

## Diel patterns of fanning activity, egg respiration, and the nocturnal behavior of male three-spined sticklebacks, *Gasterosteus aculeatus* L. (f. *trachurus*)

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Observations of male three-spined stickleback (*Gasterosteus aculeatus* L.) parental care were made in a salt-marsh tide pool at 3-h intervals over 24-h cycles, to determine if male behavior varied in association with diel changes in water temperature and dissolved oxygen concentration. An analysis of egg metabolism in situ revealed positive correlations between rate of egg oxygen consumption and egg age, water temperature, and dissolved oxygen levels, while there was a slight but significant negative correlation with the number of eggs per nest. We found no significant difference when we compared the proportion of time males spent fanning their nests during the day with night levels. However, nocturnal fanning bouts were significantly longer and less numerous than diurnal ones. In contrast to the rest of the 24-h cycle, the males remained inactive between fanning bouts at night, except when exposed to hypoxic conditions

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Des épinoches à trois épines (*Gasterosteus aculeatus* L.) mâles ont été observées à toutes les 3 h au cours de cycles de 24 h, alors qu'elles étaient en période de reproduction dans des cuvettes de marée. L'objectif était de déterminer si leur comportement parental variait en fonction des changements circadiens qui surviennent au niveau de la température de l'eau et de sa teneur en oxygène dissous. Nous avons trouvé des corrélations significatives positives entre le taux de consommation d'oxygène et les variables suivantes: l'âge des oeufs, la température de l'eau et l'oxygène dissous. Une faible corrélation négative mais significative a été observée entre le nombre d'oeufs par nid et le taux de consommation d'oxygène. Nous n'avons remarqué aucune différence significative entre le jour et la nuit dans la proportion de temps consacré par chaque mâle à la ventilation de son nid. Par contre, les séquences de ventilation furent significativement plus longues et moins nombreuses la nuit. Contrairement au reste du cycle de 24 h, les mâles sont demeurés inactifs entre les séquences de ventilation pendant la nuit, sauf lorsqu'ils furent exposés à des conditions d'hypoxie.

### Introduction

The male three-spined stickleback is well-known for its elaborate reproductive behavior. After establishing a territory, building a nest, courting females, and then fertilizing the eggs laid in its nest by one or several females, a male enters the parental phase of its reproductive cycle and fans its eggs during their development (Wootton 1976). This parental activity has been the subject of many behavioral studies in both laboratory (van Iersel 1953; Sevenster 1961; van den Assem 1967; Sargent and Gebler 1980) and field (Wootton 1972; Kynard 1979; FitzGerald 1983) conditions. However, these authors only reported daylight observations; the nocturnal behavior of the parental male remained unknown. Moreover, little is known of the diel activity of sticklebacks, especially in a natural situation. From observations on diel patterns of captures in minnow traps, Worgan and FitzGerald (1981a) suggested that nonterritorial males were quiescent at night but the authors made no direct observation of territorial males' nocturnal activity.

Ward and FitzGerald (1983) recorded sharp nocturnal decreases in water temperature and dissolved oxygen concentrations within the tide pools of our study site. In one pool, water temperature and oxygen content fell from 30°C and 15 mg/L in the afternoon to 8°C and 1 mg/L, respectively, just before dawn. Large numbers of three-spined sticklebacks breed in these pools (FitzGerald 1983), but it is not known how they

react to these nocturnal environmental conditions. Low nocturnal oxygen concentrations in the water may adversely affect their behavior (see Jones 1952) and possibly interfere with parental care. The environmental day–night fluctuations may also affect fish-egg metabolism (Braun 1978). The intensity of a male stickleback's fanning is influenced by egg metabolic rate (van Iersel 1953; Sevenster 1961), thus the nightly environmental changes in the marsh might be expected to alter paternal behavior. For example, if low temperatures slow down egg metabolism at night, the male may fan less because of lower egg oxygen requirements. However, the concurrent low dissolved oxygen concentrations may mean that less oxygen is available to the eggs, and males might then be expected to increase their levels of fanning. Both increases (Albrecht 1969) and cessations (Moyer and Bell 1976; Ross 1978) of fanning have been observed during the night in other fishes exhibiting parental care. Here we report our quantitative and qualitative observations of male parental behavior over the 24-h cycle, as well as data on diel rates of stickleback egg metabolism.

### Materials and methods

The study site was the salt marsh of the Isle Verte National Reserve, located 25 km east of Rivière-du-Loup, Québec, on the south shore of the St. Lawrence estuary. Three species of sticklebacks, three-spined *Gasterosteus aculeatus*, nine-spined *Pungitius pungitius*, and black-spotted *Gasterosteus wheatlandi*, breed there in shallow (<0.5-m) tide pools during May and June. The pools are located in the *Spartina patens* zone and are flooded twice a month. They are usually free of algae in May but growths of *Enteromorpha* spp., *Percursaria* spp., and *Ruppia maritima* commonly occur in June. Minimum and maximum values of pool-water temperature were recorded daily from May 9 to July 2, 1982. The minimum temperature usually occurred just

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before dawn and varied between 2.8 and 16.0°C (mean = 8.7). Maximum values varied between 12.8 and 30.0°C (mean = 22.7) and usually occurred in the middle of the afternoon. Other details of the study site are given in Worgan and FitzGerald (1981b) and Ward and FitzGerald (1983).

We conducted our work in May and June 1982. To determine rates of egg metabolism, clutches of at least 50 eggs were collected and each clutch individually incubated within their tide pools, in a 136- or 223-mL black bottle for 30 min. The eggs in each clutch were stuck together in a mass, and care was taken to leave the mass in one piece. Each bottle was gently and regularly agitated during the incubation period. Oxygen concentration and water temperature in each bottle were determined (Yellow Springs Instrument Co. (YSI) model 56 oxygen meter) before and after the incubation period. No filtration was performed on the water used for the incubations, but controls (i.e., water without eggs) were run concurrently to correct for any oxygen uptake the water might have had. Usually there was no change between the initial and final oxygen concentration of the controls. After one incubation period the eggs were preserved in 10% formalin for later counting, and determination of the stage of egg development. Egg stage was classified as 1, 2, 3, or 4 based on descriptions given in Wootton (1976). Under laboratory conditions these stages roughly correspond to the following periods: (1) fertilization–30 h; (2) 30.1–60 h; (3) 60.1–90 h; (4) >90 h.

We made 74 trials during night periods, and 160 spread out over daylight hours. Clutch size varied between 52 and 2553 live eggs. Egg stage of development showed little intraclutch variation, but varied among clutches from freshly fertilized to nearly hatched. Water temperature and dissolved oxygen concentrations ranged from 6.0 to 26.7°C and 3.3 to 16.6 mg/L, respectively, during the trials.

These data were analysed using the maximum  $R^2$  improvement for the dependent variable (multiple regression) technique as described in the Statistical Analysis Systems (SAS) package (Helwig and Council 1979). Oxygen consumption was the dependent variable while the stage of egg development, number of eggs per clutch, incubation water temperature, initial oxygen concentration, and hour of the day were the independent variables. The dependent variable was  $\sqrt{x + 0.5}$  transformed which rendered it normal.

For behavioral observations, we located the nests of five fanning males, all in the same part of a large (>50 m<sup>2</sup> surface area) pool. This pool was chosen among the many available because its high banks minimized water rippling as a result of the wind, and enabled us to make uninterrupted observations. The territories of all males adjoined one another and covered a total area of about 2.0 m<sup>2</sup>. All the nests were at depths of 10–17 cm and were between 40–150 cm from the bank. The minimum internest distance was 50 cm. One male (male E) had its nest totally surrounded by algae and was completely concealed from the sight of other fish. Two nests (males C and D) were half surrounded by algae and two nests (males A and B) were in the open.

Every 3 h from 1700 June 8 to 1400 June 10 these five males were observed individually for 10 min in a fixed order (A, B, C, D, E). Male C was also observed from 1700 June 5 to 1400 June 6. This gave us a total of 11 diel patterns of activity. We also made daily observations of all males at 1100 and 1400 after June 10 until no more fanning was shown by a given male. This occurred on June 10, 11, 17, 18, and 18 for males C, A, D, E, and B, respectively.

We made all observations from behind a blind installed on the bank of the pool. Night observations were made using a night vision scope (Javelin model 226) which allowed us to easily follow the fish in approximately full-moonlight levels or when a very veiled artificial source of light was present. The fish did not appear to alter their behavior in the artificial light source. Behavioral data were recorded with a Datamyte 800 (Electrogenic Corp.) event recorder. For each fish we noted (i) percent of time spent fanning, (ii) mean length of fanning bouts, (iii) number of fanning bouts, (iv) number of aggressive interactions with other fish, (v) number of times the male left the nest, and (vi) total time spent away from nest. Fanning bouts were often interrupted by "boring," an activity in which the male enlarges the entrance of its nest with its snout, or pushes additional holes in its

nest (van Iersel 1953). Unlike van Iersel's (1953) study, we considered fanning bouts separated by boring as different events. Though boring still flushes some water through the nest it has other functions than aeration, it is not an efficient aerator and is mutually exclusive with fanning. Aggression was classified as either chase, roundabout, or spine fighting (Wootton 1976). Threat displays (van Iersel 1953) were not quantified as aggression because we were unable to identify them at night in the narrow field of the Javelin night vision scope. A fish was classified as being away from its nest when it went more than 20 cm away. Movements up to this distance were centered around the nest, and the male often oriented toward the nest during these displacements. At further distances the males were usually fully occupied with territorial defense or courtship. Water temperatures and dissolved oxygen concentrations were measured with the YSI oxygen meter immediately after each observation period in a water sample taken 2 cm below the pool surface.

The rest of the statistical procedures follow Kirk (1968) and Sokal and Rohlf (1981). Our behavioral data were not amenable to parametric analysis despite attempts to find a suitable transform. Consequently we opted for a Friedman one-way ANOVA for random blocks with the time of day considered as treatment effects and individual fish as blocks. The Friedman technique is appropriate for an experimental design using repeated measures on the same subject, providing treatment levels are administered in a random fashion (Kirk 1968). We violated this assumption and used a fixed order in our observations because of weather conditions, logistical considerations, and because when time is a variable it is not easily manipulated. However, we have little reason to postulate a fixed relationship between fish activity and time of day (Walsh and FitzGerald 1984). Physical conditions in the habitat are largely unpredictable (Ward and FitzGerald 1983) so when a fish performs behavior 1 at time  $T$  there is no reason to presuppose behavior 2 at  $T + 1$ . For these reasons, and because nonparametric procedures are usually quite robust (Siegel 1956), we feel our violation would have at worst a minor effect on the probability of accepting or rejecting the null hypothesis.

We tested differences between times of day with nonparametric multiple comparisons STP (simultaneous test procedure). Unless otherwise indicated the level of significance is 95%. Kendall's tau correlations, which are appropriate for multiple measures and do not assume independence, were also calculated.

## Results

### *Egg metabolism*

The independent variables in the multiple regression analysis accounted for 48% of the variance in egg oxygen consumption ( $R^2 = 0.48$ , Table 1). The stage of egg development accounted for most of the explained variance, while the number of eggs per clutch, incubation temperature, and oxygen concentration at the start of the trial made smaller but significant contributions. Including the hour at which a trial was conducted in the model did not significantly increase the amount of explained variance.

Partial correlation coefficients indicated that, over the range of values tested, oxygen consumption per egg increased with increases in age of eggs, water temperature, and dissolved oxygen concentration. There was a slight but significant negative correlation between oxygen concentration and number of eggs per clutch. We interpret this latter observation as a crowding effect (see McQuinn *et al.* 1983). Stickleback eggs within a nest are stuck together in a large mass. In these situations, as the number of eggs in a nest increases some eggs probably experience oxygen deprivation, depending on the shape of a given mass of eggs.

### *Male behavior*

All five males continued fanning their nests at night (Fig. 1).

TABLE 1. Results of the multiple regression analysis of egg oxygen consumption on stage of egg development (X1), number of eggs in the clutch (X2), water temperature (X3), dissolved oxygen levels at the start of an incubation (X4), and time of day (X5). Partial correlation coefficients are given, as well as the sums of squares (SS, type II) which would be added to the error term if any given variable was not included in the model ( $R^2 = 0.48$ )

	Partial correlation coefficients	Type II SS	F	P
X1	0.4651	72.64	140.06	<0.01
X2	-0.0006	22.88	44.11	<0.01
X3	0.0365	5.08	9.79	<0.01
X4	0.0562	4.77	9.20	<0.01
X5	0.0000	0.01	0.02	NS

	df	SS	F	P
Regression	4	141.63	68.5	<0.01
Error	293	151.45		
Total	297	293.08		

NOTE: NS, not significant.

On average, males spent 49% of their time fanning at night (2300 and 0200) and this was slightly but not significantly higher than the proportion (42%) shown during the day (1100 and 1400). The percentages of time spent fanning were not equal throughout the 24-h cycle ( $\chi^2 = 26.3, P < 0.005, df = 7$ ) but the only significant differences we found were in comparisons of the value recorded at 0200 with those recorded during the evening (1700 and 2000). Oxygen concentration reached its lowest level at 0200 (Fig. 1), while the evening was the time when we observed the peak of nest leavings and aggressive interactions. Most of this aggression was directed at feeding schools of females, which were very active in the evening and early morning; the majority of the nest leavings were associated with the need for males to attack intruding females at the boundaries of their territories. Over the 24-h cycle, the percent of time spent fanning was negatively correlated with both the level of aggression and the oxygen concentration (Table 1).

The time of day was related to fanning-bout length ( $\chi^2 = 52.6, P < 0.001, df = 7$ ) and frequency ( $\chi^2 = 27.2, P < 0.001, df = 7$ ). Fanning bouts were significantly longer and less numerous at night than during the day (Fig. 1). In general, the shortest fanning bouts occurred when the fanning bout frequency was maximum, whereas the longest ones corresponded in time to the lowest fanning bout frequency. These maxima and minima paralleled maximum and minimum values of water temperature and oxygen content, and occurred at either the end of the night (0200 and 0500) or the middle of the afternoon (1400), depending on which fanning-bout characteristic is considered (Fig. 1). Both fanning-bout length and frequency were correlated with both water temperature and dissolved oxygen over the 24-h cycle (Table 2), though it is difficult to determine which one of these two environmental factors may actually affect the fanning regime because they both covary to a certain extent over the 24-h cycle. We observed the bouts of fanning to be frequently interrupted, during the day, by aggression and by nest-directed activities such as boring. Qualitatively, the nest-directed activities seemed to

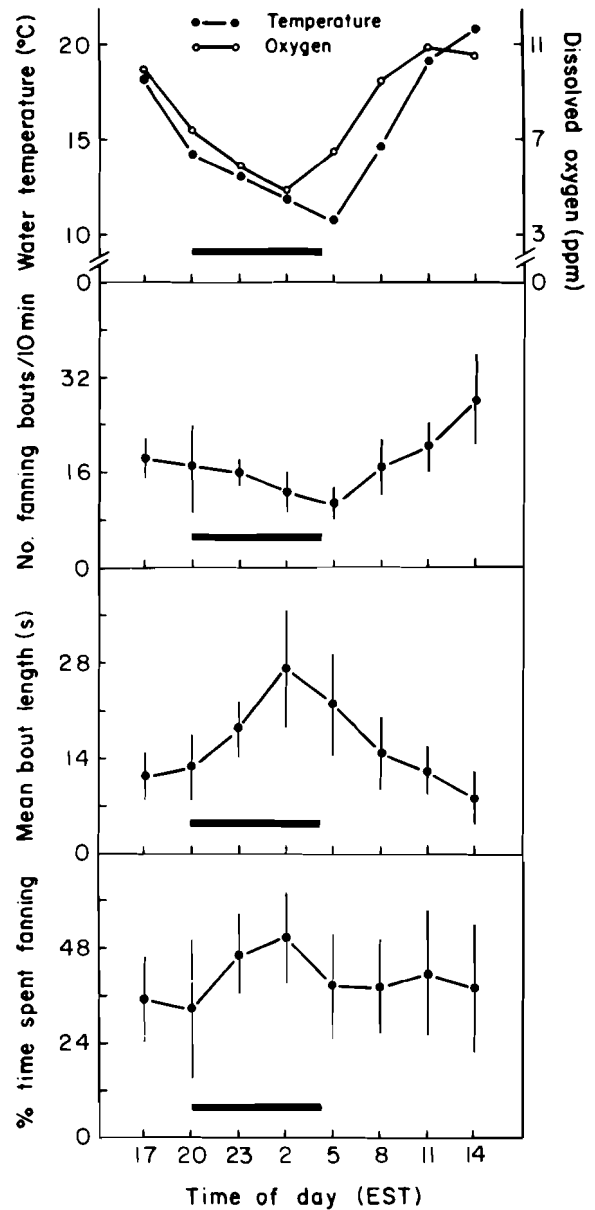


FIG. 1. Diel fanning activity of parental male three-spined sticklebacks (June 6, 9, and 10, 1982). Eleven similar patterns are plotted together as means  $\pm 1$  SD. Mean diel variations in water temperature and oxygen content are also given. The horizontal black bars represent the interval between sunset and sunrise.

occur more often at 1400 when temperature reached its highest level.

All the correlations involving temperature and aggression persisted on a day-to-day basis (Table 2). For the fish we observed daily from June 8 to 18, the peaks in fanning-bout frequency, the corresponding lows in fanning-bout length, and the peaks in water temperature occurred on the same days (Fig. 2). Although water temperature and oxygen concentration tended to covary, only water temperature was significantly correlated with both fanning-bout length and frequency (Table 2). Aggression was negatively correlated with both total fanning time and with fanning-bout length, but there was no correlation between water oxygen content and the proportion of time spent fanning (Table 2).

The males were relatively inactive at night between the fanning bouts. In contrast to the observations from the rest of the

TABLE 2. Correlation coefficients (Kendall's tau) between fanning activity and selected behavioral and environmental variables, based on 10-min observation periods per fish. On the diel basis, observations were made on five fish at 3-h intervals from 1700 to 1400; on the daily basis, they were made on three fish at 1100 and 1400 each day from June 8 to 18, 1982

	Diel observations <sup>a</sup>			Observations on successive days <sup>b</sup>		
	% time spent fanning	Fanning-bout frequency	Fanning-bout length	% time spent fanning	Fanning-bout frequency	Fanning-bout length
Dissolved oxygen concentration	-0.331**	0.280**	-0.453**	0.000	0.207	-0.278*
Water temperature	-0.138	0.233**	-0.286**	-0.109	0.334**	-0.239*
No. of aggressive interactions	-0.438**	0.069	-0.373**	-0.514**	0.192	-0.392**

NOTE: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .

<sup>a</sup> $n = 73$ .

<sup>b</sup> $n = 43$ .

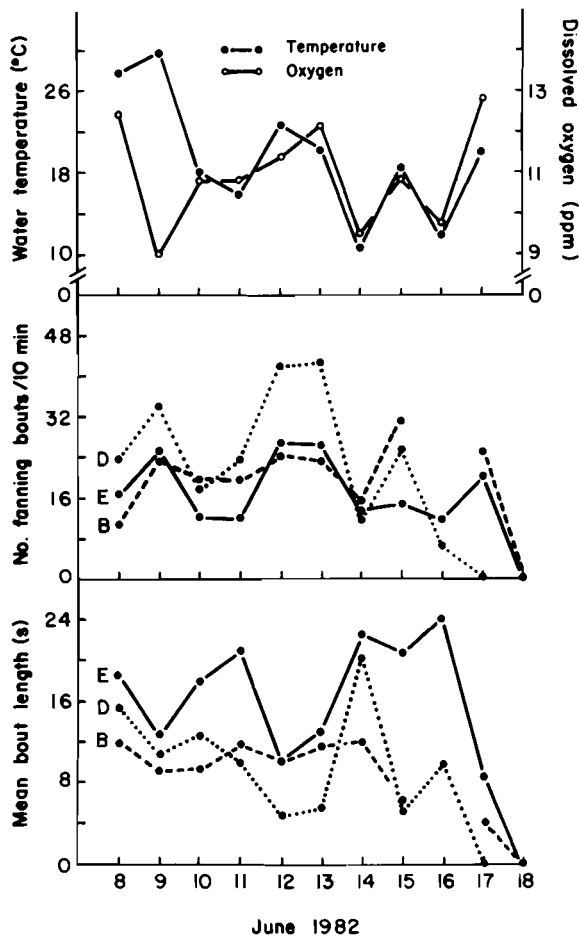


FIG. 2. Day-to-day variations in water temperature, dissolved oxygen concentration, and in the fanning-bout length and fanning-bout frequency displayed by three male (B, D, and E) three-spined sticklebacks breeding in a tide pool. Each point represents the mean of two 10-min observations made daily at 1100 and 1400. June 16 data for male B are missing.

24-h cycle, the males never interacted aggressively and very seldom left the nest at 2300 and 0200; there was also no boring. Males usually swam slowly within 20 cm of the nest, sometimes holding station over it. We witnessed only two exceptions to this nocturnal inactivity. The first one took place at

0200 on June 9: dissolved oxygen concentration fell slightly below 2.8 mg/L (water temperature was 16°C) and all five males were swimming in horizontal circles between fanning bouts, ventilating their gills at a high rate. We counted a minimum of 150 opercular openings/min in the only fish for which this could be done with accuracy. Jones (1952) reported normal rates of 96 respiratory movements/min at 13°C for the three-spined stickleback and observed an increase up to 164/min when dissolved oxygen fell to 6.6 mg/L. Two of the males (D and E) performed these activities near the surface with their snout pointed slightly upward, evidently using the more abundant oxygen dissolved in the surface layer (aquatic surface respiration, see Lewis 1970). Since their nests were near algal clumps, the oxygen concentration near them may have been even lower than 2.8 mg/L because of the algal nighttime respiration. Despite their apparent stress, all the males continued fanning their nests. Males A and C showed the highest percentages of time spent fanning during the whole study (67 and 64%, respectively). Male D spent 54% of its time fanning and had one of the highest mean bout lengths recorded during the study (40.2 s). Male B had an intermediate value of 45% of time spent fanning, and male E spent only 35% of its time fanning. Male E, who was performing aquatic surface respiration and whose nest was completely surrounded by algae, might have been bothered by the lack of dissolved oxygen; 3 h before, with the oxygen content reaching 5.5 mg/L, it was spending 50% of its time fanning. The second exception occurred at 2300 on June 7 while we were making preliminary observations in another pool. Five females raided (see Wootton 1976) the nest of the male under observation. The male first engaged in fighting with the females but it fled soon after. Despite the egg cannibalism in the raid, 52 eggs were found in the scattered nest the next morning; however, the male had left the site.

### Discussion

What do our results mean from the point of view of the parental male stickleback? The analysis of the egg-metabolism data demonstrated that the stage of egg development, number of eggs per clutch, surrounding water temperature, and ambient oxygen levels are factors significantly affecting the rate of egg oxygen consumption (Table 1). Developing eggs demand more oxygen as they get older, and they get older with each passing minute of the parental cycle. In addition, the number of eggs

in a nest (barring predation or other mortalities) should not change. This puts a constant, and probably increasing burden on a male to fan its nest. But egg metabolism is also influenced by ambient temperature and oxygen levels.

Conditions in the salt marsh are extremely variable. Within the tide pools, water temperatures and oxygen levels often fall during the evening, but this pattern is not fixed (Ward and FitzGerald 1983). If air temperatures stay high and (or) enough wind is present, water temperatures and oxygen levels can remain high. This in turn could lead to high egg metabolism and a high 24-h demand for fanning. Low ambient oxygen levels with high temperatures and well-developed eggs would do the same. The parental fish has no control over these factors, but while the burden to fan may be present during both the day and night, we observed that the way fanning was delivered to the nest changed over these periods.

Fanning bouts were significantly longer and less numerous at night than during the day (Fig. 1). This change is mainly caused by an increase in bout-interrupting activities such as aggression and boring. The majority of aggression occurred in the morning and late afternoon as males chased foraging females out of their territories. Boring activity, in turn, can be elicited when low-oxygen water is run through a male's nest (van Iersel 1953). This suggests boring may vary proportionally with egg metabolic rate. Generally, egg oxygen consumption should be higher during the day, and this may lead in turn to interruptions of fanning to bore. This would help explain our observation that the shortest but most frequent bouts of fanning occurred during peak water temperatures at 1400. In any event, van Iersel (1953) found the time of hatching of stickleback eggs to be better correlated with average bout length than with the total time spent fanning, so the night pattern seems more favorable to rapid egg development. It further suggests that the reproductively most successful males will be those that minimize interruptions to their fanning regimes. This could be accomplished by hiding the nest or decreasing territory size to reduce aggressive encounters. Both of these patterns have already been noted (FitzGerald 1983; Sargent and Gebler 1980).

The correlation coefficients between fanning activity and behavioral or abiotic factors in Table 2, although sometimes significant, are all low. The behavior of the fish at Isle Verte seemed to show high interindividual variation. This may have been due to differences in nest cover, number of neighbours, or stage of the parental cycle, or it may be intrinsic to the nature of each fish (e.g., Rowland 1982). For example, male E often displayed much longer fanning bouts than males B and D (Fig. 2). It is possible that male E was less often disturbed since its nest was in 100% cover. Sargent and Gebler (1980) found that males nesting in concealment engaged in fewer territorial encounters per minute than did males not in concealment. However, those hidden males had a lower mean fanning-bout length and a higher number of fanning bouts per minute. More extensive studies are needed to assess the importance of nest cover and other factors on stickleback fanning behavior at Isle Verte.

Direct observations have shown that during the non-reproductive period several fish species active during the day become quiescent at night, either resting on the bottom or hiding in shelters (Ebeling and Bray 1976; Emery 1973; Helfman 1981; Hobson 1965; Luckhurst and Luckhurst 1978). Emery (1973) reported that many freshwater species living in an Ontario lake, including the nine-spined stickleback, underwent

a nocturnal torpidity, decreasing their level of activity at night and apparently becoming insensitive to external stimuli. He suggested that such a torpidity could be a way to avoid detection by nocturnal predators or to conserve metabolic energy. This last function may be important to the parental male three-spined sticklebacks in our tide pools. The decrease in metabolic activity may only be the result of the decrease in water temperature, but given the fact that a male had to attend and fan the eggs day and night during a period of at least 10 days (Fig. 2), any metabolic saving may be of importance if the parent fish is to keep in sufficiently good condition to undertake further parental cycles.

Such an inactivity must not be confounded with a state of somnolence provoked by low availability of oxygen in the water (for an example of such a somnolence, see Cichocki 1977). Even at oxygen concentrations of 2.8 ppm the fish were apparently able to correctly fan their nests. Except for male E in the night of June 8–9, on no occasion have we observed the males to significantly lower their total time spent fanning at night as compared with daytime. Moreover, parental male black-spotted sticklebacks appear to have the same diel patterns of fanning activity as male three-spined sticklebacks (personal observation, three diel patterns recorded on June 4, 5, and 6, 1982). It seems, therefore, that the nightly conditions occurring in the tide pools in early June do not seriously interfere with males' capacity to care for their eggs.

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