

A mechanism for social inhibition of sexual maturation in bluegill

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Social control of maturation is a common phenomenon in fishes, yet associated mechanisms are often unidentified. An examination of interactions between mature and juvenile male bluegill *Lepomis macrochirus* in mesocosms, isolating visual, chemical and physical interactions, suggested that chemical cues are responsible for inhibiting maturation of juvenile males.

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Individual variation in timing of maturation is a fairly common phenomenon in fishes and can be influenced by social interactions among individuals (Borowsky, 1978, 1987; Bushmann & Burns, 1994; Danylchuk & Tonn, 2001). In particular, interactions between large, dominant males and smaller, subordinate males appear to influence temporal and quantitative patterns of gonadal investment by the smaller males (Borowsky, 1978; Bushmann & Burns, 1994; Jennings *et al.*, 1997). Bluegill *Lepomis macrochirus* Rafinesque exhibit extensive variation in life-history strategies and timing of maturation, and the social structure of the population can influence the timing of maturity of juvenile males; juvenile males postpone maturation and energy investment in gonads in the presence of large, mature males (Jennings *et al.*, 1997; D.D. Aday, unpubl. data). As with other fish species, however, the proximate mechanisms linking these male–male interactions with maturation patterns remain unidentified. To examine potential mechanisms associated with male inhibition of maturation, a mesocosm experiment was designed using parental male and juvenile male bluegill.

Several mechanisms associated with social control of maturation have been proposed, including visual cues, chemical cues and agonistic behaviour (Bushman & Burns, 1994). To assess a potential role for each of these cues among bluegill,

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maturity rates of juvenile males subjected to interactions with large, mature males were measured in separate treatments isolating visual, chemical and physical interactions. Specifically, 20 2 m diameter circular tanks were randomly assigned to one of the following five treatments (four tanks per treatment): chemical cues, visual cues, chemical plus visual cues, physical cues and controls. Two halves of equal area were created in each tank by a plexiglass division that was either translucent or opaque, depending on the treatment group to which the tank was assigned. Translucent plexiglass was used to separate juvenile and large, adult male bluegill in the visual cue treatment tanks. The plexiglass was surrounded by a water-tight seal, thus allowing visual interactions among fish but preventing water flow between each half. For chemical cue treatment tanks, opaque plexiglass, pre-drilled with small (4.0 mm) holes to allow water flow between each half but prevent visual interactions, was used to separate juveniles and adults. A small pilot experiment with juvenile bluegill indicated that fish were unable to detect activity through the 4.0 mm holes. The chemical plus visual cues treatment consisted of tanks divided with clear plexiglass containing 4.0 mm holes. Finally, for the physical cues treatment, juvenile and adult males were placed together on one side of the tanks. Control treatment tanks contained no adult male bluegill. Tanks in the 'physical' and 'control' treatments were also divided with opaque plexiglass to maintain equal tank area per individual in all treatments. Biomass was equalized on each side of all tanks (mean \pm S.E. total biomass per side: 72.9 \pm 6.16 g) by reducing the number of individuals in the physical treatments (Table I). Because the presence of females might be necessary for males to initiate gonad development, two mature female bluegill were placed on both sides of each treatment tank. This experimental design allowed close examination of the influence of visual and chemical cues on growth and maturation of juvenile males through the *a priori* establishment of contrasts. To examine the importance of visual cues, all treatments containing visual cues were contrasted with those that did not involve visual cues (visual contrast: visual, visual plus chemical, physical treatments *v.* chemical, controls). Likewise, to more closely examine chemical cues, all treatments involving chemical cues were contrasted with those that did not involve chemical cues (chemical contrast: chemical, visual plus chemical, physical treatments *v.* visual, controls).

TABLE I. Experimental design of tank experiment. Treatments are chemical cues, visual cues, chemical plus visual cues, physical cues and controls. Values indicate numbers of mature males (MM), juveniles (J) and mature females (MF) on the juvenile and adult sides of tanks in each treatment. Numbers of fish were chosen to equalize biomass on each side of all tanks

Treatments	Juvenile side			Large male side		
	MM	J	MF	MM	J	MF
Chemical cues	0	6	2	2	0	2
Visual cues	0	6	2	2	0	2
Chemical plus visual cues	0	6	2	2	0	2
Physical cues	1	3	2	1	3	2
Control	0	6	2	0	6	2

These preplanned comparisons increase sample size and are valuable for contrasting individual treatment groups, providing better protection against Type I statistical error than random *post-hoc* pooling of the data to generate hypotheses (Freund & Wilson, 1993).

Juvenile individuals (130–155 mm total length, L_T) were collected from Lake Charleston, Illinois (42°29' N; 88°08' W), in early April, prior to initiation of seasonal spawning in that population. At the initiation of the experiment, there was no difference (ANOVA, $P > 0.151$) in L_T or mass of juveniles used in the various treatments. To ensure that all juvenile bluegill were indeed immature, several steps were taken. First, bluegill were collected at a size range known to encompass only immature individuals based on several years of sampling Lake Charleston. Second, only fish exhibiting no secondary sexual characteristics were collected. Finally, a sub-sample of collected fish was euthanized with MS-222 and dissected in the field to examine gonads. In all cases, no gonadal development was detected. Large, mature males (190–210 mm L_T) and females (175–190 mm L_T) were collected from a separate population (Lincoln Trail Lake, Illinois, 39°20' N; 87°43' W) and only individuals in spawning condition were used.

The experiment was conducted over a period of 12 weeks. Bluegill in all tanks were fed a daily ration of brine shrimp *Artemia* spp. at a rate of 10% total bluegill mass per tank, a rate well above maintenance rations used in previous laboratory experiments with bluegill (Aday *et al.*, 2000). At the conclusion of the experiment, all juvenile fish were measured, weighed, euthanized with MS-222, and dissected for removal of gonads. For each juvenile male, a gonado-somatic index (I_G) was calculated by determining the ratio of gonad mass to body mass.

There was no difference in final I_G of juvenile males across treatments [(ANOVA, $P = 0.13$); Fig. 1(a)]. Juveniles in the treatments that involved chemical cues however, had significantly lower ($F_{1,69} = 6.8$; $P = 0.01$) I_G than juveniles in the treatments without chemical interactions with large, mature males [chemical contrast; Fig. 1(b)]. Conversely, there were no differences in I_G of juvenile males in treatments with and without visual cues (visual contrast, $P = 0.49$). To ensure that I_G differences observed in the chemical contrast were not due to variation in size, the L_T of fish in the chemical treatments was examined relative to the other treatments. No difference was found ($P = 0.96$). In addition, there was no treatment effect on growth of juveniles over the course of the experiment; average growth in L_T (2.9 ± 0.91 mm) and mass (3.6 ± 1.5 g) was similar ($P > 0.37$) across treatments. These results suggest a chemical mechanism for social suppression of maturation in bluegill.

Although the actual chemicals responsible for suppression of maturation are unknown, pheromones secreted by the mature males are the most likely candidate. Pheromonal interactions have been shown to influence a variety of interactions that may be relevant to spawning bluegill. For example, pheromones have been linked to recognition of individuals in dominance hierarchies and aggregations of related individuals (Sorensen & Caprio, 1997). Bluegill are colonial spawners, and social interactions within colonies and associated activities may be dictated by pheromonal signals. In addition, pheromones are strongly linked to reproductive activities and have been shown to be exuded by sexually mature individuals of a wide variety of fish species (Sorensen & Caprio, 1997). Although this experiment was not designed to identify the actual substance involved in chemical interactions,

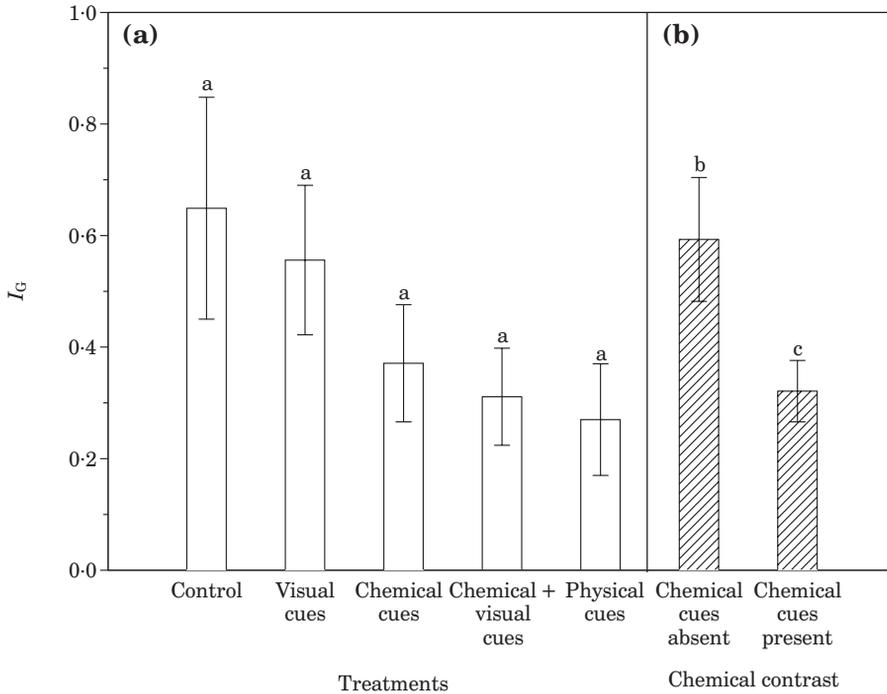


FIG. 1. (a) Treatment effects and (b) contrast between treatments with chemical cues (chemical, chemical plus visual, physical) and those without (visual, control) on mean \pm s.e. gonado-somatic indices. Different letters in (b) indicate a significant difference ($P = 0.01$).

reproductive activities of male bluegill have been linked to 11-ketotestosterone (Kindler *et al.*, 1989), and this steroid may play a role in male–male interactions. In addition, although visual cues alone did not prevent maturation, the combination of visual and chemical cues resulted in a low (although not statistically different) I_G of juveniles [Fig. 1(a)]. This suggests that visual cues may reinforce chemical cues in interactions between large males and juveniles. Small sample size resulted in some statistical limitations and may have contributed to the lack of differences among treatments. Despite the small sample size, however, the importance of chemical cues to social interactions among male bluegill was apparent in this experiment.

Because of life-history trade-offs, the timing of maturation is a critical trait that can have consequences for growth, fecundity and survival of individuals (Stearns, 1992). Timing of energy allocation to gonads based on social interactions, therefore, would be dependent on a reliable cue to ensure that maturation schedules maximize fitness in a given environment. As the ‘universal solvent’, water provides an excellent medium for chemically-directed interactions. In addition, fishes have highly evolved chemosensory systems, allowing their olfactory and gustatory senses to distinguish exceptionally complex chemical mixtures (Sorensen & Caprio, 1997). As a result, chemical cues may provide a more dependable inhibitory signal than visual cues, especially in the turbid, freshwater environments that bluegill often inhabit.

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