

1378

Diel cycles in *Hoplosternum littorale* (Teleostei): entrainment of feeding activity by low intensity colored light

Thierry Boujard¹, Yann Moreau & Pierre Luquet
INRA Hydrobiologie, B.P. 709, 97387 Kourou Cedex, Guyane, France
¹ Present address: INRA Hydrobiologie, B.P. 3, 64310 Ascaïn, France

Received 19.6.1991 Accepted 15.1.1992

Key words: Fish, Circadian rhythms, Vision

Synopsis

Low intensity colored light is very often used to observe or manipulate fish during the scotophase. According to data on fish vision, most species can perceive these wavelengths of light since their cone pigments have maximum absorption peaks around 455, 530 and 625 nm. To test whether *Hoplosternum littorale* can detect low intensity red or blue light, we attempted to entrain feeding activity, known to be nocturnal and synchronized by the circadian light/dark alternation, to such light. Feeding activity was entrained with either red or blue light, indicating that these fish can perceive these lights. In all cases, the fish fed during the darker phase of the light cycle.

Introduction

Experimental observations or manipulations of fish during the night generally require the use of a low intensity background light. To minimize the effect of such lighting a red light is typically used because this color is supposedly rapidly absorbed by water.

Nevertheless, the visual spectra in most freshwater fishes would allow them to perceive these wavelengths. For example, a peak absorption around 625 nm has been found in *Carassius auratus* (Cronley-Dillon & Muntz 1965, Yager 1967, Beauchamp & Rowe 1977), *Scardinius erythrophthalmus* (Muntz & Northmore 1970), *Perca fluviatilis* (Cameron 1982), *Hemichromis bimaculatus* (Bell 1982), *Oncorhynchus mykiss* (Douglas 1983) and *Haplochromis burtoni* (Allen & Fernald 1985). Thus, one might argue that another color should be used as a source of low intensity light. However, most freshwater fish have trichromatic vision, with

maximum cone absorption peaks around 455 nm (blue), 530 nm (green) and 625 nm (red) (Marks 1965, Harosi & MacNichol 1974, Loew & Lythgoe 1978, Beauchamp et al. 1979, Fernald & Liebman 1980). In addition, a fourth absorption peak in the near UV range (361–398 nm) is found in *Carassius auratus* (Hawryshyn & Beauchamp 1985), *Rutilus rutilus* (Avery et al. 1982, Douglas 1986), and *Tribolodon hakonensis* (Harosi & Hashimoto 1983). As in many invertebrates (Wehner 1976), some species can also perceive polarized light: *Carassius auratus* (Kleerekoper et al. 1973, Hawryshyn & McFarland 1987), *Oncorhynchus nerka* (Dill 1971), *Zenarchopterus dispar* (Waterman & Forward 1972).

Thus, light that is bright enough to be seen by a human observer might also be perceived by the fish, and the effect of such light on the fish should be tested.

Fish activity is known to be synchronized by circadian oscillations of environmental cues, and

ORSTOM Fonds Documentaire

N° 4

40468

30 SEPT 1994

M

Cote 4

B

& 4

P5

Table 1. Spectral characteristics of the light, expressed in % of transmission, of Mazda 15 W dark red sphere B22 and Mazda 15 W dark blue sphere B22, between 400 and 700 nm, according to the manufacturer.

	blue spheres	red spheres
400	85.2	-
20	70.5	-
40	53.6	-
60	28.5	-
80	10.2	-
500	1.3	-
20	0.2	-
40	-	-
60	-	-
80	-	0.8
600	-	30.0
20	-	78.9
40	-	86.1
60	-	88.1
80	0.5	88.9
700	17.8	89.5

light is generally thought to be the main factor (Ali 1964, Stickney 1972, Richardson & McCleave 1974, Varanelli & McLeave 1974, Katz 1978, Mantefel et al. 1978, Müller 1978, Bachman et al. 1979, Steele 1984, 1985). Entrainment of activity by red light has already been shown for one fish species, *Salmo trutta* (Molina-Borja et al. 1990). Much less is known about the entrainment of feeding by light in fish, but most authors report strong circadian patterns of feeding (Hoar 1942, Landless 1976, Eriksson & Van Veen 1980, Boujard et al. 1990, 1991, Boujard & Leatherland 1992a, b).

In this study we attempt to determine whether a

low intensity background red or blue light affects the circadian feeding activity of fish. For the study we used the catfish locally called atipa, *Hoplosternum littorale* Hancock, a nocturnal air-breathing fish of economic importance in French Guiana (Carter & Beadle 1931, Kramer & McClure 1982, Winemiller 1987, Boujard et al. 1988).

Materials and methods

The experiment was carried out in French Guiana (5° N 52' 30 W). For each series of observations, fish were siblings reared in ponds and transferred to the laboratory where they were fasted for some days prior to the trials. These trials were performed in recirculated water in 200 l tanks held in a light-tight room. Water temperature ranged from 28 to 30° C, the oxygen content ranged from 7 to 8 mg l⁻¹ and neither of those two parameters was cycled.

The photoperiod, mimicking the natural photoperiod, was 13.5 h light and 10.5 h dark, artificial dawn being at 05 h 45 min and artificial dusk at 19 h 15 min. The light intensity was 2.1 $\mu\text{E m}^{-2} \text{s}^{-1}$ at the surface of the water and was provided by 4 fluorescent tubes (50 W philips, 1.5 m above the surface of the water), and/or 4 low intensity dark red or dark blue lights (the light intensity was, respectively, 0.17 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 0.12 $\mu\text{E m}^{-2} \text{s}^{-1}$ at the surface of the water when dark red or dark blue lights were used alone; spectral characteristics of these lights are given in Table 1).

Each tank was equipped with demand feeders which delivered 1 g of food each time the fish

Table 2. Presentation of the experiments.

Experiments Groups	1 (Control 1)			2 (Red 1)		3 (Red 2)		4 (Blue)		5 (Control 2)			
	1	2	3	1	2	1	2	1	2	1	2	3	4
Colour of low intensity background light	red			red		red		blue		-			
Number of fish	30	30	30	4	8	5	5	5	5	10	10	10	10
Mean weight (g)	49	41	45	101	99	48	51	50	49	46	50	52	48
Start date	26. 5.1988			27. 7.1989		20.11.1989		25. 9.1989		18. 1.1990			
Day D	2. 7.1988			16. 8.1989		7.12.1989		17.10.1989		-			
End date	15. 8.1988			24. 8.1989		18.12.1989		28.10.1989		30. 1.1990			

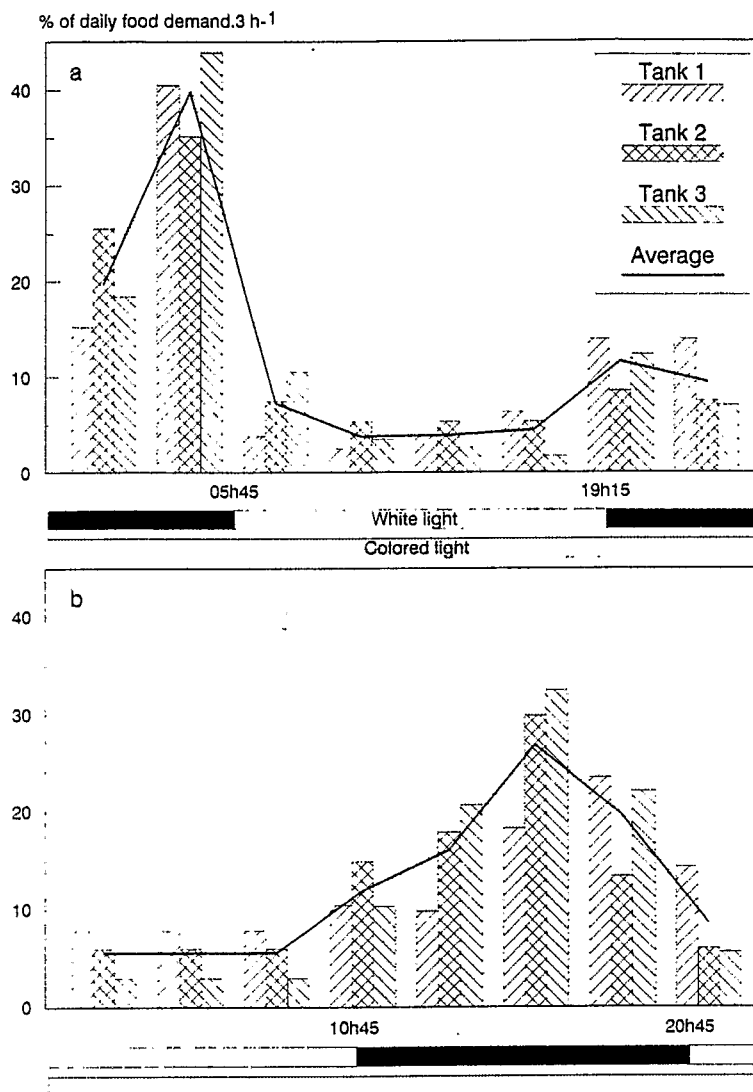


Fig. 1. Diel changes in the pattern of feeding demand in *Hoplosternum littorale* during the experiment 1 (redrawn from Boujard et al. 1990): a – before day D, the white lights are switched on between 5 h 45 min and 19 h 15 min, and a red light is switched on all the time (average from the 5 days preceding day D); b – after day D, the lighting remains the same, but dawn and dusk are advanced by 9 h (average from the 5 days following day D).

pushed a rod. Except for experiment 1, in which the demand feeders were weighed every 3 hours, these devices were connected to a computer recording the time, the day and the number of the tank from which each food demand originated. Five experiments were performed with two, three or four tanks each time (Table 2).

Experiment 1 (control 1): The red lights were left on continuously. The white lights were turned on to

simulate the natural photoperiod. To test whether the feeding demand is synchronized by light/dark alternation, after a 5 week adaptation period the onset of dusk (and dawn) was advanced by 9 hours. In this paper, and for all the experiments this day will be called day D. The results of these control trials are already published (Boujard et al. 1990), but are referred to here to clarify the findings of the other experiments.

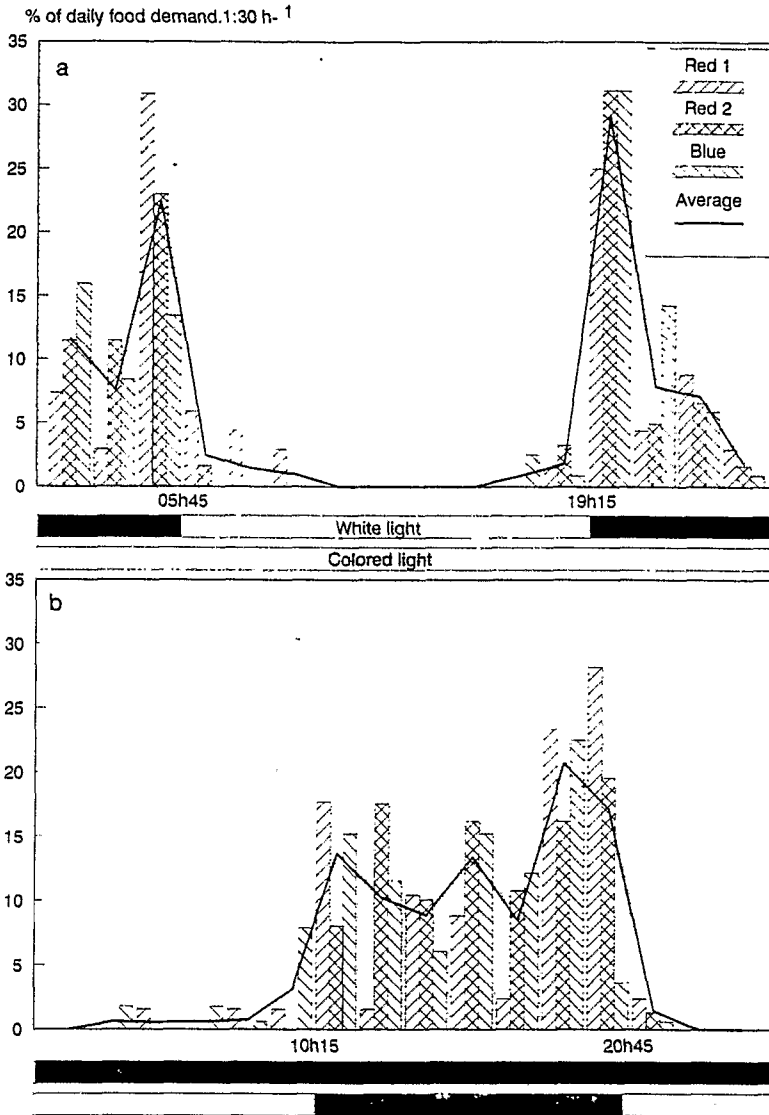


Fig. 2. Diel changes in the pattern of feeding demand in *Hoplosternum littorale* during the experiment 2 (Red 1), 3 (Red 2) and 4 (Blue); the 2 tanks used in each experiment being pooled together: a – before day D, the white lights are switched on between 5 h 45 min and 19 h 15 min, and a red (or blue) light is switched on all the time (average from the 5 days preceding day D); b – data from the 1st and 2nd day following day D, the white lights are switched off, and a red (or blue) light is switched on between 20 h 45 min and 10 h 15 min, i.e. the schedule of the lighting is the same as in experiment 1, after the day D, but a low intensity colored light is replacing the white light; c – data from the 4th and 5th day following day D, the lighting remain the same as in B; d – data from the 7th and 8th day following day D, the lighting remain the same as in B and C.

Experiments 2 and 3 (Red 1 and Red 2): Before day D, the red lights were left on continuously, and the white lights were turned on to simulate the natural photoperiod as in experiment 1. At day D, the white light was switched off. Thereafter, the red light was switched off at 10 h 15 min, and

switched on at 20 h 45 min, i.e., as in the first experiment, the onset of dusk (and dawn) was advanced by 9 hours at day D, but the photophase was produced by low intensity red lights and during the scotophase the fish were maintained in total darkness.

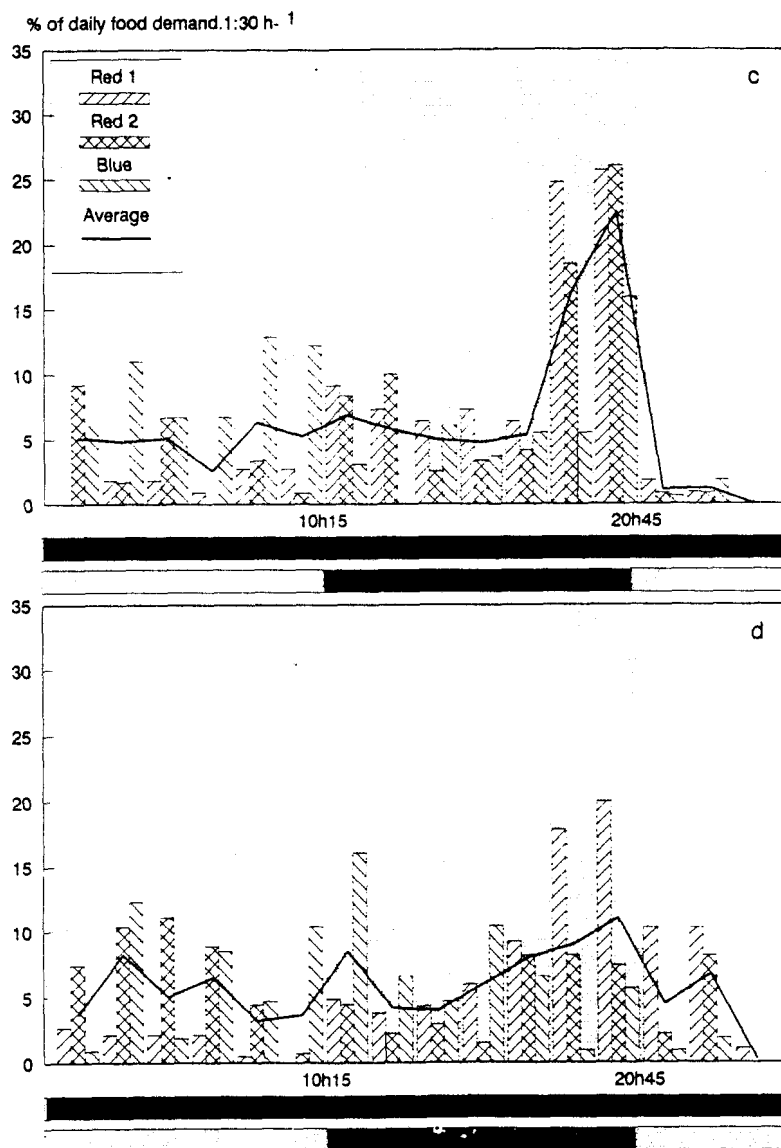


Fig. 2. Continued.

Experiment 4 (Blue): This trial was the same as Red 1 and Red 2, but it was carried out with blue instead of red low intensity bulbs.

Experiment 5 (Control 2): The red lights were permanently switched off. The white lights were switched on to simulate the natural photoperiod.

Results

During experiment 1, the three groups of catfishes

showed a nocturnal pattern of demand feeding. When the lighting simulated the natural photoperiod, i.e., before day D (Fig. 1a), 60% of the daily feeding demand took place during the 6 last hours of the scotophase. During the photophase feeding activity was erratic. A slight peak also occurred at dusk, but it was not clearly defined since the feeders were weighed only once every 3 hours. At day D, an immediate shift of the feeding rhythm occurred when dawn was advanced by 9 hours, and

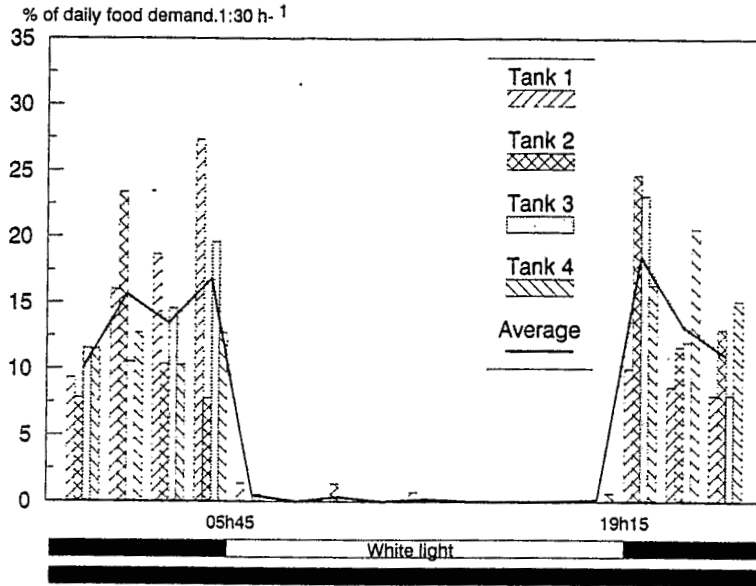


Fig. 3. Diel changes in the pattern of feeding demand in *Hoplosternum littorale* during experiment 5. The white lights only are switched on, between 5 h 45 min and 19 h 15 min (average from the 5 last days of the experiment).

the fish still fed mainly during the second part of the scotophase (Fig. 1b).

During experiments 2, 3 and 4, the fish also showed a strong circadian pattern of feeding activity before day D (Fig. 2a), with an average of 44% of their daily food demand occurring during the last 6 hours of the scotophase, a marked peak at dusk (29% of the daily food demand within the first 1.5 hours of darkness), and almost no feeding activity during the photophase. After day D (Fig. 2b), all fish still behaved in a similar way to that of fish in

experiment 1. When the light/'low intensity colored light' alternation was replaced by a 'low intensity colored light'/dark alternation the food demand was entrained by the shift of dawn, and occurred mainly during the second part of the dark phase, when the fish were held in total darkness. However, under this light regime the circadian pattern of feeding demand became less marked 4 days after day D (Fig. 2c), with a progressive increase of food demand during the period of low intensity coloured

Table 3. Comparison between the daily food demand during the 5 days before and after the day D (mean \pm SD; expressed in % of live weigh).

		Before day D	After day D	Student-Fisher test ($p > 0.01$)
Experiment	1 (Control 1)	5.2 \pm 3.8	4.9 \pm 3.5	n.s.
	2 (Red 1)	7.2 \pm 5.1	26.3 \pm 15.1	***
	3 (Red 2)	2.6 \pm 2.0	15.9 \pm 10.6	***
	4 (Blue)	3.1 \pm 1.8	11.2 \pm 8.4	***
	2, 3 & 4	4.3 \pm 7.2	17.8 \pm 12.9	***
	5 (Control 2)	3.9 \pm 2.6	—	—
Student-Fisher test ($p > 0.01$)		n.s.	*** (between control 1 and red 1, red 2, blue)	

light. The circadian pattern disappeared entirely 7 days after day D (Fig. 2d).

In experiment 5 (Fig. 3), i.e., without any background light, the feeding activity was nocturnal, with 61% of the demand during the 6 last hours of darkness, and 18% during the 1.5 h following dusk. There was almost no food demand during the photophase.

During all experiments the average self-distributed food per day ranged between 2.6 and 7.2% of live weight (Table 3) when the lighting mimicked the natural photoperiod and was not significantly different ($p > 0.01$, Student-Fisher test) for background red or blue light or no background light. After day D, a significant increase in food demand was observed in experiments 2, 3 and 4, i.e., when a colored light was the only source of light, but not in experiment 1, i.e., when the white light was still used after the day D.

Discussion

The circadian pattern of food demand was the same in all the experiments, when the light/dark alternation simulated the natural photoperiod, whether or not a background red or blue light was used. The fish were eating during the darker phase of the cycle, with a first peak at dusk and a second peak during the second half of the scotophase, and it has to be emphasized here that atipa, like most catfish, have a strongly developed tactile sense which they might use to find food in dark conditions (Boujard et al. 1988). The use of a low intensity colored light did not change the feeding pattern at all, because regardless of the source of light used, the darker phase was perceived as the scotophase by the fish. The entrainment of feeding demand in *Hoplosternum littorale*, by shifting the time of artificial dawn and dusk confirms the major role of light on the synchronization of feeding in fish. The entrainment of feeding demand, by a red or a blue low intensity light show that as for the activity rhythm (Gibson & Keenleyside 1966, Chaston 1968, Hesthagen 1980, Molina-Borja et al. 1990), the difference in intensity between the scotophase and the

photophase is more important than the intensity or the spectral characteristics of the light used.

The exception was experiment 1, in which the feeding demand was not negligible during the photophase and in which the peak associated with dusk was less marked. These two apparent discrepancies can readily be explained. The level of feeding demand during the scotophase could be a consequence of the higher number of fish maintained in each tank, which was much higher during the first experiment ($N = 30$) than during the other experiments ($N = 5-10$). Quite likely the chance of unintentional contact with the feeding trigger rod would increase with the number of fish per tank. The peak associated with dusk could be masked in experiment 1, since the feeders were not connected to a computer, but were weighed every 3 hours. The time for weighing the feeders around dusk was 17 h 15 min and 20 h 15 min, dusk being at 19 h 15 min. Consequently, this interval of time pooled 2 hours of light with 1 hour of dark.

The increase of the food demand observed when the light/'low intensity colored light' alternation was replaced by the 'low intensity colored light'/dark alternation can be interpreted as a stress effect due to the drastic change in the lighting regime on day D, rather than a depressive effect of the white light. Indeed, this hypothesis is supported by some direct observations: when the food demand increased, a large part of the food remained uneaten by the fish. Furthermore, in experiment 5, when no background light was used, the amount of daily food demand did not differ from that observed in the first part of the other experiments.

According to the results of the present experiments, a low intensity colored light can be used for nocturnal observations of this fish. But the results also show that red or blue light were perceived by the fish, and are capable of entraining the feeding activity. Thus, when an experiment needs constant darkness, a low intensity colored light should not be used since the fish might perceive this luminous environment as a long scotophase or a long photophase.

Acknowledgements

This work was funded by a grant from GCS.BBA. We are grateful to J.F. Leatherland and D.L.G. Noakes for having read and criticized this manuscript, and to Kirsten Rerat, who translated the first version of this manuscript into English.

References cited

- Ali, M.A. 1964. Diurnal rhythm in the rates of oxygen consumption, locomotor and feeding activity of yearling Atlantic salmon (*Salmo salar*) under various light conditions. Proc. Indian Acad. Sci. B 60: 249-263.
- Allen, D.M. & R.D. Fernald. 1985. Spectral sensitivity of the African cichlid fish, *Haplochromis burtoni*. J. Comp. Physiol. (A) 157: 247-253.
- Avery, J.A., J.K. Bowmaker, M.B.A. Djamgoz & J.E.G. Downing. 1982. Ultra-violet sensitive receptors in a freshwater fish. J. Physiol. 334: 23P-24P.
- Bachman, R.A., W.W. Reynolds & M.E. Casterlin. 1979. Diel locomotor activity patterns of wild brown trout (*Salmo trutta* L.) in an electronic shuttlebox. Hydrobiologia 66: 45-47.
- Beauchamp, R.D. & J.S. Rowe. 1977. Goldfish spectral sensitivity: a conditioned heart rate measure in restrained or curarized fish. Vision Res. 17: 617-624.
- Beauchamp, R.D., J.S. Rowe & L.A. O'Reilly. 1979. Goldfish spectral sensitivity: identification of the three cone mechanisms in heart-rate conditioned fish using colored adapting backgrounds. Vision Res. 19: 1295-1302.
- Bell, D.M. 1982. Physiological and psychophysical spectral sensitivities of the cichlid fish, *Hemichromis bimaculatus*. J. Exp. Zool. 223: 29-32.
- Boujard, T. & J.F. Leatherland. 1992a. Demand-feeding behaviour and diel pattern of feeding activity in *Onchorhynchus mykiss* held under different photoperiod regimes. J. Fish Biol. [in press]
- Boujard, T. & J.F. Leatherland. 1992b. Circadian rhythms and feeding time in fishes. Env. Biol. Fish. 35: 109-131.
- Boujard, T., P.-Y. Le Bail & P. Planquette. 1988. Données biologiques sur quelques espèces continentales de Guyane Française d'intérêt piscicole. Aquat. Living Resour. 1: 107-113.
- Boujard, T., P. Keith & P. Luquet. 1990. Diel cycle in *Hoplosternum littorale* (Teleostei): evidence for synchronization of locomotor, air breathing and feeding activity by circadian alternation of light and dark. J. Fish Biol. 36: 133-140.
- Boujard, T., Y. Moreau & P. Luquet. 1991. Entrainment of the circadian rhythm of food demand by infradian cycles of light/dark alternation in *Hoplosternum littorale* (Teleostei). Aquat. Living Resour. 4: 221-225.
- Cameron, N.E. 1982. The photopic spectral sensitivity of a dichromatic teleost fish (*Perca fluviatilis*). Vision Res. 22: 1341-1348.
- Carter, G.S. & L.C. Beadle. 1931. The fauna of the swamps of the paraguayan chaco in relation to its environment. II. Respiratory adaptations in the fishes. J. Linn. Soc. Lond. Zool. 37: 327-368.
- Chaston, I. 1968. Influence of light on activity of brown trout (*Salmo trutta*). J. Fish. Res. Board Can. 25: 1285-1289.
- Cronley-Dillon, J.R. & W.R.A. Muntz. 1965. The spectral sensitivity of the goldfish and the clawed toad tadpole under photopic conditions. J. Exp. Biol. 42: 481-493.
- Dill, P.A. 1971. Perception of polarized light by yearling sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 28: 1319-1322.
- Douglas, R.H. 1983. Spectral sensitivity of rainbow trout (*Salmo gairdneri*). Rev. Can. Biol. Exp. 42: 117-122.
- Douglas, R.H. 1986. Photopic spectral sensitivity of a teleost fish, the roach (*Rutilus rutilus*), with special reference to its ultraviolet sensitivity. J. Comp. Physiol. (A) 159: 415-421.
- Eriksson, L.-O. & T. VanVeen. 1980. Circadian rhythms in the brown bulhead, *Ictalurus nebulosus* (Teleostei). Evidence for an endogenous rhythm in feeding, locomotor, and reaction time behaviour. Can. J. Zool. 58: 1899-1907.
- Fernald, R.D. & P.A. Liebman. 1980. Visual receptor pigments in the African cichlid fish, *Haplochromis burtoni*. Vision Res. 20: 857-864.
- Gibson, R.J. & M.H.A. Keenleyside. 1966. Responses to light of young Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 23: 1007-1024.
- Harosi, F.I. & Y. Hashimoto. 1983. Ultraviolet visual pigment in a vertebrate: a tetrachromatic cone system in the dace. Science 222: 1021-1023.
- Harosi, F.I. & E.F. MacNichol. 1974. Visual pigments of goldfish cones. J. Gen. Physiol. 63: 279-304.
- Hawryshyn, C.W. & R. Beauchamp. 1985. Ultraviolet photosensitivity in goldfish: an independent U.V. retinal mechanism. Vision Res. 25: 11-20.
- Hawryshyn, C.W. & W.N. McFarland. 1987. Cone photoreceptor mechanisms and the detection of polarized light in fish. J. Comp. Physiol. A 160: 459-465.
- Hesthagen, I.H. 1980. Locomotor activity in the painted goby, *Pomatoschistus pictus* (Malm, Pisces), in relation to light intensity. Sarsia 65: 13-18.
- Hoar, W.S. 1942. Diurnal variations in feeding activity of young salmon and trout. J. Fish. Res. Board Can. 6: 90-101.
- Katz, H.M. 1978. Circadian rhythms in juvenile American shad, *Alosa sapidissima*. J. Fish Biol. 12: 609-614.
- Kleerekoper, H., J.H. Matis, A.M. Timms & P. Gensler. 1973. Locomotor response of the goldfish to polarized light and its e-vector. J. Comp. Physiol. 86: 27-36.
- Kramer, D.Z. & M. McClure. 1982. Aquatic surface respiration, a undespread adaptation to hypoxia in tropical freshwater fishes. Env. Biol. Fish. 7: 47-55.
- Landless, P.J. 1976. Demand-feeding behaviour of rainbow trout. Aquaculture 7: 11-25.

- Loew, E.R. & J.N. Lythgoe. 1978. The ecology of cone pigments in teleost fishes. *Vision Res.* 18: 715-722.
- Manteifel, B.P., I.I. Girska & D.S. Pavlov. 1978. On rhythms of fish behaviour. pp. 215-224. *In*: J.E. Thorpe (ed.) *Rhythmic Activity of Fishes*, Academic Press, London.
- Marks, W.G. 1965. Visual pigments of single goldfish cones. *J. Physiol.* 178: 14-32.
- Molina Borja, M., E. Perez, R. Pupier & B. Buisson. 1990. Entrainment of the circadian activity rhythm in the juvenile trout, *Salmo trutta* L., by red light. *J. interdiscipl. Cycle Res.* 21: 81-89.
- Müller, K. 1978. Locomotor activity of fish and environmental oscillations. pp. 1-20. *In*: J.E. Thorpe (ed.) *Rhythmic Activity of Fishes*, Academic Press, London.
- Muntz, W.R.A. & D.P.M. Northmore. 1970. Vision and visual pigments in a fish, *Scardinius erythrophthalmus* (the rudd). *Vision Res.* 10: 281-298.
- Richardson, N.E. & J.D. McLeave. 1974. Locomotor activity rhythms of juvenile atlantic salmon (*Salmo salar*) in various light conditions. *Biol. Bull.* 147: 422-432.
- Steele, C.W. 1984. Diel activity rhythms and orientation of sea catfish (*Arius felis*) under constant conditions of light and darkness. *Mar. Behav. Physiol.* 10: 183-198.
- Steele, C.W. 1985. Absence of a tidal component in the diel pattern of locomotory activity of sea catfish, *Arius felis*. *Env. Biol. Fish.* 12: 69-73.
- Stickney, A.P. 1972. The locomotor activity of the juvenile herring (*Clupea harengus harengus* L.) in response to changes in illumination. *Ecology* 53: 438-445.
- Varanelli, C.C. & J.D. McCleave. 1974. Locomotor activity of Atlantic salmon parr (*Salmo salar* L.) in various light conditions and in weak magnetic fields. *Anim. Behav.* 22: 178-186.
- Waterman, T.H. & R.B. Forward. 1972. Field demonstration of polarotaxis in the fish *Zenarchopterus*. *J. Exp. Biol.* 180: 33-54.
- Wehner, R. 1976. Polarized-light navigation by insects. *Sci. Amer.* 235: 106-115.
- Winemiller, K.O. 1987. Feeding and reproductive biology of the currito, *Hoplosternum littorale*, in the Venezuelan llanos with comments on the possible function of the enlarged male pectoral spines. *Env. Biol. Fish.* 20: 219-227.
- Yager, D. 1967. Behavioural measures and theoretical analysis of spectral sensitivity and spectral saturation in the goldfish, *Carassius auratus*. *Vision Res.* 7: 707-727.