

EFFECT OF SEASON AND SEX ON THE PHOTOPIC SPECTRAL SENSITIVITY OF THE THREE-SPINED STICKLEBACK

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It is now almost two decades since Tinbergen and his associates (Tinbergen, 1951; Tinbergen & van Iersel, 1947; van Iersel, 1953) identified the relevant features of visual displays which trigger sexual and parental behaviour in the three-spined stickleback. Specifically, they showed that during the breeding season, when the female's abdomen is swollen with eggs, the latter serves as a visual sign stimulus which triggers appropriate sexual behaviour in the sensitized male. In addition, the red colouring which the belly of the male acquires during this period was found to elicit, in other sensitized males, the complex behaviour patterns which characterize the defence of territory in this species.

Presumably, these complex behaviour patterns arise during the spawning period because sexual hormones are released into the circulation and bring about the sensitization of specific circuits within the central nervous system. However, it is perhaps as well to emphasize that the present evidence does not permit a distinction to be made as to whether the action of the sexual hormones is (1) a 'facilitatory' one which operates by lowering the firing threshold of appropriate neural circuits; or (2) whether the action is inhibitory upon certain activity, raising the threshold of circuits which are inappropriate; or yet indeed (3) whether the hormones provide a specific combination of facilitation and inhibition within the centres. These questions, though of interest, are difficult to resolve experimentally.

In spite of the inherent difficulties confronting all studies relating to this problem, the present authors entertained the possibility that perhaps the sexual hormones which are released into the circulation during the spawning period may conceivably also act upon the retina itself where they may initiate detectable changes that lead to a shift in overall spectral sensitivity of the retina towards the longer wavelength end of the spectrum. This would effectively lower the visual threshold for red objects and perhaps contribute to the effectiveness of red stimuli in releasing appropriate sexual behaviour. Two possibilities were considered. First, if *Gasterosteus* belongs to the group of fishes that possess a paired visual pigment (i.e. their visual pigment contains a mixture of retinene₁ and retinene₂) then a shift in spectral sensitivity towards the longer wavelength end of the spectrum might rise because the action of the sexual

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hormones resulted in a greater proportion of visual pigment based on vitamin A₁ being replaced by one based on vitamin A₂.

Our second hypothesis considered the possibility that increased sensitivity to red visual stimuli might occur because certain synapses along the visual pathways from the red-sensitive photoreceptors might be selectively sensitized by the action of circulating hormone.

Initially we considered our first hypothesis to be the more favourable because changes in vitamin A pattern within the retina have been found to occur in several species of fish which spend part of their life-cycle in a salt-water environment, but migrate to fresh streams to spawn (Dartnall, 1962, 1967; Crescitelli, 1958; Bridges, 1956, 1964). The rainbow trout, for example (Bridges, 1956), spends part of its life-cycle in salt water, where the greater part of its visual pigment is based on vitamin A₁; however, at some stage the fish migrates upstream to spawn in fresh water and the vitamin A pattern within the retina changes in favour of A₂. Furthermore, since, according to Wald (1941), the majority of euryhaline fishes possess a paired pigment system, the suggestion that seasonal changes in the vitamin A pattern in sticklebacks might contribute to the effectiveness of red objects as releasing stimuli during certain times of the year seemed a promising hypothesis.

In the present study we employed the fish's innate tendency to follow moving stripes to determine the behavioural spectral sensitivity. The threshold for each of several spectral lights was determined by finding the lowest intensity to which the fish would display a detectable optomotor response to the moving stripes. Spectral sensitivity curves were determined separately for males and females at different times of the year, and our experiments were designed to reveal if spectral sensitivity of the three-spined stickleback was affected by: (1) season, (2) the sex of the individual.

METHODS

Sticklebacks were obtained from a local pond on the outskirts of Edinburgh, Scotland (latitude 55° 57' N.). In captivity they were fed daily on *Tubifex*.

The method and apparatus used to determine the behavioural spectral sensitivity of sticklebacks was the same as that employed in a previous study with goldfish (Cronly-Dillon & Muntz, 1965). Each stickleback was placed in a white bucket containing water to a depth of 4 in., and a 500 W. projection lamp (colour temperature approx. 3100° K.) projected the image of the spokes of a slowly rotating wheel onto the sides of the bucket. The axis of rotation of the stripes was orthogonal to the base of the bucket and coincided with its centre. Spectral lights were produced by ten interference filters (Balzers Lichtenstein) and the intensity of the light was controlled with neutral-density filters. Table 1 gives the characteristics of the interference filters and the correction factors necessary to equate the quantum energies of the different spectral lights. The correction factors were calculated from the manufacturers' specification of the colour temperature of the projection lamp and the spectral transmission curves of the interference filters which were determined with the aid of an automatic recording spectrophotometer.

Background illumination was produced by stray light from the projector and diffuse illumination from an ordinary 60 W. tungsten bulb. The level of background

Illumination as reflected from the sides of the bucket was measured with an S.E.I. photometer. In addition, the light reflected from the sides of the bucket, when no filters interrupted the projector beam, was measured and was 1.4 ft.-lamberts.

Table 1. *Characteristics of the interference filters used in the experiments*

λ max (nm.)	Half-maximum band width (nm.)	Logarithm (correction factor)
418	8	0
444	8	-0.30
469	14	-0.314
489	8	-0.518
516	8	-0.409
541	12	-0.479
567	8	-0.526
594	8	-0.445
630	12	-0.602
668	6	-0.214

THRESHOLD CRITERIA

Although different individuals often differed from one another in the manner with which they would respond to the moving stripes we were soon able to distinguish when an animal was displaying an optomotor response and when it was not. Some fish would attempt to follow a particular stripe and swim around the bucket at approximately the same speed as the stripes, others would start to follow the stripe, overtake it, then double back, then attempt to follow another stripe, and so on, repeating the cycle indefinitely. However, with practice it soon became apparent that the movements which a fish makes when it starts to follow a stripe are different from those which it makes when it starts to double back, and in addition we were able to note whether the swimming pattern was altered when the direction of rotation of the stripes was reversed.

During a threshold determination at a particular wavelength the light intensity was reduced with the aid of neutral-density filters in steps of approximately 0.2. At each intensity the fish were given ten trials, and the direction of rotation of the stripes was varied randomly during the test sequence. A time limit of 5 min. was set for each trial. A particular intensity was considered above threshold if the fish gave a clear optomotor response in 80% of the trials.

The different spectral lights were tested in random order. We attempted to select a presentation sequence where the spectral separation between succeeding test wavelengths was as great as possible as a precaution against the possibility that the thresholds might be affected as result of selective adaptation introduced by tests for the colour previously used.

RESULTS

Spectral sensitivity curves were determined for seventeen fish and the results were grouped according to the season in which the tests were run, and also according to the sex of individual fish. In order to get the fish fully light-adapted before starting an experiment they were all subjected for 1 hr. to a bright light produced by a 60 W. tungsten bulb.

Summer

Spectral sensitivity curves were determined within the period of 8–12 July for one group of four males and another group consisting of four females.

According to Hoar (1955), who studied seasonal changes in the reproductive activity of female sticklebacks, spawning occurs during the relatively short period between May and the end of July. Thus our experiments were conducted in the latter half of the spring spawning season. All males in this first series of experiments had red belly markings, and all the females had an abdomen swollen with eggs.

Table 2. *Results for individual sticklebacks during summer spawning (8–12 July)*

λ	Males						Females				
	I	I	III	IV	V	Mean	I	II	III	IV	Mean
418	2.87	2.77	2.69	2.77	2.88	2.79	2.78	2.88	2.77	2.78	2.80
444	3.38	3.03	3.17	2.94	2.94	3.09	2.97	2.91	2.91	2.83	2.90
469	4.64	4.22	3.87	4.61	4.22	4.31	3.46	3.71	3.90	3.66	3.68
489	5.71	5.02	4.69	4.81	5.52	5.15	4.26	4.64	4.02	4.25	4.29
516	5.29	5.34	4.72	5.03	5.30	5.17	4.41	4.86	5.05	5.14	4.86
541	4.52	4.29	3.72	3.88	4.48	4.18	3.76	4.20	4.51	4.51	4.24
567	3.94	3.47	3.30	3.30	3.70	3.56	3.50	3.81	4.49	4.49	4.07
594	4.69	4.78	4.09	4.37	4.59	4.50	5.43	5.63	6.32	6.11	5.87
630	3.62	4.31	3.37	3.62	3.61	3.71	4.08	4.28	4.48	4.73	4.39
668	3.06	2.80	2.06	2.75	3.00	2.73	2.55	3.34	3.54	3.56	3.25

The results for individual fish are given in Table 2, and the average spectral sensitivity curve for each of these groups is shown in Fig. 1. It is apparent that each curve displays characteristic maxima. In males, these wavelength maxima occur at approximately 502 nm. (λ_1) and 594 nm. (λ_2). Spectral sensitivity curves were plotted for individual fish and the position of the shorter wavelength (λ_1) peak was determined from the graphs for each animal (Table 5).

When spectral sensitivity curves of males and females were compared it was found that the visual threshold at the longer wavelength end of the spectrum was considerably lower in females than in males. In females the peak at 594 nm. rises considerably above the peak at 512 nm. (i.e. the optomotor threshold at 594 nm. was almost 1 log. unit below that at 510 nm.). On the other hand, with males the threshold at 594 nm. was always higher than the corresponding 502 nm. peak under the same experimental conditions. This difference in the value of the optomotor threshold between males and females was examined statistically. First, we obtained the difference between the optomotor threshold at the shorter and the longer wavelength peak (O.D.₁ – O.D.₂) for each individual (Table 4). The data were then grouped according to sex (Table 4) and subjected to analysis by the Wilcoxon test. The result of our analysis confirmed that females in the summer are significantly more red-sensitive than males ($\rho = 0.01$).

Winter

Table 3 gives the results for individual fish, and Fig. 2 shows the average spectral sensitivity curve for five males and four females determined within the period of

25 October–26 November. Within this period reproductive activity in sticklebacks is at a minimum (Hoar, 1955, 1957).

Table 3. Results for individual sticklebacks during the winter period (25 October–26 November)

λ	Males					Females				
	I	II	III	IV	Mean	I	I	III	IV	Mean
418	2.88	3.03	3.02	2.97	2.97	2.87	2.45	2.88	2.88	2.77
444	3.06	3.70	3.48	3.41	3.41	3.48	2.92	2.92	3.15	3.11
469	3.99	5.13	5.12	4.24	4.74	4.19	3.72	3.67	3.91	3.87
489	5.72	6.61	6.93	6.26	6.38	5.73	5.71	5.71	5.83	5.74
516	6.24	5.93	6.82	6.41	6.66	6.42	6.90	7.10	7.30	6.95
541	5.09	5.86	5.40	5.45	5.45	4.85	5.13	4.67	5.29	4.98
567	4.22	4.90	4.20	4.56	4.47	3.99	3.80	3.70	3.70	3.79
594	6.07	6.40	6.19	6.20	6.22	5.62	5.41	6.30	6.07	5.85
630	4.50	4.80	5.10	4.64	4.76	4.36	4.60	4.61	4.62	4.55
668	2.99	2.88	2.99	2.94	2.95	2.99	3.00	2.81	2.81	2.90

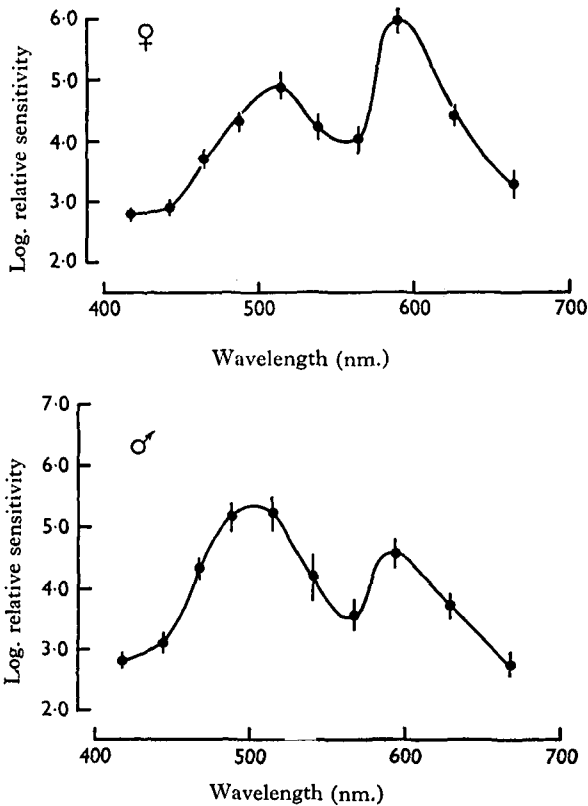


Fig. 1. Spectral sensitivity of female and male sticklebacks during the latter part of the spring spawning period (8–12 July). Background illumination 0.09 ft.-lambert.

In this series of experiments we had no previous knowledge before each test of the sex of individual fish, since all external features and colouring which had previously permitted us to distinguish between males and females had by now disappeared. Therefore after running the spectral sensitivity tests for each fish, its sex was determined by dissection.

Here again there were two peaks evident in each curve (Fig. 2). In males, peaks occurred approximately at 502 nm. and 594 nm. respectively, and the general shape of the curve did not differ appreciably from that found in the summer. In females also,

Table 4. *Difference between the optomotor threshold at the shorter wavelength peak and that at the longer wavelength peak in individual fish (measured in units of optical density) (O.D.₁ - O.D.₂)*

Male		Female	
Summer	Winter	Summer	Winter
+1.05	+0.30	-1.0	+1.15
+1.25	+0.80	-1.25	+0.75
+0.75	+0.55	-0.85	+0.95
+0.65	+0.20	-0.85	+0.90
+0.80			

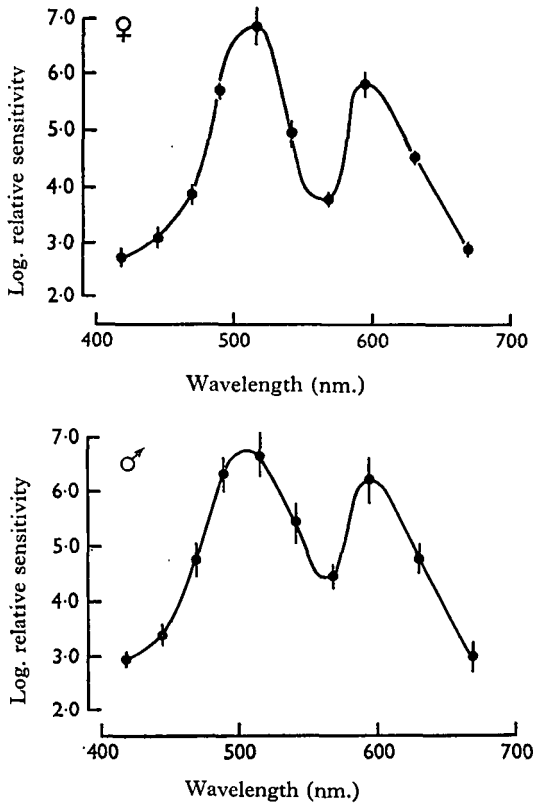


Fig. 2. Spectral sensitivity of female and male sticklebacks during the winter (25 October-26 November). Background illumination 0.05 ft.-lamberts.

λ_{\max} occurred at similar positions to those found in the summer (c. 510 and 594 nm.), but here, unlike the males, the shape of the curve underwent a change from that found in the summer. The threshold at 594 nm. relative to the 512 nm. peak was now considerably raised. Unfortunately, due to an error in setting up the apparatus, the background illumination for the winter months was lower than in experiments performed during the summer, consequently thresholds were generally lower than might

be expected had the level of background illumination been the same as that used in the summer. However, since, within any particular season, both groups were tested under the same conditions of background illumination, any difference which occurred between the sexes was not attributable to a difference in conditions of background illumination.

Finally, the wavelength position of the shorter wavelength peak was determined from the individual spectral sensitivity curves and this data is shown in Table 5. We then conducted a simple analysis of variance on the data and found that the latter departed significantly from homogeneity ($P = 0.01$). In addition, we found that the value of λ_1 in females was significantly greater than the corresponding value for males ($P = 0.01$).

Table 5. Wavelength position (λ_{max}) of the shorter wavelength peak (λ_1) determined from the spectral sensitivity curve of individual fish

Males		Females	
Winter	Summer	Winter	Summer
504	498	510	514
500	498	510	512
504	502	508	506
504	504	508	507
	498		

DISCUSSION

Our results did not show a seasonal shift in the wavelength position of the peaks of the spectral sensitivity curves. Thus the suggestion that seasonal changes in the vitamin A pattern within the retina of sticklebacks might contribute to the effectiveness of red objects as releasing stimuli was not confirmed. On the other hand, we found, independently of season, what appears to be a consistent difference (of the order of 10 nm.) between males and females in the wavelength of the shorter wavelength (λ_1) peaks. For male sticklebacks the shorter wavelength (λ_1) peak occurred at approximately 502 nm., whereas in females the first peak occurred at approximately 510 nm.

One suggestion that might account for the difference in spectral sensitivity between male and female sticklebacks is that it is due to a permanent difference in vitamin A pattern within the retina. We recall that since sticklebacks are euryhaline the retina probably contains both vitamin A_1 and vitamin A_2 chromophores (Wald, 1941).

Another possibility is that the difference in spectral sensitivity may be due to a difference in the structure of the opsin moiety of the visual pigment. This is similar to a suggestion of Bridges (1965), who advanced the hypothesis that there may be a whole range of discrete opsins with structures differing from one another in a regular stepwise fashion, each opsin having the ability to influence the light-absorbing properties of the chromophore, and each step-change resulting in shift of approximately 10 nm. in λ_{max} . This hypothesis was originally advanced to explain the fact that the absorption peaks of extracted visual pigments, from different species of fish, tend to form groups or clusters about certain preferred positions within the spectrum.

Our second finding in the present experiments was that the optomotor threshold for the red end of the spectrum (594 nm.) in females was considerably lower during

the spawning season than the corresponding threshold for males, where spectral sensitivity appears to remain virtually unchanged throughout the year. On the other hand we found evidence that this difference between the sexes disappears during winter months when reproductive activity is at a minimum.

Since the overall spectral sensitivity of female sticklebacks tested during the summer seemed to display no wavelength shift but only a selective lowering of the optomotor threshold at the longer wavelengths it suggests that the effect was not due to a change in vitamin A pattern. The evidence suggests rather, that selective sensitization may have taken place within visual pathways or at synapses concerned with transmitting impulses from red-sensitive photoreceptors to the brain. Perhaps, also, it may be argued that the same effect could have been produced by selective inhibition of pathways and structures concerned with transmitting impulses from receptors sensitive to the shorter wavelengths. However, this alternative seems unlikely because the visual threshold in females at 510 nm. and that of males at 502 nm. were approximately of the same magnitude; furthermore, in males, there is apparently no seasonal variation in spectral sensitivity. These observations, taken together, support the hypothesis that selective facilitation of certain visual pathways occurred rather than inhibition.

SUMMARY

1. The photopic spectral sensitivity of the three-spined stickleback *Gasterosteus aculeatus* was obtained behaviourally using the optomotor response as the index of sensitivity. Our experiments were designed to determine whether spectral sensitivity was affected (i) by the sex of the fish, and (ii) by season.

2. In females λ_{\max} occurred at approximately 510 nm. and 594 nm. In males the corresponding values were 502 nm. and 594 nm. Thus there appears to be a small but consistent difference of the order of 10 nm. between the shorter wavelength peaks of males and females.

3. In males the spectral sensitivity appears to remain virtually unchanged throughout the year. By contrast, during the summer months when reproductive activity is highest, the optomotor threshold for females at the red (594 nm.) end of the spectrum is considerably lower than the corresponding threshold value for males. This difference between the sexes disappears during winter months when reproductive activity is at a minimum.

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