebrate behavioral evolution (Bshary and Brown, 2014; Lucon-Xiccato and Bisazza, 2017). Furthermore, for some teleost species, the wide range of genetic tools available (Kirchmaier et al., 2015) in conjunction with the high degree of genetic homology with humans (Howe et al., 2013; Kinoshita et al., 2012) enable comparative research on normal and dysfunctional behavior (Lin et al., 2016; Signore et al., 2009). A paradigm that is often used to study learning ability is associative learning using food rewards. In fish, one approach to study feeding behavior and learning is the use of self-feeders (da Silva et al., 2016). Fish are able to activate self-feeders through a “trigger” sensor placed in the water, a learning process based on operant conditioning associated with a food reward (Benhaïm et al., 2017; da Silva et al., 2016). Self-feeders have successfully been used with several fish species mainly to study daily rhythms of feeding behavior and nutrient selection (da Silva et al., 2016; del Pozo et al., 2011). When fish are allowed to self-feed, they display a daily rhythm of behavior entrained by the light/dark (LD) cycle, with a preference for activity during either the light or the dark phase. In most cases this diurnal versus nocturnal feeding behavior is species specific (López-Olmeda, 2017). However, some species can switch

¹Institute of Biological and Chemical Systems, Biological Information Processing (IBCS-BIP), Karlsruhe Institute of Technology, Hermann-von-Helmholtz Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

²Department of Physiology, Faculty of Biology, University of Murcia, 30100 Murcia, Spain

³Institute for Applied Computer Science, Karlsruhe Institute of Technology, Hermann-von-Helmholtz Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

⁴Department of Life Sciences and Biotechnology, University of Ferrara, Via Luigi Borsari 46, 44121 Ferrara, Italy

⁵School of Life Sciences, Lanzhou University, No.222 South Tianshui Road, 730000 Lanzhou, PR China

⁶These authors contributed equally

⁷Lead contact

*Correspondence: felix.loosli@kit.edu (F.L.), nicholas.foulkes@kit.edu (N.S.F.)

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the phase of their feeding behavior over the course of their lifespan, a phenomenon called dualism (Sánchez-Vázquez et al., 1998). In addition, self-feeders have been applied to research on social interactions and learning (Benhaïm et al., 2017; Millot and Bégout, 2009).

We have focused on the medaka (*Oryzias latipes*), a teleost species that represents a well-established vertebrate genetic model (Takeda and Shimada, 2010) and in which photoperiod has a profound effect on physiology and behavior (Koger et al., 1999; Takeda and Shimada, 2010). In contrast to other vertebrate photoperiodic models (Chemineau et al., 2007; Dardente et al., 2010), its economic husbandry, short generation time, and high fecundity render medaka a particularly attractive vertebrate model to study photoperiodism. Medaka is a seasonal breeder with a marked photoperiodic control of reproduction. It requires a long photoperiod for reproductive activity with reducing daylength resulting in behavioral and metabolic changes that culminate in gonadal hypotrophy and the absence of mating (Koger et al., 1999).

We studied the behavior of groups of medaka in a learning paradigm whereby they have to activate a sensor to obtain a food reward (Pylatiuk et al., 2019). Fish were tested under either long (14 h Light:10 h Dark, 14:10 LD) or short (10 h Light:14 h Dark, 10:14 LD) photoperiod conditions that affect reproductive behavior in medaka (Kobayashi et al., 2012; Ono and Uematsu, 1968). We specifically examined whether male and female medaka perform equally well under different photoperiodic conditions in our learning paradigm.

RESULTS

Food-on-demand paradigm

We developed a food-on-demand paradigm where medaka can activate a food dispenser by swimming into a specific sector of the tank (Pylatiuk et al., 2019). Each aquarium was equipped with a centrally located infrared photocell that recorded locomotor activity and a second photocell located in a top corner that, upon activation, triggered the food dispenser and recorded the food-demanding activity (Figure 1A, Video S1). This setup allowed fish to choose when and how much food was delivered once they associated swimming into this sector with the food reward.

Daily locomotor and feeding patterns of medaka

We used this setup for the continuous, long-term analysis of mixed groups of medaka, consisting of seven males and seven females, under standard conditions associated with normal reproductive activity (26°C, 14:10 LD; Figure S1). We observed distinct diurnal patterns of both locomotor and feeding activity (Figure 1B). Specifically, a characteristic diurnal pattern of locomotor behavior was observed with a gradual increase in daily activity beginning 2 h before lights on. Feeder activations were scarce during the first 4 days, whereas after 5.1 ± 1.8 days medaka groups showed a significant increase of diurnal feeder activation (Figure 1B).

Two-sensor test shows associative learning

To test whether the increase in feeder activation was random or due to learning, we offered the fish a second, identical dummy sensor, which was not connected to the feeder (Figure 1A). At first, naive fish visited both sensors indiscriminately with equal frequencies but the preference for the feeder-activating sensor started to increase from day 2 onward (Figure 1C). Following day 15, fish displayed a stable and statistically significant preference for the feeder-activating sensor, largely ignoring the dummy (t test, $p = 0.031$). At day 21, the connection was reversed, such that the feeder-activating sensor became the dummy and vice versa. On the second day following this contingency reversal, the distribution of visits to the two sensors switched, and from the fifth day onward the fish displayed a significant preference for visits to the new feeder-activating sensor (t test, $p = 0.0013$) (Figure 1C). This result reveals that visits to the feeder-activating sensor were not random but directly correlated with fish receiving a food reward. We therefore conclude that fish learned the novel feeding behavior and flexibly adjusted it based on changes in reward contingency. This is consistent with reports revealing clear associative learning and reversal learning capacities, including the use of self-feeding systems, in other teleost species (Benhaïm et al., 2017; Brown et al., 2011; del Pozo et al., 2011; Laland et al., 2003; Lucon-Xiccato and Bisazza, 2014; Wodinsky and Bitterman, 1957).

Sex difference in learning

To address sex differences in learning, we recorded male and female fish separately under standard conditions (group size 14 fish, 26°C, 14:10 LD). Female-only groups showed strictly diurnal locomotor and

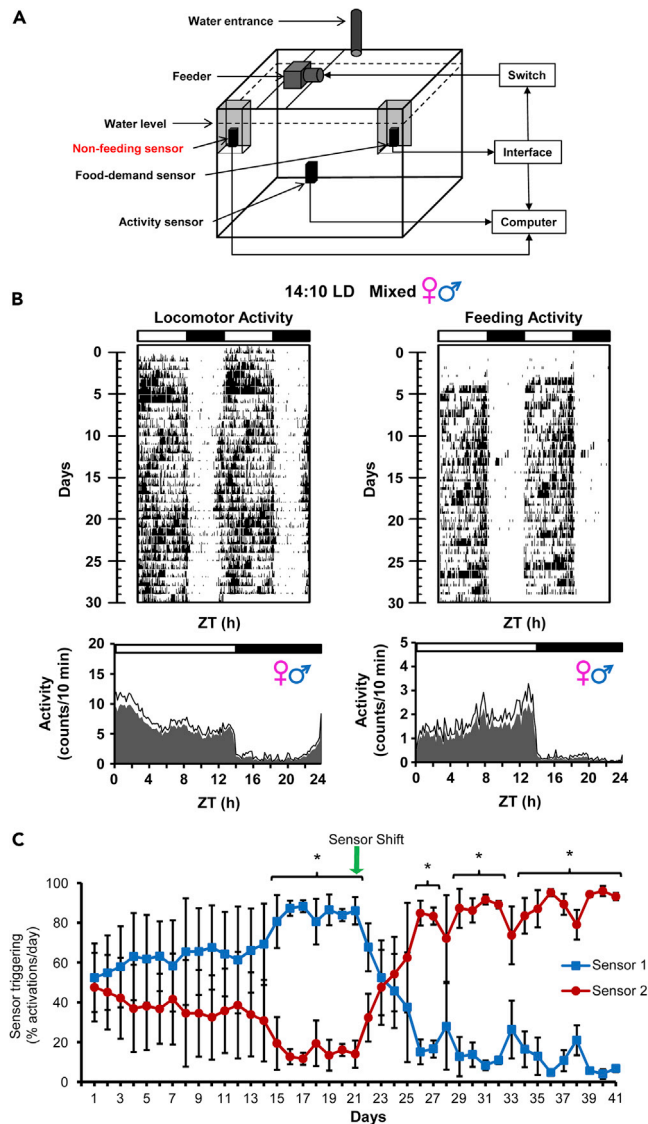


Figure 1. Learning of novel feeding behavior by medaka

(A) Diagram of experimental setup to detect locomotor and feeding activities. Each aquarium was equipped with a centrally located infrared photocell with a large motion detection range to record locomotor activity. A food-demand sensor was located at one upper corner of the aquarium. Its activation triggered the feeder to release food. To prevent random sensor activation, the sector for activation was enclosed by an alcove accessible only from below. The scheme shows the two-sensor setup with the dummy sensor indicated in red.

(B) Actograms and mean waves of mixed medaka groups. Representative actograms of locomotor (left panel) and self-feeding (right panel) activities of a group of seven males and seven females during a 1-month self-feeding experiment. Actograms were double plotted (timescale, 48 h) for better visualization, the height of each point representing the number of sensor activations/10 min. The light and dark phases are indicated by white and black bars (x axis top, 14:10 LD). Number of days is indicated on the y axis. Below each actogram is the average mean wave of the locomotor and feeding activity during the recording period, calculated as mean \pm SD using the data from all experimental groups (n = 8, data were obtained from two independent trials). The vertical axis shows activity (interruptions/10 min) and the horizontal axis zeitgeber time (h). The gray area represents the mean values and the continuous line the SD.

(C) Two-sensor learning test. As illustrated in A, two infrared photocells were installed simultaneously at both top front corners. Initially sensor 1 activated the feeder, whereas sensor 2 served as the dummy. Time points of connection reversal are indicated by a green arrow (sensor 2 activates feeder and sensor 1 is the dummy). Percentages of activations of sensor 1 and sensor 2 on each day were presented as the mean \pm SEM and were compared using Student's t test (*P < 0.05) (n = 5, data obtained from two independent experiments).

See also [Figures S1](#) and [S2](#).

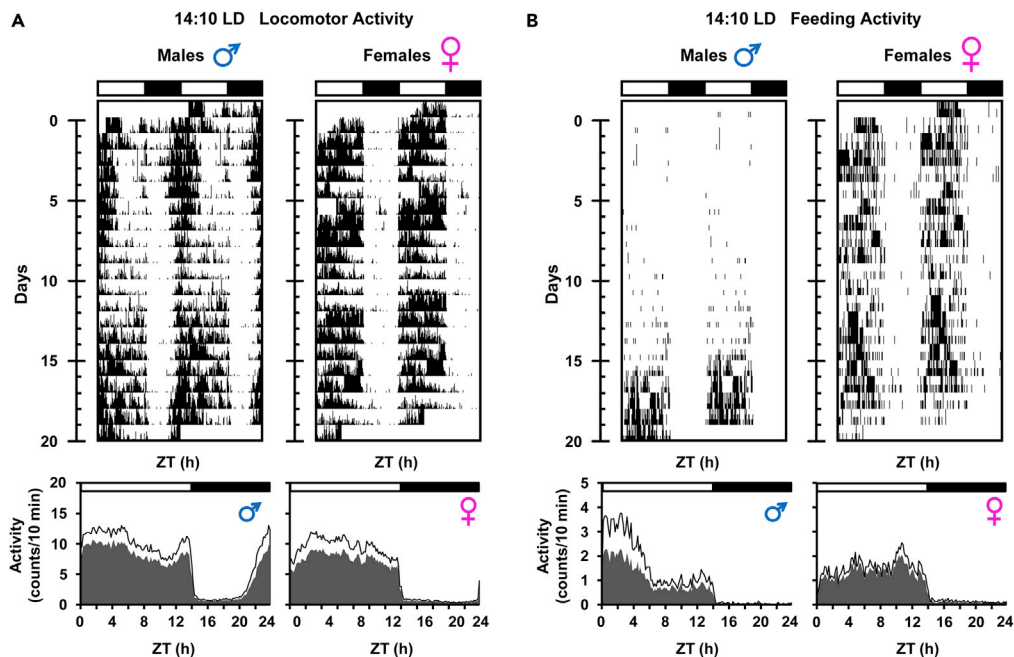


Figure 2. Sex differences of locomotor and feeding activity

Representative actograms and mean waves of locomotor (A) and self-feeding (B) activities of separated females-only and males-only groups during a 20-day recording period. The female-only and male-only groups showed similar diurnal locomotion, with the exception that male-only groups exhibited strong anticipatory activity 2 to 4 h before the onset of the light period, whereas females showed a strict diurnal pattern (A). Female-only groups showed robust diurnal self-feeding around day 3, whereas male-only groups started to show feeder activation around day 18; the feeding frequency was significantly lower in male-only groups than in female-only groups (B). Representative actograms belonging to one of the experimental groups, whereas the mean waves were calculated using the data from all replicates ($n = 6$, data were obtained from two independent experiments). Actograms and mean waves including mean values and SD are represented as described in Figure 1B.

See also Figures S1 and S2.

feeding patterns (Figures 2 and S1), whereas male-only groups displayed a distinct peak of locomotor activity preceding the dark-light transition (Figure 2A). It is surprising that the number of feeder activations of male-only groups was considerably lower than that of the female-only or mixed groups (Figure 2B). The overall activity of male-only groups was not decreased, indicating that the lower frequency of feeder activation was unlikely to be the result of a generally reduced locomotor activity (Figure S2). The learning time of males to operate the self-feeding device was significantly longer than that of females (12.8 ± 2.5 days and 3.8 ± 1.1 days, respectively) and that of the mixed-sex groups (5.1 ± 1.8 days) (one-way ANOVA, $p = 0.029$) (Figure 4). No significant differences were observed between female-only and mixed-sex groups. Thus, male-only groups showed slower learning and overall lower feeder activation than the other groups.

In nature, medaka males and females cohabit, and so we analyzed whether females also performed better in the mixed groups at 14:10 LD. Sexual dimorphisms of medaka allow the unambiguous identification of sexually mature males and females (Egami, 1959). We next used visual inspection combined with video monitoring data to quantify male and female visits to the activation sensor in mixed groups maintained under a 14:10 LD photoperiod (Video S1). Under these conditions, nearly all the feeder activations were made by females ($93.25\% \pm 5.50\%$) (Student's *t* test, $p = 4.271 \times 10^{-5}$). This shows that irrespective of group composition, males exhibited a much lower frequency of feeder activation. Thus, our results point to a perturbed learning performance of males under long photoperiod conditions.

Photoperiod length affects male learning

Given the sex-specific difference in learning and the known strong effects of photoperiod on reproductive activity and physiology in this species, we next tested whether day length might also influence male

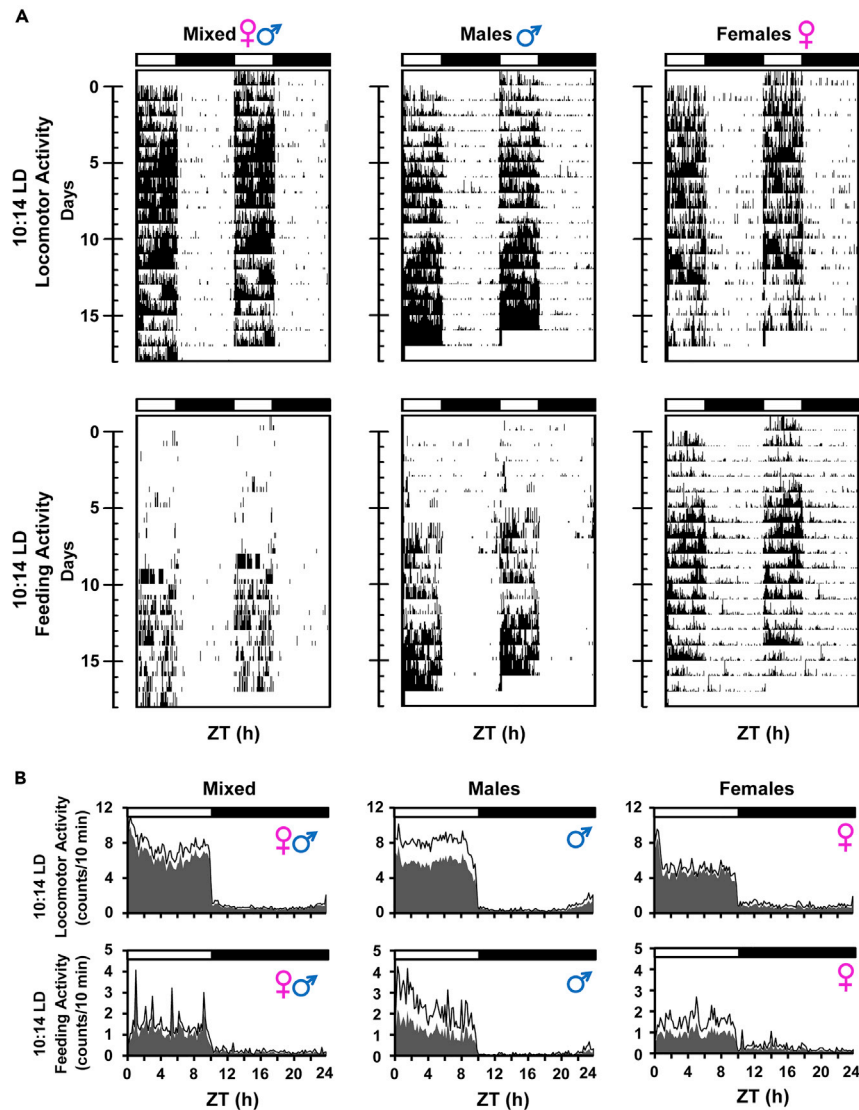


Figure 3. Locomotor and feeding activity under non-mating conditions

Representative actograms from one experimental group (A) and mean waves (B) of locomotor and self-feeding activities, calculated using data from all experimental replicates, of mixed (left panels), separated male-only (middle panels) and female-only (right panels) groups during an 18-day recording period. Locomotor activities of all groups exhibited a strict diurnal pattern without anticipation before the onset of the light period. All groups showed very similar diurnal self-feeding activities, with no significant difference in terms of learning time ($n = 5$, data were obtained from two independent experiments). Actograms and mean waves including mean values and SD are represented as described in Figure 1B. See also Figures S1 and S2.

cognitive performance. We therefore tested naive males and females in a short photoperiod (10:14 LD; Figure S1). We initially confirmed adaptation of the fish to short photoperiod conditions by the observation that none of the female fish possessed fertilized eggs, indicating absence of mating (Koger et al., 1999). We then recorded the locomotor and self-feeding activity of male-only, female-only, and mixed groups under these conditions. Analogous to the long photoperiod, all groups under a 10:14 LD short photoperiod showed diurnal locomotor activity. However, the mixed and male-only groups lacked the increase in locomotor activity, which preceded lights on under 14:10 LD conditions (Figure 3 and left panels in Figures 1B and 2A, respectively). In addition, medaka reared under a 10:14 LD photoperiod showed levels of locomotor and self-feeding activity during the light phase that were comparable with those of medaka maintained under 14:10 LD conditions (t test, $p > 0.05$) (Figure S2). Of importance, all groups including the male-only

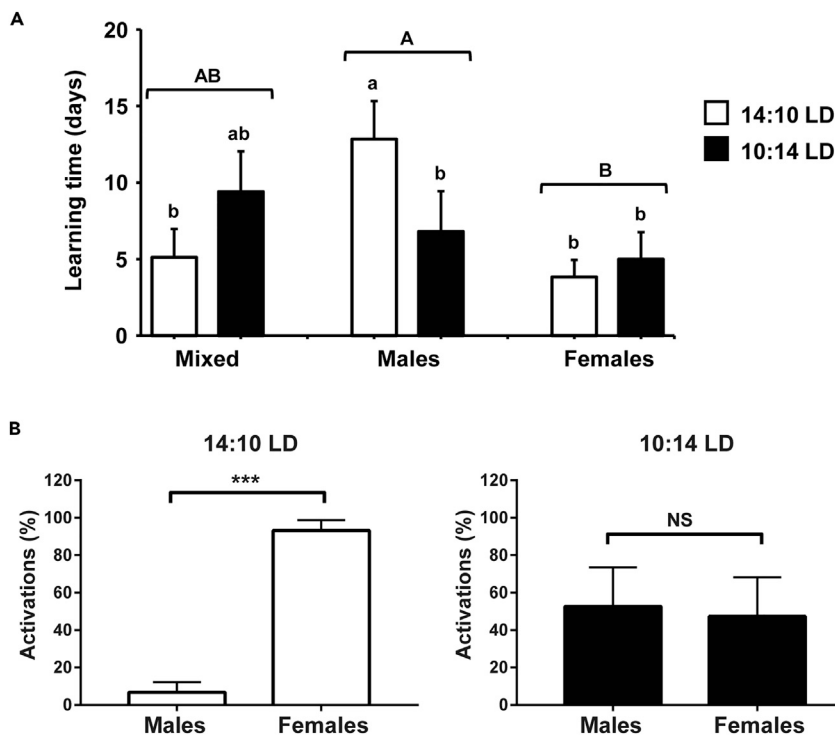


Figure 4. Photoperiod influences associative learning and self-feeding activity

(A) Learning time analysis. Learning time for association of mixed, female-only, and male-only groups in 14:10 (white bars) and 10:14 LD conditions (black bars) were calculated based on the raw numbers of total daily self-feeding activity, using a Heaviside step function, and represented as mean \pm SD ($n = 8$ for mixed-sex at 14:10 LD; $n = 6$ for male-only and female-only at 14:10 LD, and for mixed-sex at 10:14 LD; and $n = 5$ for male-only and female-only at 10:14 LD, data were obtained from two independent experiments; see Figure S1 for further details). Different lowercase letters indicate significant differences between the experimental groups (one-way ANOVA, $p = 0.029$). Different uppercase letters indicate significant differences between sexes (two-way ANOVA, $p = 0.047$). As the interaction between sex and photoperiod was significant (two-way ANOVA, $p = 0.040$), differences found between sexes depended on photoperiod. No significant differences were found between photoperiods (two-way ANOVA, $p = 0.83$).

(B) Visual inspection of feeder activation under 14:10 (left) and 10:14 LD conditions (right). Three aquaria with seven males and seven females were observed at three time points each day, i.e., morning, noon, and afternoon, for at least 3 days, each observation lasting for 1 h. Three independent experiments were used for statistical analysis. Male activation and female activation percentages, represented as mean \pm SD, were compared using Student's *t* test; statistical differences of $p < 0.05$, $p < 0.01$, $p < 0.001$ are represented by *, **, or ***, respectively ($n = 6$, data were obtained from two independent experiments).

See also Figure S1.

groups showed robust diurnal feeding rhythms (Figure 3) and there was no significant difference in learning time between the male-only, female-only, and mixed-sex groups (6.8 ± 2.6 , 5.0 ± 1.8 and 9.4 ± 2.6 days, respectively) (*t* test, $p > 0.05$) (Figure 4). We next quantified how often males and females in mixed groups activated the feeder. By visual inspection we demonstrated that feeder activation by males occurred to the same extent as by females ($52.65 \pm 20.89\%$ and $47.35 \pm 20.89\%$, respectively) (binomial test, $p = 0.772$). Thus, also in the presence of females, males efficiently activated the feeder sensor such that there was no significant difference between male and female activation frequency under a short photoperiod.

DISCUSSION

By using an experimental paradigm to study self-feeding behavior, we have revealed a significant impact of seasonal environmental conditions and sex on learning performance in medaka (Figure 4). Specifically, under long photoperiod males performed poorly as measured by sensor activations and learning time, whereas under short photoperiod conditions, male performance improved, reaching a level equivalent to that of females. The sex-specific difference in learning under long photoperiod conditions was masked at the level of mixed-sex groups by the females' feeding behavior.

The influence of sex on learning has been described in diverse vertebrate species, including humans (Andreano and Cahill, 2009), rodents (Dalla and Shors, 2009; Jonasson, 2005), and teleost fishes (Carbia and Brown, 2020; Lucon-Xiccato and Bisazza, 2017). In fish, most of the sex-specific cognitive differences have been observed in tropical species that reproduce across the entire year (Lucon-Xiccato et al., 2019; Wallace et al., 2020) or potential effects of seasonality were not investigated (Keagy et al., 2019). One explanation for the sex-specific learning differences in medaka may be based on the allocation of energetic resources to reproduction. Carbia and Brown (2020) found that, in the case of the intertidal Cocos Frillgoby, *Bathygobius cocosensis*, males are less successful than females at navigating a maze during the breeding season. They suggested that the reduction in cognitive ability in turn reduces male brain metabolic costs. The liberated resources can then be allocated to reproductive activities such as nest guarding. Our data obtained under long photoperiod conditions are consistent with the presence of intense reproductive-related behaviors in medaka. Indeed, mixed-sex groups showed a characteristic increase in locomotor activity before the end of the night period, in agreement with the fact that medaka mate in the morning (Kobayashi et al., 2012; Ono and Uematsu, 1957). This increase in locomotor activity prior to lights-on was also observed in the male-only groups, where it was even more pronounced than in mixed groups, suggesting reproductive behaviors such as male-male competition or searching for mating partners. As suggested by Carbia and Brown (2020), male medaka may exhibit a shift in brain metabolic allocation resulting in the observed sex-specific learning difference.

An explanation for the observed sex-specific learning differences based on some adaptive significance may not necessarily require differential energetic allocation (Rowe and Healy, 2014). Alternatively, these learning differences may reflect the prioritizing of reproductive versus feeding behaviors in males. Male medaka reproductive behavior is rather complex and includes male-male competition (Weir, 2013; Yokoi et al., 2016), searching for and chasing females (Nakayama et al., 2004), as well as an intricate courtship display to ensure reproductive success of the male (Ono and Uematsu, 1968). Some of these behaviors, notably male-male competition, are also likely to be expressed in male-only groups, as suggested by studies with low operational sex ratios (Clark and Grant, 2010). Consistent with these observations, we have documented an increase of locomotor activity in male-only groups prior to lights-on that we interpret as mating-associated (see above). We speculate that the engagement of males in reproduction-related activity negatively impacts upon their learning performance via either reduced attention, reduced motivation to feed, or a reduction in the chances to learn because of the reduced feeding activity. In line with this explanation, in another teleost fish, the guppy (*Poecilia reticulata*), males have evolved a foraging strategy based on short feeding windows in order to prioritize courtship behavior for the remaining part of the day (Dussault and Kramer, 1981). Similar prioritization effects have also been reported for some invertebrate species (Ryan et al., 2014). In addition, female gametogenesis (oogenesis) is highly demanding in terms of energy and broodstock nutrition influences fecundity and oocyte quality (Bobe and Labbé, 2010). This could also cause greater feeding motivation in females compared with males. It cannot be excluded that, during the reproductive period, males are able to compensate for their reduced foraging activity thanks to the advantages of living as part of a group. As this species forms mixed-sex shoals, males can exploit resources identified by females as observed in many fish species, whereby uninformed individuals follow informed individuals during foraging (Reader and Laland, 2000; Reebs, 2001). Indeed, social learning of uninformed individuals using visual cues during foraging activities has been demonstrated for medaka (Ochiai et al., 2013). Thus, male medaka could scrounge food from female food-demands, freeing themselves from the cognitive burden of learning directly efficient foraging behavior and allowing them to devote more resources to mating.

A proximate mechanism for the observed sex-specific differences seems likely to include the participation of the endocrine system. In rodents, sex-specific differences in learning have been associated with reproductive status (Galea et al., 1996) and its correlated hormonal changes (Galea et al., 1995). Epinephrine, glucocorticoids, and insulin as well as sex steroid hormones have all been shown to play key roles in regulating learning and memory in rodents (Frick et al., 2015). Changes in photoperiod trigger alterations in endocrine function via the brain-pituitary-gonadal axis (Cowan et al., 2017; Dardente et al., 2016). In turn, this affects the downstream production of sex steroid hormones that influence learning and memory (Frick et al., 2015). Similarly, in mammals, hormones from the RFamide family (e.g., kisspeptins and the gonadotropin-inhibitory hormone) are modulated by photoperiod and strongly influence reproductive and feeding behavior (Bechtold and Luckman, 2007; Simonneaux et al., 2013). Therefore, it is tempting to speculate that photoperiod-dependent changes in neuroendocrine axis function are a central contributor to the observed effects of day length and sex on learning in medaka, although further research is required to elucidate this hypothesis.

Here, we demonstrate seasonal dependence of sex-specific differences in learning and that day length is an important environmental signal regulating cognitive plasticity. This finding highlights the need to consider the potentially confounding effects of light conditions and sex when analyzing learning behavior in medaka and other seasonal breeding models. Our results indicate that social mechanisms may compensate for a learning deficit of one sex. These compensatory mechanisms lead to the tolerance of a form of sexual conflict in medaka (Chapman et al., 2003) and might have provided evolutionary potential for the appearance of sex-specific differences in learning. Medaka and other teleost fish display complex social structures and skills (e.g., Cattelan et al., 2019; Schürch et al., 2010; Wang and Takeuchi, 2017), as well as remarkable sex-specific cognitive differences (reviewed in Lucon-Xiccato and Bisazza, 2017). Therefore, this taxon could play an important role in unraveling the mechanisms of cognitive evolution in males and females of social animals.

Medaka is a powerful genetic model for investigating complex disease phenotypes (Kirchmaier et al., 2015; Takeda and Shimada, 2010). Therefore, studies based on medaka are not simply relevant for understanding fish biology but may also provide important new insight to improve human health. A number of studies in humans have reported seasonal variation in nervous system function and disease susceptibility, which is often sex dependent (Amons et al., 2006; Lim et al., 2017; Rajajärvi et al., 2010). Therefore, our description of this fascinating behavioral pattern in medaka may ultimately lead to the identification of the fundamental genetic and molecular factors underlying seasonal and sex-specific differences in human cognition.

Limitations of the study

In the present research, we focused on behavioral parameters. We speculate that the effects of photoperiod on the reproductive and endocrine systems may in turn modulate learning and behavior. Thus, future research on the relationship between factors from the reproductive axis, especially hormones, and learning will provide important insight into the underlying mechanisms.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2021.102784>.

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AUTHOR CONTRIBUTIONS

The authors have made the following declarations about their contributions:

N.S.F., F.L., J.F.L.-O., and H.Z. conceived and designed the experiments. J.F.L.-O. and H.Z. performed the experiments. N.S.F., J.F.L.-O., H.Z., F.L., M.R., and T.L.-X. analyzed the data. N.S.F., F.L., C.P., and J.F.L.-O. contributed reagents/materials/analysis tools. N.S.F., F.L., H.Z., J.F.L.-O., and T.L.-X. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
Japanese Medaka (<i>Oryzias latipes</i>) iCab inbred strain	Loosli et al. (2000)	N/A
Software and algorithms		
Automated Feeding and Motion Recording Software	Pylatiuk et al. (2019)	N/A
El Temps	Prof. Díez-Noguera, University of Barcelona http://www.el-temps.com/principal.html	N/A
SPSS software v19	SPSS Inc., IBM, Chicago, IL, USA	N/A
RStudio	https://rstudio.com/	N/A
fBasics package for R	https://www.rdocumentation.org/packages/fBasics/versions/3011.87	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Nicholas S. Foulkes (nicholas.foulkes@kit.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Behavioural recordings were performed by means of custom made software, reported previously in Pylatiuk et al. (2019). This software is available upon request. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The medaka (*Oryzias latipes*) inbred strain iCab was maintained at the medaka facility of the Institute of Biological and Chemical Systems, Biological Information Processing (IBCS-BIP) (Loosli et al., 2000) and at the Faculty of Biology, University of Murcia. Animal husbandry and experimental procedures were performed in accordance with European (2010/63/EU) and local animal welfare standards (Tierschutzgesetz §11, Abs. 1, Nr. 1, AZ35-9185.64/BH, AZ 35-9185/G-179/13 for Germany; RD 53/2013 and Law 32/2007, license number A13191003 for Spain). The facilities are under the supervision of the local representatives of the animal welfare agencies.

Mixed-sex groups (1:1 sex ratio) of the isogenic inbred iCab strain were reared in 6-litre stocking tank systems with recirculating water, at a density of 1.66 fish/litre (10 fish per tank). Systems were equipped with mechanical and biological filters and the water quality was checked weekly. The water conditions were kept at pH 7 and conductivity 400 μ S/cm. Photoperiod was set at a 14:10 h light:dark (LD) cycle and the water temperature at 26°C. Fish were fed manually during the light phase, two times a day with a commercial feed (Tetramin flakes, Tetra) and once a day with live brine shrimps.

To study the influence of sex on the experimental results female only, male only and mixed (male and female) groups were tested (see [method details](#) and [Figure S1](#)).

METHOD DETAILS

All iCab fish used for the experiments were 5 months old. Medaka of the iCab strain, that were used throughout the experiments, reach sexual maturity after 2-3 months under the maintenance conditions used (14:10 LD at 26°C). Sexual maturity of the fish was confirmed by visual inspection: the presence of fertilized egg clusters attached to females after successful mating and the observation of the stereotypic mating behaviour (mating dance; Koger et al., 1999; Ono and Uematsu 1957; Ono and Uematsu 1968). iCab fish (male-, female-, or mixed- sex groups according to experimental design) that were identified as sexually mature were randomly selected from the stocking tank.

Fish were randomly assigned to the different experimental groups and photoperiods (Figure S1). Half of the medaka groups were maintained in the same conditions as during husbandry with long photoperiod (14:10 LD) and water temperature of 26°C. The remaining medaka groups were transferred to a different room with a short photoperiod set at a 10:14 LD and water temperature of 26°C. Absence of mating in the short photoperiod was verified by visual inspection (absence of mating dance and females without egg clusters). The fish were then left to acclimatize at a density of 1.4 fish/litre (14 fish per tank) for three weeks in the recording 10-litre tank before recording was initiated. During this acclimatization period, the feeding regime was the same as during the husbandry.

Recording tank set-up

Feeding and locomotor activity of fish was recorded in 10-litre tanks (tank size 26x35x15 cm, 11 cm water depth) with two infrared photocells as described (Pylatiuk et al., 2019) (Figure 1A, Video S1). The photocell used to record locomotor activity (E3S-AD62 Omron, Japan) was attached centrally to the aquarium front wall and the sensitivity adjusted to cover the entire length of the aquarium. The photocell used as a food-demand sensor was placed at one upper front corner, 2 cm below the water surface and 1 cm from the side. This photocell was connected by an interface to a commercial feeder (Eheim, Germany). To prevent random activation of the food-demand sensor, its sensitivity was adjusted to a range of 1 cm, covering an area shielded by transparent plastic and accessible only from below to ensure that fish have to swim actively inside this alcove to activate the sensor. Its activation triggered the feeder, which was adjusted to dispense about 0.002 g flakes/biomass (Tetramin flakes, Tetra). Interruption of the infrared beam by swimming fish was recorded and stored as 10 minutes bins using custom-made software (Pylatiuk et al., 2019). During the experimental phase, feeders were activated by the researchers twice every day to ensure their functionality. This also ensured that fish which had not yet learned to operate the feeder received a small amount of food. Each biological replicate (single medaka group) consisted of 14 untrained (naïve), 5 month old medaka acclimated for 3 weeks to the recording tank. After the 3-week acclimatization period, both locomotor and feeding activity were recorded from day 1 onwards in each experiment (Figure S1). Data were recorded from two independent experiments for each photoperiod (14:10 and 10:14 LD) and sex distribution (mixed, male-only and female-only). Each independent experiment had 2-4 biological replicates, which were pooled together for the statistical analyses and results, resulting in the following replicate numbers: n=8 for mixed sex at 14:10 LD; n=6 for all-males and all-females at 14:10 LD; and n=5 for all treatments at 10:14 LD and the two-sensor experiment.

QUANTIFICATION AND STATISTICAL ANALYSIS

The analysis of locomotor activity records, representation of actograms and calculations of mean waveforms were done using the chronobiology software El Temps (version 1.291, © Prof. Díez-Noguera, University of Barcelona). Statistical analyses were performed using SPSS software v19 (SPSS Inc., Chicago, IL, USA) and RStudio (<https://rstudio.com/>). p values < 0.05 were considered statistically significant. Data subjected to statistical tests were firstly checked for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). For the two-sensor experiment, data were expressed as the percentage of activations of the feeder sensor and the dummy sensor for each day within each tank. Then, statistically significant differences were assessed for each day between feeder and dummy activations by means of a two-tailed Student's t-test. For the male or female activations in the manual inspection or video recordings, data were expressed as percentages and then subjected to a two-tailed Student's t-test to check for significant differences between sexes in each photoperiod. Data from the day of learning were subjected to a one- and two-way ANOVA, followed by a Duncan's *post hoc* test, to detect significant differences between all groups (one-way ANOVA) and between sex, photoperiod and the combination of both factors (two-way ANOVA).

Learning time analysis

We defined learning time as the time taken by fish to shift from random activations to an intentional operation of the self-feeding device as a result of associative learning. In order to define this time objectively, we used a Heaviside step function (Blöchl et al., 2011). First, the total number of activations in each experimental day was calculated for each tank; then, these series of data were adjusted to a Heaviside function (fBasics package for R, <https://www.rdocumentation.org/packages/fBasics/versions/3011.87>). Learning day was defined as the day after the inflection point calculated by this function.

Visual inspection and video recording of feeding activity

We used visual inspection and camera monitoring to determine whether males or females of mixed groups triggered the sensors. After stable associative learning was achieved, visual inspection and camera monitoring were done under 14:10 and 10:14 LD conditions. Each condition was tested in two independent experiments. For each condition and experiment, three tanks (n=3) with 7 males and 7 females were observed (N=6 for each experimental condition). Data for each fish group was calculated from the average of a series of one-hour observations done at 3 time points each day, i.e. morning, noon and afternoon, for 3 days. Video recording was performed concomitantly with manual observations using a commercial video system (Video S1). A camera recorded the photocell and alcove area, each recording lasted at least one hour and was edited using video editing software.