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Environmental influence on maturation and dominance relationships in the three-spined stickleback (*Gasterosteus aculeatus* L.): temperature competes with photoperiod for primacy

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Abstract

In this study, the influence of a combination of different photoperiods and temperatures on the final maturation and social interactions in three-spined sticklebacks was investigated. Water temperature appears to be the principal signal affecting gonadal development and breeding activity of sticklebacks in pre-spawning and spawning periods. Males can mature independently of photoperiod and a stimulatory effect of high temperature is not diminished by light deprivation. On the other hand, low temperature can inhibit the development of secondary sexual characters in males exposed to long day or constant light. In females, lighting seems to be more decisive for

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complete maturation and the lack of light delays the maturation rate, even in high temperature. While kept under the same conditions, males mature quicker than females. The presence of light and visual information are crucial to establish the social position of individuals in the group. In light, a rigid social hierarchy with one dominant, sexually active male is observed. In constant darkness, however, several males in the group demonstrate every sign of sexual activity.

INTRODUCTION

In seasonally breeding fish, the annual reproductive cycle is regulated by cyclic changes in environmental factors such as photoperiod and temperature. In sticklebacks, the combined effect of photoperiod and temperature on sexual maturation and breeding has been demonstrated by various authors (Borg, Ekström 1981; Borg 1982a,b; Borg, Van Veen 1982; Borg et al. 1987; Baggerman 1989; Bornestaf, Borg 2000; Bornestaf et al. 2001). The prevalent view promoted photoperiod as a major environmental factor controlling the maturation and development of secondary sexual characters in this species (Baggerman 1957, 1989; Borg 1982b; Mayer et al. 1997; Borg et al. 2004). The role of water temperature in the control of reproductive activity and spawning has been considered to be of secondary importance or has even been ignored. There are several studies pointing to temperature as an important factor controlling breeding and maturational processes in sticklebacks (Craig-Bennett 1931; Borg 1982a; Borg, Van Veen 1982; FitzGerald et al. 1986; Whoriskey et al. 1986; Lachance et al. 1987). In accordance with Craig-Bennett (1931), rising temperature affects the development of sexual characters in males and the duration of sexual activity in sticklebacks is inversely proportional to the temperature. The effect of temperature is different depending on the season: high temperature stimulates maturation of both sexes in spring, but diminishes sexual activity at the end of the breeding season in late summer or early autumn (Borg 1982a; Borg, Van Veen 1982). Temperature influences stickleback's reproductive behavior (FitzGerald et al. 1986; Whoriskey et al. 1986; Lachance et al. 1987) and various reproductive processes, including maturation of eggs per clutch, interspawning intervals and breeders' condition (Boulé, FitzGerald 1989; Guderley, Foley 1990). Also, in other teleost species, higher water temperature strongly accelerates gametogenesis, maturation and spawning time (Breton et al. 1980a,b; Davies, Bromage 2002). But, on the other hand, extreme temperatures may have a deleterious effect on ovulation, fertility and egg survival (Pankhurst et al. 1996; Pankhurst, Thomas 1998). In view of these data, there is still a question that needs to be answered: which environmental factor - temperature, lighting regime, or both - is decisive for the maturation and successful reproduction of sticklebacks? There is still a lack of data about maturation of fish while exposed to very low or gradually increasing water temperature together with lengthening photoperiod, i.e. environmental

conditions mimicking what the fish experience in the field at the onset of breeding. The influence of constant darkness on maturation and sexual behavior in this species has also never been tested. These issues are addressed in this paper.

During the pre-spawning and spawning phase, stickleback males demonstrate characteristic territorial and aggressive activities, building nests, courting females and taking parental care of the eggs and offspring (Wootton 1976; Gaudreault, FitzGerald 1985; Candolin, Salesto 2006). The pattern of behavior is determined, to some extent, by social relationships in the fish group. It is well established that reproductive success in sticklebacks depends largely on the interrelations within the group (Ketele, Verheyen 1985; Candolin 1999, 2000; Le Comber et al. 2003). However, there are no data on the influence of photoperiod and temperature on social relationships in this species. Thus the question arises whether the interrelations in fish groups can affect breeding.

In this paper, we have examined the influence of temperature and photoperiod on the final phases of maturation of males and females. Also an attempt has been made to distinguish between the effect of temperature from that of light. The experiments were carried out at controlled water temperature and photoperiod regimes, which were applied in various combinations. Changes in appearance and behaviour of fish, as well as social interrelations, were monitored. The stage of sexual development was determined on the basis of sexual characters and specific behaviour and then verified by histological analysis of gonads.

MATERIALS AND METHODS

Sampling

Adult three-spined sticklebacks were caught in the Dead Vistula River (5-6 PSU salinity) on three occasions in spring: 22 March 2000 (in 3ºC ambient water temperature and 12L:12D natural photoperiod), 30 April 2001 (13ºC, 13L:11D) and 9 March 2002 (3ºC, 12L:12D) and assigned to Experiment 1, Experiment 2 and Experiment 3, respectively.

Prior to each experiment, from the total captured fish, 20 were chosen from each assignment to evaluate current maturity of gonads. These fish were killed by decapitation immediately after capture, and the ovaries and testes were dissected. The gonads were fixed in Bouin's fluid, embedded in paraffin, cut and stained with hematoxylin and eosin for histological analysis. Stages of gonad development were evaluated according to the classification by Sokołowska and Kulczykowska (2006) and briefly reported in Table 1.

Table 1

Stages of ovaries and testes maturity in three-spined sticklebacks (see: Sokołowska, Kulczykowska 2006).

Phase	Stage	Histological description of gonads
FEMALES		
Previtellogenesis	1	The nucleoli are located peripherally of the nucleus
	2	The nucleoli disperse over the nucleus
Endogenous vitellogenesis	3	Initial vacuolisation of the peripheral part of the cytoplasm
	4	Intermediate vacuolisation of the peripheral and central part of the cytoplasm
	5	Complete vacuolisation of the cytoplasm
Exogenous vitellogenesis	6	Initial accumulation of secondary yolk in the periphery of the oocyte
	7	Most or entire cytoplasm fill up with secondary yolk
Ovulation	8	Ovulating oocytes full of yolk are spent and postovulatory follicles are present
Regression	R	Advanced regression of all oocytes, cytoplasm and layers are granulated and disintegrated
MALES		
Intermediate spermatogenesis	1	Spermatocytes (SC), spermatids (ST) and spermatozoa (SZ) in a large number. Single spermatogonia (SP) are present.
	2	SZ in a large number. SC and ST or ST are present, but in a smaller amount than in stage 1.
Completed spermatogenesis	3	SZ dominate. Dense SZ fill the lateral seminiferous tubules, but not the central duct. The single SC, ST and SP are present.
	4	SZ dominate. Single layers of SP around the periphery of tubules.
Spawning, releasing of sperm	5	The seminiferous tubules are filled by diluted SZ, or partly or completely empty after spawning. SZ are present in the central tubule and in the spermatic ducts. Intermediary numbers of SP and SC or SC only are present.
Commencement of spermatogenesis	6	Quiescent phase. Seminiferous tubules are filled with numerous SP. Resorption of residual SZ.
	7	SP and SC dominate. The interstitial tissue is reduced.
Intensive spermatogenesis	8	SP, SC and ST in large numbers. Only few SZ are present.
	9	SC and ST in large numbers. A small number of SZ. Single SP are present.

Experimental procedures

Fish were acclimated to the laboratory conditions at the ambient environmental photoperiod and temperature in a 300 l tank filled with fresh water for 2 days prior to experiments. The water was constantly circulated between the tank and external filter (Eheim Classic), where it also was aerated.

Combined regimes of photoperiod and temperature which were used in the three experiments are presented in Table 2. Fish were assigned randomly to one of the following experimental groups: Experiment 1: LL+21ºC, 16L:8D+21ºC, 8L:16D+21ºC, DD+21ºC, LL+(9-21ºC), 16L:8D+(9-21ºC), 8L:16D+(9-21ºC), DD+(9-21ºC) (see Figure 1); Experiment 2: LL+18ºC, 16L:8D+18ºC, 8L:16D+18ºC, DD+18ºC (see Figure 2); Experiment 3: 12L:12D+18ºC, 12L:12D+(4-14ºC), 12L:12D+3ºC and LL+5ºC (see Figure 3).

Table 2

Light and temperature treatments in Experiment 1, Experiment 2 and Experiment 3.

	LL constant light	16 L:8D lights on at 4 a.m.	12 L:12D lights on at 6 a.m.	8 L:16D lights on at 8 a.m.	DD constant darkness
Experiment 1	21°C 9-21°C [(a)]	21°C 9-21°C	×	21°C 9-21°C	21°C 9-21°C
Experiment 2	18°C	18°C	×	18°C	18°C
Experiment 3	5°C	×	18°C 4-14°C [(b)] 3°C	×	×

[(a)] temperature increasing from 9 to 21°C in rate of about 1°C per day until the 11-th day, with the minor increase afterwards

[(b)] temperature increasing from 4 to 14°C in rate of about 1°C per day until the 8-th day, to remain stable afterwards

Each group was maintained in a 30 l glass aquarium with stone-sandy bottom without plants. As there were no outward signs allowing one to distinguish the male from the female, a sex ratio was not determined prior to the experiment.

In each variant, observations of appearance and behavior of fish were made daily between 7:00 and 19:00, every third hour for several minutes. The most active fish that competed to breed in particular variants (dominant and subordinate individuals, gravid females) were easily recognised by characteristic external features (e.g. nuptial colors, size and shape of the body/tail/fins) during the experiment. In contrast, immature fish formed separate groups and hid themselves behind stones, filters or in the corners. Variation in the intensity of nuptial coloration of mature sticklebacks was evaluated according to the 6-point scale of the development of the secondary sexual characters proposed by Craig-Bennett (1931). The males with most pronounced red and blue colors were classified as 4th stage. Aggression in males was assessed on the basis of attack frequencies: the number of bites, hits and chases aimed at intruders within defended territories per minute. The biting was suggested to be an adequate measure of aggression in sticklebacks (Peeke et al. 1969, Wootton 1971). Territoriality was recognised by tracking the swimming route of the male within the area where aggressive behavior occurred. Territorial males, which built nests, showed advanced aggressive behavior and red coloration of undersides, were considered as dominants in each treatment group.

Cotton threads for the nests were put into the aquarium. Water was circulated between the aquarium and internal filter (Junior Bio) where it also was aerated, and temperature was recorded daily at midday. The light regime

was regulated automatically by clocks. Fluorescent tubes (Philips TL 8W/840) served as light sources, with the light intensity approximately 5000 lux at the water surface. The fish were fed daily at 3 p.m. *ad libitum* with frozen *Tubifex*, *Mysis mixta* or living *Crangon crangon*. The fish were killed by decapitation at the end of experiments and the ovaries and testes were taken and fixed for histological analysis. In addition, in Experiment 2 nuptial males were taken for analysis of testicular maturity on the first and eighth day of the exposure.

Statistical analysis

Variables of social structure (percentage of mature fish, percentage of dominants, duration of the dominance relationship, sex ratio of mature fish, and time of maturity) were of qualifying type and were not normally distributed. The statistical analysis was carried out using non-parametric independent group comparisons by Kruskal-Wallis's ANOVA rank test followed by Mann-Whitney's *U*-test. Differences were considered to be significant if $p<0.05$. The relation between temperature and/or photoperiod conditions and variables of social structure were performed using Kendall's rank correlation at a level of significance $p<0.05$. Statistical analyses were carried out using *Statistica 7.1* software (Sokal, Rohlf 1995).

RESULTS

Experiment 1 (Figure 1)

Sticklebacks from the control group, exposed to 3ºC and 12L:12D, were immature: the 6th stage of oogenesis was recorded in females, and the 3rd or 4th stage of spermatogenesis in males. There were no morphological differences between males and females.

Sticklebacks of both sexes matured in all variants of the experiment and there was no statistical difference in percentage of mature fish between experimental groups treated by long and continuous light or long and continuous darkness under both temperature conditions (Mann-Whitney's *U*-test). However, the number of dominants and time of maturation varied between treatments. There were dominant males (grey bars) and subordinate males (white bars) among mature sticklebacks in the 5th stage of maturity. The intensity of nuptial coloration and aggressive and territorial behavior depended on temperature and photoperiod conditions, and presence of conspecifics. Fish maintained at a constant temperature of 21ºC and LL, 16L:8D, 8L:16D, or DD matured quicker than the fish exposed to increasing temperatures from 9 to 21ºC in the corresponding light regimes (Mann-Whitney's *U*-test, $p<0.05$). Males subjected to a combination of high

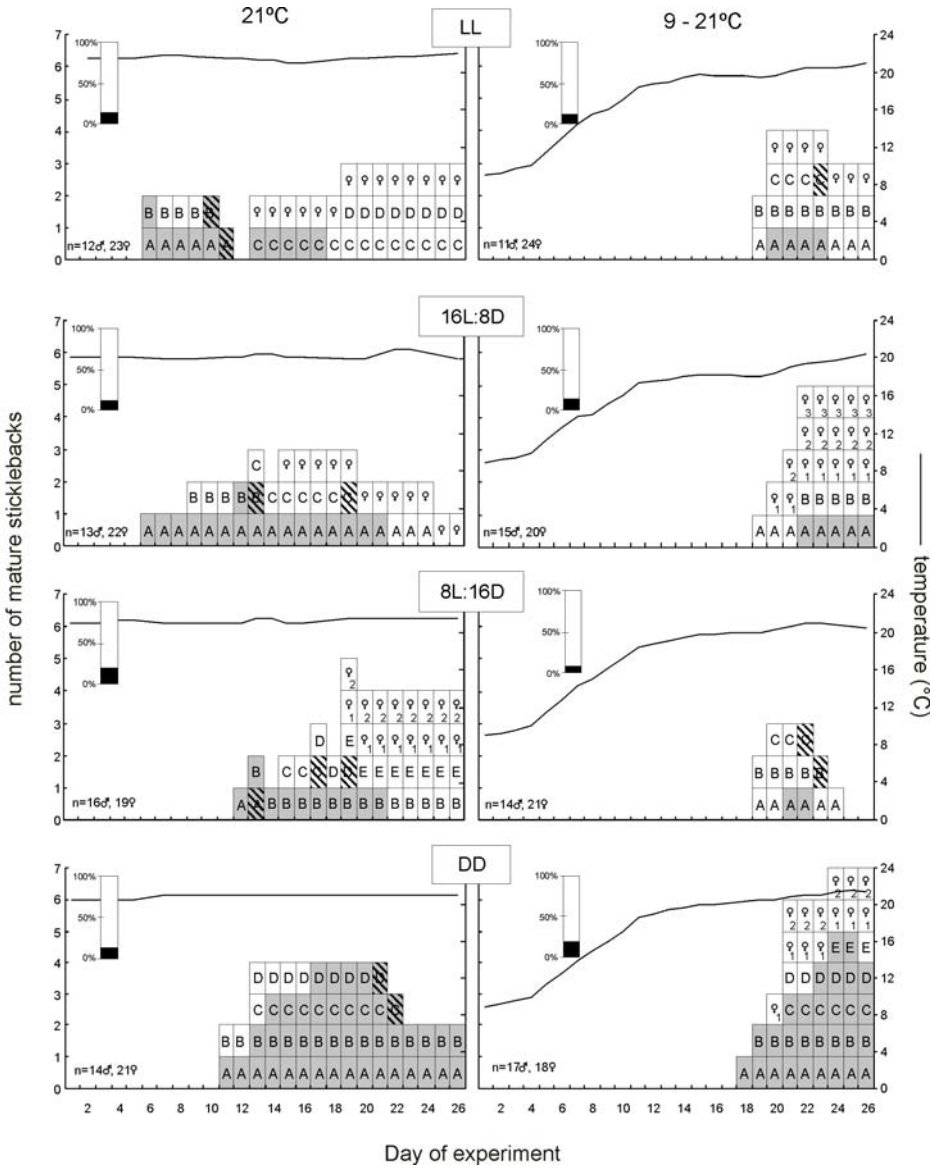

Fig. 1. The number and percentage of mature sticklebacks after exposure to a combination of high (21°C-left column) and increasing water temperature (9-21°C-right column) and different photoperiods (LL, 16L:8D, 8D:16L, DD). Letters from A to E show dominant males with distinct nuptial features (grey bars) and subordinate males with slight nuptial features (white bars). Striped bars: dead males, ♀: mature females, n: number of fish.

temperature and long or constant light regimes (16L:8D, LL) were the first to demonstrate secondary sexual characters such as blue/red nuptial colors and aggressive/territorial behavior (Mann-Whitney's *U*-test, $p<0.05$).

Male-male aggressive competition resulted in one individual becoming a leader, while others lost their nuptial signs and took submissive positions. Only one dominant was present at any given time in the group exposed to light. In certain cases the dominant male killed the weaker rival (♂B exposed to high temperature in LL and 16L:8D regime). In experimental variants with increasing temperatures, sticklebacks matured no earlier than 5-6 days after the temperature reached 20ºC. Dominant males occurred here less frequently and kept their dominant position for a shorter time while exposed to 24 h, 16 h and 8 h of light, than after exposure to adequate light regimes at a constant high temperature (Mann-Whitney's *U*-test, $p<0.05$). Sticklebacks maintained in 16 h of darkness lost their nuptial features before the end of experiment. Fish also matured in complete darkness (DD), but only at high temperatures close to 20ºC. In DD, several males matured at the same time and maintained signs of sexual activity throughout the experiment. All mature males behaved as dominants presenting red nuptial colors and building nests. There was no aggression in the group. Percentage of dominants increased significantly with the lengthening of the dark period (Kendall's rank correlation, $p<0.05$) and was significantly higher in DD than in light (Mann-Whitney's *U*-test, $p<0.05$).

Mature females were in an ovulation phase (stage 8). In the experiment, there were more mature males than females (Mann-Whitney's *U*-test, $p<0.01$). Females matured later than males in both temperature variants (Mann-Whitney's *U*-test, $p<0.05$).

Experiment 2 (Figure 2)

Sticklebacks from the control group, exposed to 13ºC and 13L:11D, were immature: the 6^{th} or 7^{th} stage of oogenesis in females and the 3^{rd} stage of spermatogenesis in males were noted. There were no morphological differences between males and females.

Mature sticklebacks of both sexes appeared in all photoperiod regimes after exposure to high temperatures. There was no statistical difference in percentage of mature sticklebacks between long/continuous light and long/continuous darkness regimes (Mann-Whitney's *U*-test). There were, however, differences in time of maturation and dominance relationships between treatments. In males subjected to 16 h of darkness, the nuptial features temporarily vanished.

Dominant males (grey bars) and subordinate males (white bars) reached the 5^{th} stage of maturity. Males A showed an intensive red coloration of undersides immediately after transfer to tanks. In light regimes their nuptial colors came

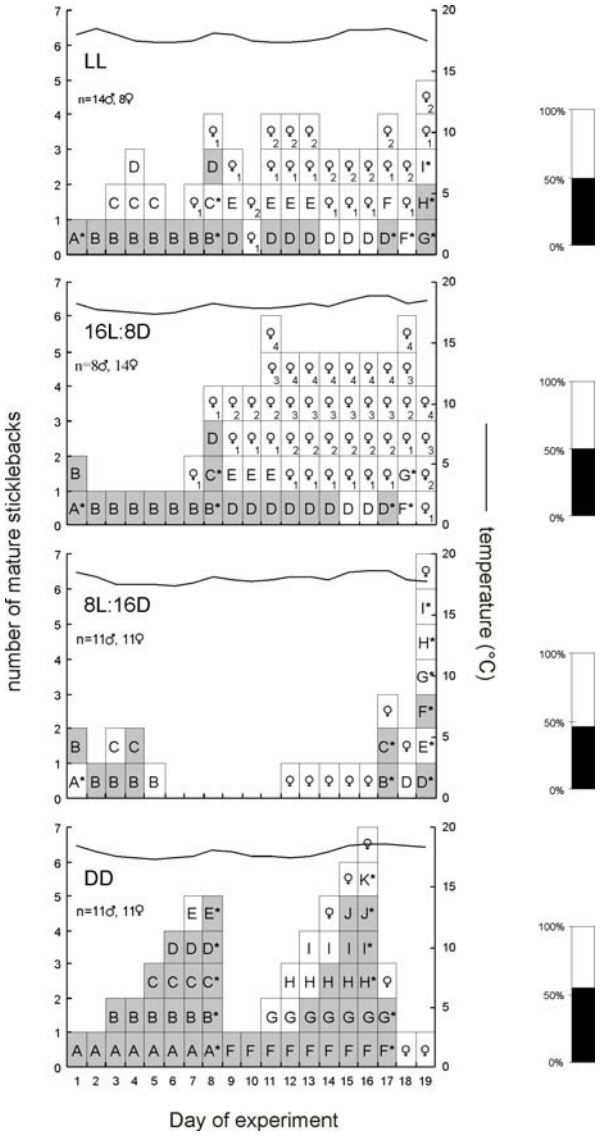

Fig. 2. The number and percentage of mature sticklebacks after exposure to a combination of high water temperature (18ºC) and different photoperiods (LL, 16L:8D, 8L:16D, DD). Letters from A to K show dominant males with distinct nuptial features (grey bars) and subordinate males with slight nuptial features (white bars). Star: nuptial males taken for analysis of testicular maturity, ♀: mature females, n: number of fish.

together with aggressive and territorial behavior. The dominants A were taken off (marked with stars) to determine the stage of testes' development. Upon removing them, males B took a leading role immediately and started to demonstrate intensive nuptial features. Other mature males (♂C or ♂D) remained passive and non-territorial, showing slight nuptial colors. Similarly, after taking mature males (stars) for analyses on the eighth day, other mature males (♂D in LL, 16L:8D; ♂F in DD) became leaders. Generally, each male removed for analysis was replaced by a successor within several minutes. Nuptial color intensity might change very quickly and inversely. In light regimes, male-male competition most often resulted in submission of one of the rivals. Mature sticklebacks became pale (♂D in LL, 16L:8D) or lost their nuptial signs (♂E in LL, 16L:8D). Only one dominant was always present at a given time in the group exposed to light. However, in DD, several males showed signs of sexual activity at the same time. They matured in succession, built nests and kept the nuptial features throughout the experiment. There were no dominants in DD, although all males matured. Differences between variables were not statistically analysed due to low amounts of data.

In the experiment, there were fewer mature females and they matured slower than males (Mann-Whitney's *U*-test, $p<0.05$); the first mature females were observed after 7 days, while kept at long day or continuous light.

Experiment 3 (Figure 3)

Sticklebacks from the control group, exposed to 3ºC and 12L:12D, were immature: the 5th or 6th stage of oogenesis in females and the 3rd stage of spermatogenesis in males were observed. There were no morphological differences between males and females.

In all experimental treatments, mature males in the 5th stage were noticed, but their number and intensity of the nuptial features depended on the conditions. A combination of high temperature (18ºC) and natural photoperiod (12L:12D) strongly stimulated maturation in males. All males (♂A, ♂B, ♂C) in this group started to behave aggressively and territorially as early as the third day of the experiment. The high temperature significantly stimulated the percentage of mature males and time of attaining maturity (Kendall's rank correlation, $p<0.05$). Male A, which was more expansive and slightly colored, became a dominant (grey bars) and built a nest on the 7th day. This one remained a dominant throughout the experiment, despite poor coloration. Mature males B and C lost nuptial features (white bars), left previously occupied territories and joined other subordinate fish. Sticklebacks exposed to increasing temperatures from 4 to 14ºC started to behave aggressively as soon as the temperature reached 12ºC. Male A began to show dominant features

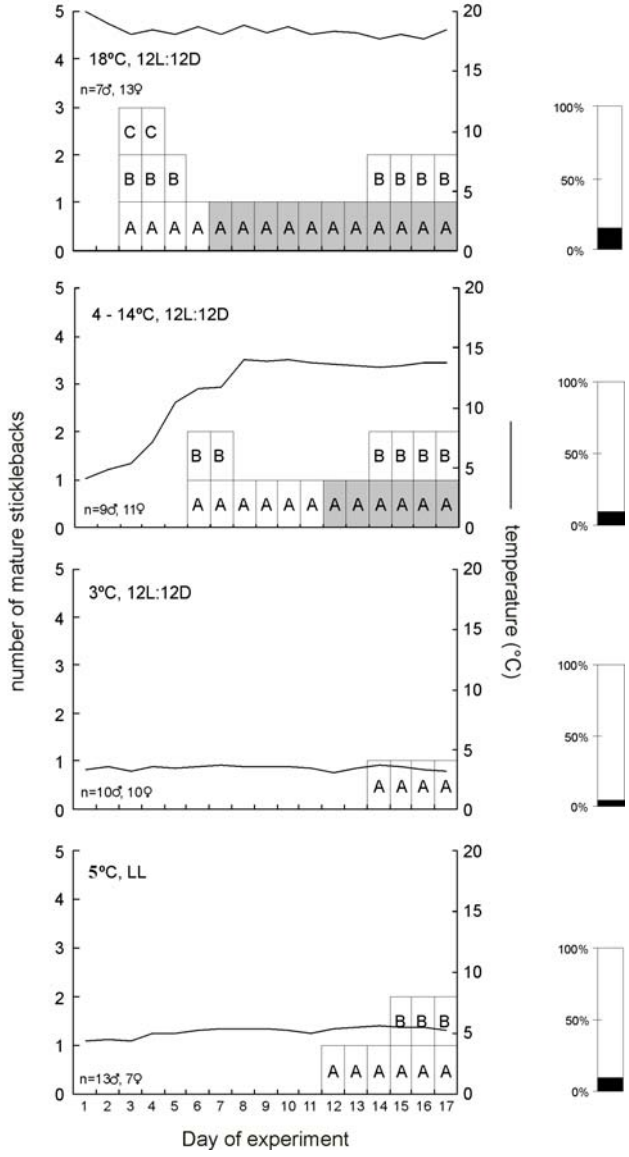

Fig. 3. The number and percentage of mature sticklebacks after exposure to a combination of different temperatures (18ºC, 4-14ºC, 3ºC, 5ºC) and photoperiods (LL, 12L:12D). Letters from A to C show dominant males with distinct nuptial features (grey bars) and subordinate males with slight nuptial features (white bars), n: number of fish.

while the temperature was as high as 13ºC, whereas the non-red male B became subordinate. In the groups exposed to low temperatures (3ºC, 5ºC), males were pale and non-territorial. There was no competition between males and females. Slight blue-green nuptial coloration of ventral sides of the body and head appeared only in a few individuals at 3ºC, 12L:12D and at 5ºC, LL. Low temperatures markedly delayed maturation in males, even though they were subjected to long or constant light regimes (12L:12D, LL).

At 18ºC and 12L:12D several females were at the pre-spawning stage (7), but not one attained an ovulation phase. In other treatments females remained in the 5th or 6th stage of maturity.

DISCUSSION

The role of temperature and photoperiod in maturation and successful breeding of sticklebacks is still in dispute. In this paper, an attempt has been made to distinguish the influence of temperature from that of light, on the final phase of sexual development in the three-spined stickleback. The studies were performed in spring, when the reproductive activity of this long-day breeder is observed.

Our results, which indicate that long photoperiod and high temperature are extremely effective for maturation of sticklebacks, agree with other investigations (Baggerman 1957, 1985; Borg 1982a; Borg, Van Veen 1982; Borg et al. 1987). However, many studies on sticklebacks' reproduction, pointed to light as a decisive environmental factor influencing the final phase of maturation and development of secondary sexual characters in this species (Borg 1982b, Baggerman 1989, Mayer et al. 1997, Borg et al. 2004).

Our studies show that in spring the sticklebacks mature independently of photoperiod, and their reproductive activity is affected by temperature. In early spring, after winter's long dark phase, the photosensitivity of fish is growing and the response time to external light stimulation becomes probably shorter. Most likely the rapidly rising temperature is the signal triggering final maturation in sticklebacks. Our earlier studies show that beginning of sexual activity in sticklebacks in spring coincides with a marked increase in water temperature in this season (Sokołowska et al. 2004). It suggests that relatively short days in early spring do not seem to be the limiting factor in maturation in this species, as long as water temperature is high enough. Baggerman (1985) also reports a seasonal photoreactivity threshold in sticklebacks and demonstrates that even short photoperiods from January to March are sufficient to promote breeding in sticklebacks. According to Borg and Ekström (1981), ovarian maturation and male kidney hypertrophy in sticklebacks also proceed in spring independently of photoperiod, but in high temperature.

Maturation of gonads in both sexes of sticklebacks depends on temperature. The high water temperature evidently stimulates the final stages of development in these fish in March, as has been shown by Borg (1982a) and Borg and Van Veen (1982). In addition, a crucial role of temperature in maturation and development of secondary sexual characters was highlighted in sticklebacks by Craig-Bennett (1931). There is also support for a primary role of temperature in breeding of many fish species. Burger (1939) and Matthews (1939) indicated that temperature, with photoperiod as only a minor influence, was a primary environmental factor regulating spermatogenesis in *Fundulus heteroclitus*. Also male bitterlings (*Rhodeus amarus*) and lake chub (*Couesius plumbeus*) became sexually mature while kept at higher temperature, irrespective of the length of the day (Verhoeven, Van Oordt 1955; Ahsan 1966). Temperature played a crucial role in reproduction of both sexes of *Gambusia affinis* (Medlen 1951). Killifish females (*Fundulus confluentus*) matured quicker when kept at high temperature, and showed no evident responsiveness to the length of the day (Harrington 1959). De Vlaming (1975) indicated that a long photoperiod could not induce the final phase of testicular and ovarian maturation in *Notemigonus crysoleucas* while kept at low temperature. Moreover, the water temperature above 10ºC was the main stimulus for the gametogenesis, final maturation and spawning in both sexes of tench (Breton et al. 1980a, b) and rainbow trout (Nakari et al. 1987).

Our studies strongly suggest a pivotal role of temperature in the final phase of maturation in sticklebacks. Andersson et al. (1992) reported that just temperature, not photoperiod, influenced GnRH (gonadotropin-releasing hormone) binding in the pituitary of sticklebacks in the breeding season. In spring and summer, both males and females showed the highest capacity of GnRH binding sites in response to high temperature.

We have found that the response of males and females to the same temperature/photoperiod conditions is different and in general males matured quicker than females. In males, kept at 12L, 16L or LL regimes, the final phase of maturation, defined by stage of gonads development, distinct secondary sexual characters and nuptial behavior, was evidently stimulated by high temperature; low temperature markedly delayed the breeding readiness. Moreover, our results clearly demonstrated that males subjected to DD or 16D regimes became mature, if only water reached a temperature close to 18ºC. This agrees with observations in other fish species: an absence of light, even for four weeks prior to the breeding, does not inhibit activity of the testis in *Fundulus heteroclitus* (Matthews 1939) and does not stop spermatogenesis in the minnow *Phoxinus laevis* (Bullough 1939). Matthews (1939) also showed a retarding influence of low temperatures on maturation of the sperm. In contrast to males, females subjected to DD or 16D in our study matured later or did not attain

maturity. Most probably light is a more decisive environmental factor in females' than in males' maturation. It is known that sticklebacks begin breeding in April and the rate of gametogenesis in both sexes is different (Sokołowska, Kulczykowska 2006). Most free-living stickleback males complete post-spawning spermatogenesis in late autumn or early winter and are ready to spawn soon after exposure to favorable temperature and photoperiod in spring. However, advanced synthesis of secondary yolk in females begins in spring and thus the lighting condition seems to be an important signal for their maturation. Hence the lack of mature females in Experiment 3 was probably due to relatively short day length at the beginning of March. Our results agree with Merriman and Schedl (1941), who showed that light influences the maturation of the oocytes, but does not affect spermatogenesis. Borg and Van Veen (1982) and Bornestaf et al. (2001) also reported that stickleback females exposed to short photoperiods matured occasionally or not at all. Similarly, four months of short photoperiod or continuous darkness greatly retarded oogenesis in the minnow (Bullough 1940). Long photoperiod is extremely effective also for the maturation of bitterling females (Verhoeven, Van Oordt 1955) and female pink salmon *Oncorhynchus gorbuscha* (Beacham, Murray 1988). We can speculate that the internal time-keeping system controlling reproduction activity of sticklebacks is variously sensitive to temperature and light in males and females.

To gain a better insight into the influence of environmental factors on the final phase of reproduction of sticklebacks, we extended our study to include the observation of social relationships in the group. It is established that only sexually active dominants, which show a broad spectrum of sexual behaviors, can successfully breed. In subordinated males that have little chance of successful courting, spermatozoa may regress in the testes at the end of breeding season. The positive response of females to a courting male is an important signal, which can influence the sexual behavior of the sticklebacks in the group. Sexual behavior can be also affected by other social factors such as density of fish, presence of nuptial males and other conspecifics in the neighborhood.

In our experiments, where fish were subjected to light regimes of 8L, 12L, 16L, and LL along with high temperatures, rigid social relationships with one dominant in the group were observed. Only one aggressive and sexually active male in nuptial colors built a nest and guarded his territory. Upon removing the dominant, one of the subordinates became a leader. Sometimes, changes in color and behavior of mature males were reversed and a leader lost their nuptial features, i.e. colors, aggressive behavior and tendency to isolate. It might be due to the unfavorable conditions (low temperature, short day) or the presence of

other conspecifics, i.e. non-gravid females, which are known to stress the spawning males (FitzGerald 1993).

In stickleback males exposed to high temperatures and constant darkness, a lack of light influenced their behavior, but did not inhibit maturation of gonads. Several males in the group showed all signs of sexual activity and full nuptial condition throughout the experiment. There were even more breeders at DD regime than at other light variants. It is apparent that fish kept in constant darkness have no eye contact with conspecifics. In males, readiness to breed is usually manifested by nuptial coloration and aggressive behavior, thus a lack of visual information on presence of potential rivals results in disappearance of territorial and aggressive behaviors and promotes the development of sexual characters in many individuals at the same time. Simultaneous maturation of several males, which would result in the coexistence of several breeders in the same small area, never occurred in the presence of light. Therefore, we have proposed that visual stimulation is crucial to establishing social relationships in sticklebacks. The prominent role of vision in the exploration of environment and successful courting was highlighted in this species (Wootton 1976; Dzieweczynski, Rowland 2004). The large optic tectum lobes and large retinal surface together with the ability to distinguish colors and shapes, support the important role of vision in sticklebacks (Cronly-Dillon, Sharma 1968; Wootton 1976). Also temperature may affect social relationships in sticklebacks by stimulation (high temperature) or inhibition (low temperature) of gonads' maturation which, in turn, can result in changed reproductive behavior. Other studies confirm that temperature affects the aggressive behavior of males (FitzGerald et al. 1986). In low temperatures, the sticklebacks stop most of their activity and hide under pool banks and in vegetation (Whoriskey et al. 1986, Lachance et al. 1987).

These studies show that an increase in water temperature is a principal signal activating a final phase of gonads' development and reproductive behavior in stickleback males. Lighting seems to be a critical factor in the maturation of females. The presence of light and visual stimuli are vital to establish social relationships in a group of sticklebacks.

REFERENCES

Ahsan S.N., 1966, *Effects of temperature and light on the cyclical changes in the spermatogenetic activity of the lake chub, Couesius plumbeus (Agassiz)*, Can. J. Zool., 44: 161-72

Andersson E., 1992, *Neuroendocrine control of reproduction in the three-spined stickleback, Gasterosteus aculeatus L.*, PhD thesis, Stockholm University, Stockholm, Sweden

Andersson E., Borg B., Goos H.J.Th., 1992, *Temperature, but not photoperiod, influences gonadotropin-releasing hormone binding in the pituitary of the three-spined stickleback, Gasterosteus aculeatus*, Gen. Comp. Endocrinol., 88 (1): 111-16

Baggerman B., 1957, *An experimental study on the timing of breeding and migration in the three-spined stickleback (Gasterosteus aculeatus L.)*, Arch. Néerl. Zool., 12: 105-317

Baggerman B., 1985, *The role of biological rhythms in the photoperiodic regulation of seasonal breeding in the stickleback Gasterosteus aculeatus L.*, Nether. J. Zool., 35 (1, 2): 14-31

Baggerman B., 1989, *On the relationship between gonadal development and response time to photostimulation of sticklebacks living under natural conditions and under constant short-day conditions for long periods of time*, Can. J. Zool., 67 (1): 126-35

Borg B., Ekström P., 1981, *Gonadal effects of melatonin in the three-spined stickleback, Gasterosteus aculeatus L., during different seasons and photoperiods*, Reprod. Nutr. Dev., 21 (6A): 919-27

Borg B., 1982a, *Seasonal effects of photoperiod and temperature on spermatogenesis and male secondary sexual characters in the three-spined stickleback, Gasterosteus aculeatus L.*, Can. J. Zool., 60: 3377-86

Borg B., 1982b, *Extraretinal photoreception involved in photoperiodic effects on reproduction in male three-spined sticklebacks, Gasterosteus aculeatus*, Gen. Comp. Endocrinol., 47 (1): 84-87

Borg B., Van Veen T., 1982, *Seasonal effects of photoperiod and temperature on the ovary of the three-spined stickleback, Gasterosteus aculeatus L.*, Can. J. Zool., 60: 3387-93

Borg B., Peute J., Reschke M., Van Den Hurk R., 1987, *Effects of photoperiod and temperature on testes, renal epithelium, and pituitary gonadotropic cells of the threespine stickleback, Gasterosteus aculeatus L.*, Can. J. Zool., 65 (1): 14-19

Borg B., Bornestaf C., Hellqvist A., Schmitz M., Mayer I., 2004, *Mechanisms in the photoperiodic control of reproduction in the stickleback*, Behaviour, 141 (11-12): 1521-30

Bornestaf C., Borg B., 2000, *Endogenous breeding cycles in male threespine sticklebacks, Gasterosteus aculeatus*, Behaviour, 137: 921-32

Bornestaf C., Mayer I., Borg B., 2001, *Melatonin and maturation pace in female three-spined stickleback, Gasterosteus aculeatus*, Gen. Comp. Endocrinol., 122 (3): 341-48

Boulé V., FitzGerald G.J., 1989, *Effects of constant and fluctuating temperatures on egg production in the threespine stickleback (Gasterosteus aculeatus)*, Can. J. Zool., 67: 1599-1602

Breton B., Horoszewicz L., Billard R., Bieniarz K., 1980a, *Temperature and reproduction in tench: effect of a rise in the annual temperature regime on gonadotropin level, gametogenesis and spawning. I.-The male*, Reprod. Nutr. Dev., 20 (1A): 105-18

Breton B., Horoszewicz L., Bieniarz K., Epler P., 1980b, *Temperature and reproduction in tench: effect of a rise of the annual temperature regime on gonadotropin level, gametogenesis and spawning. II.-The female*, Reprod. Nutr. Dev., 20 (4A): 1011-24

Bullough W.S., 1939, *A study of the reproductive cycle of the minnow in relation to the environment*, Proc. Zool. Soc. Lond., A, 109: 79-101

Bullough W.S., 1940, *The effect of the reduction of light in spring on the breeding season of the minnow (Phoxinus laevis Linn.)*, Proc. Zool. Soc. Lond., 110: 149-57

Burger J.W., 1939, *Some experiments on the relation of the external environment to the spermatogenetic cycle of Fundulus heteroclitus (L.)*, Biol. Bull., 77: 96-103

Candolin U., 1999, *Male-male competition facilitates female choice in sticklebacks*, Proc. R. Soc. Lond. B., 266: 785-89

Candolin U., 2000, *Male-male competition ensures honest signaling of male parental ability in the three-spined stickleback (Gasterosteus aculeatus)*, Behav. Ecol. Sociobiol., 49: 57-61

Candolin U., Salesto T., 2006, *Effects of increased vegetation cover on nesting behavior of sticklebacks (Gasterosteus aculeatus)*, Behav. Ecol. Sociobiol., 59: 689-93

Craig-Bennett A., 1931, *The reproductive cycle of the three-spined stickleback, Gasterosteus aculeatus*, Linn., Philos. Trans. Roy. Soc. Ser. B, 219: 197-279

Cronly-Dillon J., Sharma S.C., 1968, *Effect of season and sex on the photopic spectral sensitivity of the three-spined stickleback*, J. Exp. Biol., 49 (3): 679-87

Davies B., Bromage N., 2002, *The effects of fluctuating seasonal and constant water temperatures on the photoperiodic advancement of reproduction in female rainbow trout, Oncorhynchus mykiss.* Aquaculture, 205: 183-200

De Vlaming V.L., 1975, *Effects of photoperiod and temperature on gonadal activity in the cyprinid teleost, Notemigonus crysoleucas*, Biol. Bull., 148: 402-15

Dzieweczynski T.L., Rowland W.J., 2004, *Behind closed doors: use of visual cover by courting male three-spined stickleback, Gasterosteus aculeatus*, Anim. Behav., 68 (3): 465-71

FitzGerald G.J., Guderley H., Blouin M., 1986, *The effect of temperature upon the aggressive behaviour of male sticklebacks (Gasterosteidae)*, Nat. Can., 113: 235-40

FitzGerald G.J., 1993, *The reproductive behavior of the stickleback*, Scient. Amer., 268 (4): 50-55

Gaudreault A., FitzGerald G.J., 1985, *Field observations of intraspecific and interspecific aggression among sticklebacks (Gasterosteidae)*, Behaviour, 94: 203-11

Guderley H., Foley L., 1990, *Anatomic and metabolic responses to thermal acclimation in the ninespine stickleback, Pungitius pungitius*, Fish Physiol. Biochem. 8 (6): 465-73

Harrington R.W.Jr., 1959, *Effects of four combinations of temperature and daylength on the ovogenetic cycle of a low-latitude fish, Fundulus confluentes Goode & Bean*, Zoologica, 44 (11): 149-68

Hellqvist A., Schmitz M., Mayer I., Borg B., 2006, *Seasonal changes in expression of LH-β and FSH-β in male and female three-spined stickleback, Gasterosteus aculeatus*, Gen. Comp. Endocrinol., 145 (3): 263-69

Ketele A.G.L., Verheyen R.F., 1985, *Competition for space between the three-spined stickleback, Gasterosteus aculeatus L. f. leiura and the nine-spined stickleback, Pungitius pungitius (L.)*, Behaviour, 93: 127-38

Lachance S., Magnan P., FitzGerald G.J., 1987, *Temperature preferences of three sympatric sticklebacks (Gasterosteidae)*, Can. J. Zool., 65: 1573-76

Le Comber S.C., Faulkes C.G., Formosinho J., Smith C., 2003, *Response of territorial males to the threat of sneaking in the three-spined stickleback (Gasterosteus aculeatus): a field study*, J. Zool. Lond., 261 (1): 15-20

Mayer I., Bornestaf C., Wetterberg L., Borg B., 1997, *Melatonin does not prevent long photoperiod stimulation of secondary sexual characters in the male three-spined stickleback Gasterosteus aculeatus*, Gen. Comp. Endocrinol., 108 (3): 386-94

Matthews S.A., 1939, *The effects of light and temperature on the male sexual cycle in Fundulus*, Biol. Bull., 77: 92-95

Medlen A.B., 1951, *Preliminary observations on the effects of temperature and light upon reproduction in Gambusia affinis*, Copeia, 2: 148-52

Merriman D., Schedl H.P., 1941, *The effects of light and temperature on gametogenesis in the four-spined stickleback, Apeltes quadracus (Mitchill)*, J. Exp. Zool., 88: 413-49

Nakari T., Soivio A., Pesonen S., 1987, *Effects of an advanced photoperiod cycle on the gonadal development and spawning time of 2-year-old Salmo gairdneri R. reared in earth ponds under extreme annual water temperatures*, Aquaculture, 67 (3-4): 369-84

Pankhurst N.W., Purser G.J., Van Der Kraak G., Thomas P.M., Forteath G.N.R., 1996, *Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the rainbow trout Oncorhynchus mykiss*. Aquaculture, 146: 277-90

Pankhurst N.W., Thomas P.M., 1998, *Maintenance at elevated temperature delays the steroidogenic and ovulatory responsiveness of rainbow trout Oncorhynchus mykiss to luteinizing hormone releasing hormone analogue*. Aquaculture, 166: 163-77

Peeke H., Wyers E., Herz M., 1969, *Waning of the aggressive response to male models in the three-spined stickleback (Gasterosteus aculeatus L.)*, Anim. Behav., 17 (2): 224-28

Sokal R.R., Rohlf F.J., 1995, *Biometry*, New York, W.H. Freeman and Company, pp. 887

Sokołowska E., Kalamarz H., Kulczykowska E., 2004, *Seasonal changes in brain melatonin concentration in the three-spined stickleback (Gasterosteus aculeatus): towards an endocrine calendar*, Comp. Biochem. Physiol., A, 139 (3): 365-69

Sokołowska E., Kulczykowska E., 2006, *Annual reproductive cycle in two free living populations of three-spined sticklebacks (Gasterosteus aculeatus L.): patterns of ovarian and testicular development*, Oceanologia, 48 (1): 103-24

Verhoeven B., Van Oordt G.J., 1955, *The influence of light and temperature on the sexual cycle of the bitterling, Rhodeus amarus (preliminary communication)*, Proc. Kon. Nedl. Akad. Wetsch. Amst., C, 58: 628-34

Von Hippel F.A., 1999, *Black male bellies and red female throats: color changes with breeding status in a threespine stickleback*, Env. Biol. Fish., 55 (3): 237-44

Whoriskey F.G., FitzGerald G.J., Reebs S.G., 1986, *The breeding-season population structure of three sympatric, territorial sticklebacks (Pisces: Gasterosteidae)*, J. Fish Biol., 29: 635-48

Wootton R.J., 1971, *Measures of the aggression of parental male three-spined sticklebacks*, Behaviour, 40 (3-4): 228-61

Wootton R.J., 1976, *The biology of sticklebacks*, London, Academic Press, pp. 387