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Latitudinal variation in photoperiodic response of the three-spined stickleback *Gasterosteus aculeatus* in western North America

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Reproductive maturation in both male and female three-spined stickleback *Gasterosteus aculeatus* was strongly photoperiodic in a northern population (Alaska, 61° N) but not in a southern population (Oregon, 43° N) from western North America. Increasing reliance on photoperiod with increasing latitude is a general phenomenon among vertebrates, and is probably due to the anticipation of a narrower window of opportunity for reproduction and development at higher latitudes.

Key words: biogeography; evolution; kidney; seasonal development; sexual dimorphism.

INTRODUCTION

At temperate and polar latitudes with distinct summer and winter seasons, the ability to exploit the favourable season, to avoid or mitigate the unfavourable season and to make a timely transition between the two lifestyles are all components of fitness. Animals use the length of day, or photoperiod, to time their seasonal development, reproduction, migration and dormancy (Bradshaw & Holzapfel, 2007). Generally, the influence of day length on the seasonal activities of vertebrates increases with latitude but, compared with arthropods, there are very few experimental studies involving vertebrates and none, as far as is known, involves latitudinal variation among populations of a single species of fish. Herein, intraspecific variation in response to day length of northern and southern populations is examined in the three-spined stickleback *Gasterosteus aculeatus* L.

In fishes with long gonadal cycles, reproduction and migration are generally cued by a combination of a circannual clock whose period of oscillation is approximately annual and by increasing and decreasing day lengths that serve to set the circannual clock; fishes with short gonadal cycles are less dependent on a circannual clock and more dependent on a direct response to a single constant day length (Bromage *et al*., 2001; Bradshaw & Holzapfel, 2007). *Gasterosteus aculeatus* L. is a small fish found

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*Author to whom correspondence should be addressed. Tel.: +1 541 346 4542; fax: +1 346 2364; email: mosquito@uoregon.edu in marine, estuarine and freshwater habitats (Bell & Foster, 1994) that has long been a model for studies of ecology and behaviour (Wootton, 1976), and has recently been used for studies of the microevolution of developmental processes (Cresko *et al*., 2007). It has a weak circannual rhythm, has a strong response to constant day lengths and is distributed in the Holarctic from *c*. 35–70° N (Baggerman, 1985; Bornestaf & Borg, 2000).

The most thorough studies of photoperiodism in *G. aculeatus* use populations collected from nature, mainly the Baltic Sea (*c*. 56–59° N; 12–30° E). At this latitude, long days promote reproduction in both males and females during the late spring and early summer (Borg, 1982; Borg *et al*., 2004). In males, long days promote a sexual syndrome of colour change from drab to bright, territoriality, kidney hypertrophy and spiggin (glue for nest building) production, nest building and courtship (Borg *et al*., 2004). In early autumn, fish go through a brief refractory period (Borg, 1982). Later in the autumn, fish become reproductively responsive to long days and critical photoperiod (the median day length required to trigger breeding phenotype) declines, probably due to decreasing autumnal day lengths and temperature (Baggerman, 1972, 1985). Increasing day lengths in the spring then promote gonadal maturation in females and spiggin production in males so that, in the southern Baltic Sea, the breeding season of marine *G. aculeatus* peaks from early May to early July (Borg, 1982).

In the Rhône River delta at *c*. (43° 20′ N; 4° 58′ E), the breeding season of anadromous *G. aculeatus* peaks earlier in February and March (Crivelli & Britton, 1987). While Crivelli & Britton (1987) did not identify the environmental cues determining the migration and spawning in the Rhône Delta, the difference in phenology between the Baltic Sea and Rhône Delta populations suggests that there may be a geographic difference in response to day length between northern and southern European populations. To pursue the question of geographic variation in photoperiodic response of *G. aculeatus*, photoperiodic response of *G. aculeatus* was determined among western North American populations over a range of 18° N latitude. Previous studies in Europe have considered wild-caught fish whose photoperiodic response may be altered by their environmental history. To determine genetic difference among geographic populations, the first (G_1) or third (G_3) generation of laboratory maintained fish were reared on short days, and upon attaining adult size, were exposed to different constant day lengths at 20° C.

MATERIALS AND METHODS

COLLECTION AND LABORATORY LINES

The Alaskan stock was collected from Rabbit Slough (61°34′ N; 149°15′ W). In nature, this population has an anadromous life history with spring runs beginning in mid-May and ending in mid-June. The animals used for these experiments were G_3 outbred descendants from wild-caught individuals. The Oregon *G. aculeatus* were collected from Eel Creek (43°35′ N; 124°11′ W), which is a small perennial stream contiguous with the Pacific Ocean *via* a larger, connecting waterway. This population is probably resident within the stream system as all fish trapped in Eel Creek show a consistent low-armour phenotype (indicative of resident freshwater fish) and a unique body morphology that is a subset of that found in the larger connecting stream (Q. S. Yeates-Burghart & T. Bolle, unpubl. data). Furthermore, fish in breeding condition are found in Eel Creek only during a short time from mid-March to late

April. The animals used for these experiments were G_1 outbred descendants from wild-caught individuals.

The experimental fish were produced and hatched using standard crossing protocol (Cresko *et al*., 2004). To prevent transmission of disease or parasites, *G. aculeatus* embryos were bleached in a 6% solution of sodium hypochlorite on days 2 and 5 post-fertilization. After hatching, the fry were put in a 75·7 l aquarium at a density of 1·25 fish l^{-1} under a 10L:14D cycle at 20° C in saline adjusted to 7 with Instant Ocean (http://www.instantocean.com) using a refractometer. Juvenile *G. aculeatus* were fed dry food (Zeigler Larval AP100, 250–450 µm; www.zeiglerfeed.com) and freshly hatched brine shrimp *Artemia* sp. (www.sfbb.com). Adults were fed dry food (Nelson's silver cup fish feed, trout fry, 0·59–1·38 mm; www.silvercup.com).

Experimental fish were maintained as fry, juveniles and adults (>50 mm long) on the 10L:14D cycle for 10–12 months (Alaska fish) or 10–11 months (Oregon fish). All fish used in experiments were at least 55 mm long measured from the dorsum of the pre-maxilla to the caudal peduncle. Within each population, fish from several parental lines were pooled according to sex and paired (one male and one female) haphazardly into 4 l tanks for experiments. Several pairs of fish were exposed to different L:D cycles (Fig. 1), not a single 10L:14D cycle. Any fish that died was not replaced. At the end of 6 weeks, all surviving fish were included in the data set.

PHOTOPERIOD CABINETS AND HUSBANDRY

Experiments were run in light-tight, air-cooled cabinets located in climate-controlled rooms held at 20° C. Photoperiod cabinets consisted of six 29 cm × 40 cm × 53 cm (H × W × D) chambers with five shelves each. Each shelf accommodated three, 4 l, continuously aerated tanks and was illuminated by a twilight lamp (a single Lumex SLX-LX5093UWC/G water-cool white LED in a 3·5 cm translucent spherical reflector; www.lumex.com) and a daytime fixture (Sylvania white, nine-LED strip light, LED/UC/W/9/W, www.sylvania.com). Twilight was simulated by turning on the twilight bulb for 15 min before and for 15 min after the daylight fixture. Twilight and daylight were programmed with Chrontrol XT electronic timers (www.chrontrol.com). Fish were fed daily and the water was changed every 2 days in each tank using Instant Ocean saline solution adjusted to 7 using a refractometer.

Experiments with Alaskan fish were run in two blocks. The first block used a broader span of day lengths to identify the region of the critical photoperiod. The second block repeated day lengths at which there had been incidental death in the first block (to increase sample size) and also used a narrow span of day lengths to refine the estimate of the critical photoperiod. The data from both blocks were combined. Experiments with Oregon fish were run as a single block.

ASSAYS OF SEXUAL MATURATION

In *G. aculeatus*, ovarian growth and kidney hypertrophy are reliable indicators of sexual maturation (Mayer *et al*., 2004). Ovaries enlarge during the maturation of eggs and male kidneys enlarge during the production of spiggin. To quantify sexual development across light treatments, the ovary-somatic index (I_O) and the kidney-somatic index (I_K) were determined. Kidneys or ovaries were excised, dried to constant mass along with their respective owners at 37° C and weighed using a Mettler AT261 DeltaRange electronic balance (www.mt.com). The I_O was calculated as the ratio of ovary to total body mass and the I_K as the ratio of kidney to total body mass.

RESULTS

Sexual maturation in Alaskan *G. aculeatus* exhibited a sigmoid dose–response curve that increased with day length for both I_O (ANOVA, $F_{7,63}$, $P < 0·001$) and I_K (ANOVA, $F_{7,51}$, $P < 0·01$; Fig. 1). Sexual maturation in Oregon *G. aculeatus* did

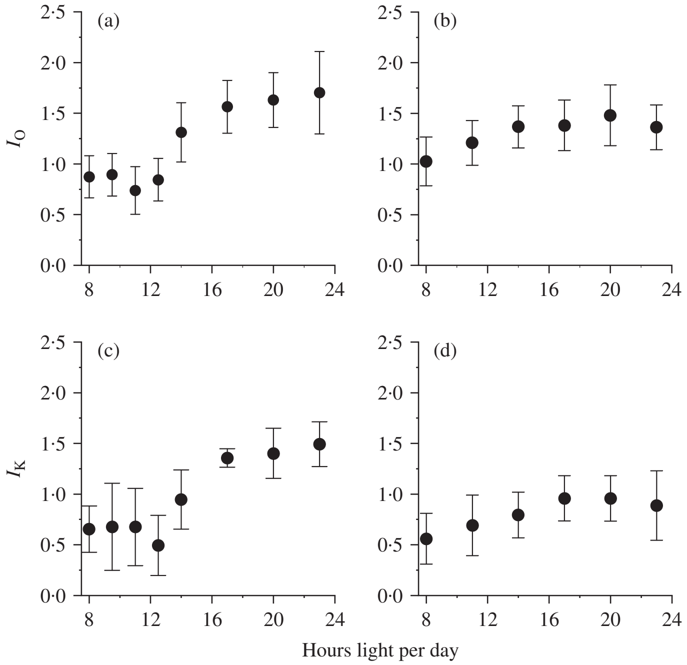

FIG. 1. Photoperiodic response of (a), (c) Alaskan ($n = 61$) and (b), (d) Oregonian ($n = 43$) *Gasterosteus aculeatus* from western North America of (a), (b) ovary (I_O) and (c), (d) kidney (I_K) to total body mass in females and males, respectively. Values are means ± 2 S.E., I_O and I_K were raised by 10^3 before log transformation to ensure positive values on a $\log_{10}$ scale.

not vary with day length for either I_O (ANOVA, $F_{5,55}$, $P > 0{\cdot}05$) or I_K (ANOVA, $F_{5,62}$, $P > 0{\cdot}05$).

DISCUSSION

While photoperiod affects the timing of seasonal activities in many organisms, intraspecific, geographic variation in photoperiodic response has been widely considered only in arthropods (Danilevskii, 1965; Tauber *et al*., 1986; Danks, 1987; Bradshaw & Holzapfel, 2007). Even among farmed finfishes where photoperiodic control of migration, maturation and reproduction is widespread (Bromage *et al*., 2001), there are, as far as is known, no explicit comparisons of photoperiodic response among populations within a species. In *G. aculeatus*, all previous studies of photoperiodic response have used wild-caught fish where the environmental influence on reproduction is unknown; none of the studies has considered photoperiodic response over a wide climatic range.

The more northern, Alaskan population of *G. aculeatus* is strongly photoperiodic, while the more southern, Oregon population shows no significant response to photoperiod (Fig. 1). Experiments were run with laboratory-reared fish in the G_1 or G_3 generation to minimize field effects; differences in photoperiodic response between Alaskan and Oregon populations represent genetic differences between them.

The focal Alaskan population used in the present study is anadromous (Cresko, 2000; Cresko *et al*., 2004; Karve *et al*., 2008), but it is unknown whether the migratory behaviour of the Oregon population is entirely within fresh water as in upstream populations in the Navarro River of California, U.S.A. (Snyder & Dingle, 1990), or involves migration from estuarine or marine habitats. A weaker photoperiodic response would be expected in resident or short-distance migrating populations (Clarke *et al*., 1994; Quinn & Adams, 1996). The lack of a discernible photoperiodic response in the Eel Creek, Oregon, population could reflect a strictly freshwater habitat where proximal cues such as food and temperature could be sufficient for timing reproduction.

Nonetheless, the increase in photoperiodic response of northern *G. aculeatus* is consistent with other vertebrates. In more northern populations of Scandinavian frogs *Rana temporaria*, where there is a strictly limited growing season, day length provides a firm, regulating cue for seasonal reproduction; in more southern populations, frogs use day length to modulate temperature-dependent processes (Laurila *et al*., 2001). In lizards, with increasing latitude, day length has an increasing effect on metabolic rate in *Sceloporus undulatus* (Angilleta, 2001), on growth rate in *Lacerta viviparia* (Uller & Olsson, 2003) and on ability to maintain a constant body temperature during the spring in *Sceloporus occidentalis occidentalis* (Lashbrook & Livezey, 1970). In mammals, with increasing latitude, short days have an increasing tendency to induce gonadal regression in mice *Peromyscus leucopus* and *Peromyscus maniculatus* (Lynch *et al*., 1981; Sullivan & Lynch, 1986; Heideman *et al*., 1999; Lowrey *et al*., 2000) or embryonic dormancy in multiple species of mustelids (Thom *et al*., 2004). Hence, the general vertebrate pattern, including that of *G. aculeatus*, is a pattern of an increasing influence of day length in the timing of important seasonal life-history events with increasing latitude among intraspecific populations. The results with *G. aculeatus* are in accord with the above authors in concluding that increasing reliance on day length by vertebrates at higher latitudes is due to an increasing use of a highly reliable, anticipatory cue to prepare in advance for an increasingly narrow window of opportunity for reproduction and development.

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