

# Manipulations of the AVT System Shift Social Status and Related Courtship and Aggressive Behavior in the Bluehead Wrasse

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Arginine vasotocin (AVT) and its mammalian homologous arginine vasopressin (AVP) influence male sexual and aggressive behaviors in many species. We tested the effects of AVT and an AVP-V<sub>1a</sub> receptor antagonist on the display of alternative male tactics in a tropical coral reef fish, the bluehead wrasse *Thalassoma bifasciatum*. We gave AVT injections to territorial and nonterritorial males of the large and colorful phenotype (terminal phase) and an AVP-V<sub>1a</sub> receptor antagonist, Manning compound, to territorial males in the field. AVT increased courtship independent of status, while its effects on territoriality and aggression were dependent upon male status. In territorial males, AVT increased courtship and tended to decrease the number of chases toward initial phase individuals. In nonterritorial males, AVT increased courtship, chases toward initial phase individuals, and territorial behavior while decreasing feeding. These are all behaviors rarely seen in nonterritorial males, so AVT made these males act like territorial TP males. The AVP-V<sub>1a</sub> receptor antagonist had opposite effects. It decreased courtship and territorial defense, making these males act more like nonterritorial males. Manipulations of the AVT system shifted males within a single phenotype from the nonterritorial social status to the territorial social status and vice versa. Since the entire suite of behaviors related to territoriality was affected by AVT system manipulations, our results suggest that the AVT system may play a key role in motivation of behaviors related to mating. © 2001 Academic Press

**Key Words:** arginine vasotocin; vasopressin; aggression; sexual behavior; territoriality; social behavior; alternative reproductive tactics; teleost.

The neuropeptides arginine vasotocin (AVT) and arginine vasopressin (AVP) have emerged as important neurohormones in the regulation of male social behavior. As neuropeptides, AVT/AVP can rapidly

alter behavior through production and release in brain areas regulating social behavior (review by DeVries, 1995). Due to these rapid effects, AVT/AVP may be used by animals to modulate behavioral responses to changing social conditions. This may be particularly important in species where such social interactions influence mating success.

Exogenous AVT/AVP stimulates courtship and/or sexual behavior in a variety of vertebrate species. Examples include the canary (Voorhuis, De Kloet, and De Wied, 1991), bullfrogs (Boyd, 1994), gray treefrogs (Semsar, Klomberg, and Marler, 1998), rough-skinned newt (Moore and Zoeller, 1979), killifish (Pickford and Strecker, 1977), and white perch (Salek, Sullivan, and Godwin, submitted for publication).

The role of AVT/AVP in the regulation of aggression appears to be dependent on a species' social system. AVT increases aggression in the colonial zebra finch (Goodson and Adkins-Regan, 1999), but decreases aggression in the territorial field sparrow (Goodson, 1998a) and violet-eared waxbill (Goodson, 1998b). In voles, AVP increases male–male aggression in monogamous, nonterritorial prairie voles, but not in promiscuous, territorial montane voles (Young, Winslow, Nilsen, and Insel, 1997). These species differences suggest that the AVT/AVP system may contribute to interspecific variation in social systems and social behavior (Goodson, 1998a,b; Young, 1999). Further support for this hypothesis includes recent transgenic experiments where changing AVP-V<sub>1A</sub> receptor density and distribution in naturally promiscuous mice increases affiliative behavior typical of monogamous voles (Young, Nilsen, Waymire, MacGregor, and Insel, 1999).

Beyond species differences, could the AVT system also be responsible for individual behavioral differ-

ences between alternative male phenotypes and across social classes? This appears to be the case between phenotypes in the plainfin midshipman, *Porichthys notatus*, where AVT differentially affects vocal-motor responses in the two fixed alternate male phenotypes (Goodson and Bass, 2000a,b). AVT decreases vocal-motor responses in the territorial Type I males which court females but has no effect in the nonterritorial Type II males that sneak on the Type I males and have vocal patterns similar to females. These direct recordings of neural activation from the vocal motor circuitry in anesthetized fish have helped to clarify how AVT may differentially affect neural pathways underlying a specific behavior which differs between phenotypes. The current study compares how AVT affects sexual, aggressive, and territorial behavior within a single sexual phenotype in freely behaving animals in their natural environment.

Our model, the bluehead wrasse (*Thalassoma bifasciatum*), is a female-to-male sex-changing fish. Among males there are two morphological phenotypes: a female-like initial phase (IP) that spawns with groups of other IP males or sneaks on pair spawns and a brightly colored terminal phase (TP) that usually defends one of the traditional spawning sites on a reef where it courts and pair spawns with females (Warner, 1984, 1988). Initial phase males are comparable to nonterritorial Type II males in the plainfin midshipman system, while TP males are comparable to the territorial Type I males. A key difference between the bluehead wrasse and the midshipman is that while morph type is fixed within individuals in the midshipman, it shows great plasticity in blueheads, with both females and IP males having the ability to become TP males (Warner, 1984; Warner and Swearer, 1991).

There is an additional level of variation in male behavior in bluehead wrasses that is the focus of this paper. Similar to other highly social fish species (e.g., *Sparisoma viride*, Cardwell and Liley, 1991; *Haplochromis burtoni*, Fernald and Hirata, 1977; Francis, Soma, and Fernald, 1993), there are two social classes within in the TP male phenotype: territorial (T-TP) and non-territorial (NT-TP; T/NT terminology from Fernald and Hirata, 1977). During the early afternoon spawning period, T-TP males act as described above: remaining primarily on and aggressively defending their spawning site, courting females, and abandoning feeding. This extremely active suite of behaviors is correlated with relatively high levels of hypothalamic AVT mRNA (Godwin, Sawby, Warner, Crews, and Grober, 2000; Grammer, 1998). In contrast, NT-TP males often become TP males before there is an open

spawning site on the reef. However, they rapidly occupy territories when these become available. NT-TP males do not defend a spawning site during the spawning period but rather move over large areas of reef and continue to feed while rarely courting females or chasing conspecifics. This lack of territorial and sexual behavior in NT-TP males is correlated with levels of hypothalamic AVT mRNA which are lower than those of T-TP males and comparable to IP males (Grammer, 1998).

This study had three goals. First, to determine whether exogenous AVT would increase courtship, aggressive, and territorial behaviors in NT-TP and T-TP males. Second, to determine whether the social status of a male affects its response to AVT. Third, to determine if an AVP-V<sub>1a</sub> receptor antagonist (Manning compound) blocks the behaviors affected by AVT.

## METHODS

### *Field Methods and Behavioral Observations*

**Field site.** We conducted this experiment on patch reefs with 8–15 TP males and 5–9 spawning sites in Teague Bay, St. Croix, United States Virgin Islands (5, 8, 11, 17, 22; for description of reefs see Gladfelter and Gladfelter, 1987). AVT administration was mainly conducted during May–July 1999, with the addition of two T-TP and two NT-TP males added in May–June 2000. The experiment testing the effects of the AVP-V<sub>1a</sub> receptor antagonist was conducted during June 2000.

**AVT administration.** We caught, measured, and tagged all the TP males on a given reef (see Warner, 1992). Males ranged in size between 75 and 95 mm standard length. To allow identification of individuals, we used floy tags inserted into the dorsal musculature under anesthesia with different external colored bead combinations (No. 11 seed beads). Two or three days after initial capture, we observed each focal male to identify its status as either T-TP or NT-TP and determine a baseline level of behavior. We recorded a suite of aggressive, sexual, and territorial behaviors (Table 1) during a full spawning period in 10 minute bouts, starting at the beginning of the spawning period and finishing 20 min after the last spawn (70–150 min; median 110 min). In addition, we recorded the number of feeding bites as both an indicator of general activity levels and an indication of a male's territorial motivation.

One to two days after the observation day, we

caught the focal male before the spawning period, ranging between 20 and 90 min depending on the ease of capture of the males and the accurate prediction of the onset of spawning, which varies slightly from day to day. We found no correlation between individual behavior scores and the elapsed time between the injection and onset of the spawning period. We then gave the focal male an intraperitoneal injection of either saline (0.9% NaCl) or AVT (Sigma, St. Louis) as described below. We standardized AVT doses to 1  $\mu\text{g}/\text{gram}$  body weight in a 1- $\mu\text{g}$  AVT/ 10- $\mu\text{l}$  saline solution. Body weight was estimated from a length-weight regression and the known length of the male. The dose was based on behaviorally effective dosages reported in studies using amphibian models (e.g., Propper and Dixon, 1997). Injection volumes for T-TP males ranged from 70 to 160  $\mu\text{l}$  (average, 115  $\mu\text{l}$ ). Injection volumes for NT-TP males ranged from 60 to 90  $\mu\text{l}$  (average, 75  $\mu\text{l}$ ). Saline treatments were matched for volume.

Once a focal male was injected, we watched the male through the entire spawning period, recording the same behaviors as on the observation day. Two or three days after the first injection, we repeated the procedure on the same focal male using the opposite treatment as that on the first injection day [ $n(\text{saline then AVT, with 2 days separating treatments}) = 10$ ;  $n(\text{AVT/saline, 2 days}) = 7$ ;  $n(\text{saline/AVT, 3 days}) = 2$ ;  $n(\text{AVT/saline, 3 days}) = 1$ .] The order of the treatments was randomized for each fish, and observers were blind to the treatment received by their focal male. Additionally, each male was watched by the same observer on the saline and AVT injection days to eliminate variation due to observer effects.

**AVP receptor antagonist (Manning compound) administration.** To inhibit endogenous AVT activity and further test the dependency of sexual and aggressive behavior on AVT, we administered Manning compound [( $\beta$ -mercapto- $\beta$ ,  $\beta$ -cyclopentamethylenepriopionyl<sup>1</sup>, *O*-Me-Tyr<sup>2</sup>, Arg<sup>8</sup>)-vasopressin, Sigma, St. Louis, MO] to T-TP males. Manning compound is a specific inhibitor of the mammalian AVP- $V_{1a}$  receptor subtype. The AVT receptor of other teleosts is most closely related to the mammalian  $V_{1a}$  receptor subtype and Manning compound inhibits AVT-induced changes in *Xenopus* oocytes expressing the teleost AVT receptor (Mahlmann, Meyerhof, Hausmann, Heierhorst, Schonrock, Zwiers, Lederis, and Richter, 1994). Additionally, we have cloned a 237-bp portion of the bluehead AVT receptor from brain mRNA. This clone shows the highest homology to the AVT receptor of

other teleosts and the AVP-  $V_{1a}$  receptor of mammals (unpublished data).

The same general design as that for AVT administration was used in the Manning experiment, with the following differences. Since saline treatments did not appear to significantly affect the behavior of males (see Results), we did not record observation days on unmanipulated males for this experiment. In addition, analyzing data from a full spawning period did not appear to provide any additional information relative to analyzing alternate 10-min time budgets through the spawning period. Therefore, each observer watched two focal males on a given day, alternating recording 10-min time budgets on each male through the entire spawning period. This produced at least four 10-min time budgets from each male each day. Only T-TP males were used since we expected Manning compound to reduce courtship and territorial behavior. Since NT-TP males do not normally display these behaviors or do so only at low levels, they were excluded from the experiment. Doses for T-TP males were standardized to 3.2  $\mu\text{g}$  Manning/gram body weight in a 3.2- $\mu\text{g}$  Manning/10  $\mu\text{l}$  saline solution, resulting in injection volumes from 110 to 165  $\mu\text{l}$  (average, 135  $\mu\text{l}$ ). All injections were given within 20 min of and prior to the onset of spawning.

### Hormone Administration

We captured the focal male by lift-netting and trapped it in the pocket of a hand net held underwater. We then placed the needle (25 gauge,  $\frac{5}{8}$  in.) of the 1-cc syringe into the abdominal cavity of the male near the vent, moved it anterior to the vent approximately 15 mm, and slowly released the hormone. Once captured, handling time and injection took no longer than 3 min, averaging approximately 2 min, and males were immediately released back onto the reef. Preliminary experiments suggested that this method was strongly preferable to transferring the fish to a boat and anesthetizing them for injection (100 mg/L MS-222 in seawater). Fish injected under anesthesia often disappeared into holes in the reef on release for 10–30 min and appeared to show lower levels of activity upon emerging. In contrast, fish injected in the water immediately after capture and then released resumed apparently normal behavior within 2 min. To verify that injected fluid was retained during and shortly after injection, we performed 10 trials with a weak solution of the vital dye Alcian blue and observed no significant loss of fluid. All experimental procedures were

approved by the North Carolina State University Institutional Animal Care and Use Committee.

### Statistical Analysis

Spawning periods vary in length and, within each spawning period, there is a broad peak of behavior (Warner and Schultz, 1992). There can be considerable variation between time budget periods due to variation in encounters with other individuals (T-TPs; number of females visiting sites, number of sneaker IP males near sites). Therefore, we assessed hormonal effects by restricting the analyses to this broad peak in behavior while averaging over several time budget periods to account for the variation described above.

Individual maximum behavior scores for each behavior type (Table 1) were calculated as follows. For the AVT administration experiment, the values from the three 10-min observation bouts in which a given male showed the highest frequency of behavior for that day were averaged. Depending on the nature of the behavioral measure, this score is an average maximum frequency per 10 min, an average maximum time spent doing a particular behavior (in seconds) per 10 min, or an average duration for individual behavioral acts. Due to the variation of these behaviors in the field, where we cannot control the encounter rate with females, we feel an average of the highest three time budgets is a better estimate of behavior than using the single highest time budget alone.

Once these maximum behavior scores were calculated, we used paired *t* tests to: (1) compare individual behavior scores for observation days and saline treatment days to determine the effect of handling and injection on behavior (two-tailed), (2) examine potential order effects (two-tailed), and (3) to compare individual behavior scores from saline and AVT treatments within each social class to determine the effect of AVT on behavior of T-TP and NT-TP males (one-tailed). Data on feeding bites were  $\log_{10}$ -transformed to eliminate heterogeneity of variance. In addition, for aggressive and courtship behavior in T-TP males, we analyzed the correlation between the magnitude of the hormone effect (difference between control and treatment scores) and the score of the saline treatment. This correlation tests whether the baseline level of behavior influences the behavioral response to hormone treatment.

For the AVP receptor antagonist experiment, we did not collect pretreatment data and therefore only compared individual scores between treatments with paired *t* tests. Since we did not have data for the entire

spawning period for each male, we calculated individual behavior scores by averaging behavior over the earliest 40 min of recorded behavior once the spawning period had begun. In the correlation between saline behavior and magnitude of change of summed duration of chases toward IPs, one individual was omitted from the analysis because it was an outlier (Dixon test on treatment effect,  $r = 2.21$ ,  $P < 0.05$ ). All statistics were performed in SAS (Cary, NC).

## RESULTS

### AVT Administration

We observed 9 NT-TP males and 11 T-TP males. There were no differences in maximum scores for any behavioral category between pretreatment and saline treatments. Therefore, the act of handling the fish and giving injections did not significantly change behavior (all comparisons,  $P > 0.05$ ). See Table 1 for descriptions of behaviors.

Both social classes responded to AVT treatment by increasing aspects of courtship behavior. NT-TP males courted both significantly more frequently and for a significantly greater total amount of time ( $P < 0.01$ ,  $P < 0.01$ ; Fig. 1A). T-TP males tended to increase courtship frequency and significantly increased courtship summed duration ( $P = 0.056$ ,  $P < 0.05$ ; Fig. 1B). However, the average duration of each courtship did not increase for T-TP males ( $P > 0.05$ ). Additionally, T-TP males with higher frequencies of courtship on saline treatment days showed a smaller response to AVT treatment ( $r = 0.63$ ,  $P < 0.05$ ). Despite these increases in courtship, neither social class responded to AVT treatment by increasing the frequency of looping (a behavior that appears to "advertise" site occupancy) or by altering their spawning frequency (all  $P > 0.1$ ).

AVT did not appear to affect the frequency or summed duration of aggression toward other TP males in either social class (all  $P > 0.1$ ). However, AVT did have significant and status-dependent effects on aggression toward IP individuals. NT-TP males given AVT chased IP individuals significantly more frequently and for a significantly greater total amount of time ( $P < 0.05$ ,  $P < 0.05$ ; Fig. 2A). In contrast, T-TP males showed a nonsignificant trend toward decreasing the amount of time they spent chasing IPs without apparently changing the frequency of those chases ( $P = 0.06$ ,  $P = 0.1$ ; Fig. 2B). This trend for reduced aggression toward IPs was accompanied by a

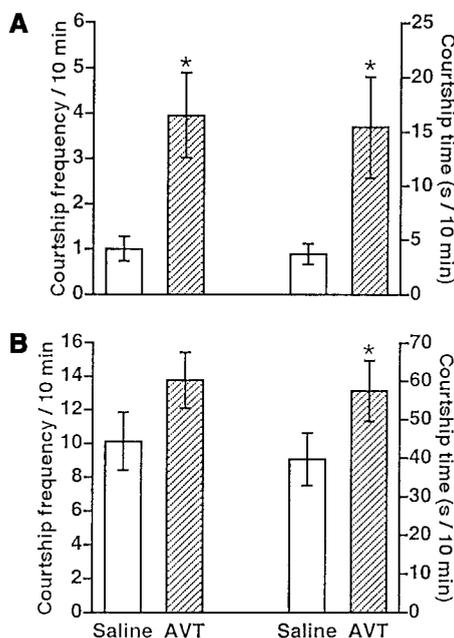
**TABLE 1**  
Behaviors of Bluehead Wrasse

Behavior	Description	Measurements
Courtship	Swimming tight circles with fluttering of pectoral fins above a female.	Frequency and time courting(s)
Aggression	Fast, directed swimming toward stationary or moving conspecifics (aggressions directed at TP and IP individuals were recorded separately).	Frequency and time chasing(s)
Spawn	Rapid, upward rush (approx. 0.5–1.0 m) with female in which gametes are released.	Frequency
Inspection	Movement towards the vent area of an IP individual where the focal males comes within half a body length and 45° of either side of the IP individual. This behavior may allow the focal individual to distinguish between IP males and females and/or to determine if females are ready to spawn.	Frequency
Loop	Motion similar to spawn but performed without the female. This behavior may serve to advertize a territorial male's presence on a spawning site. Not all males show looping behavior.	Frequency
Feeding bite	Snapping movement in water or on the bottom.	Frequency

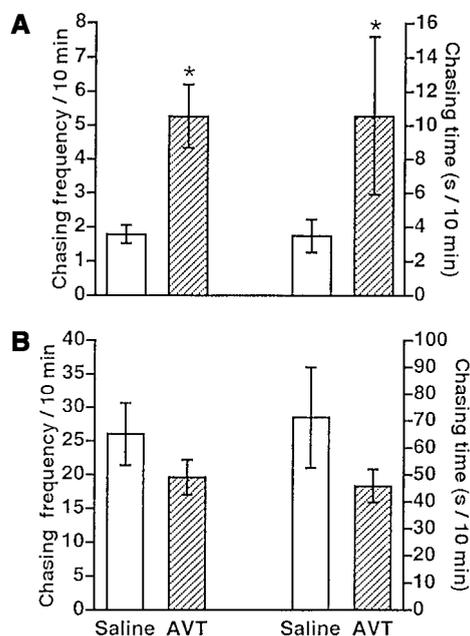
significant influence of the level of baseline behavior on a male's response to AVT treatment. The more time a male chased on the saline day, the greater the decrease in aggressive behavior with AVT treatment ( $r = 0.96$ ,  $P < 0.001$ ).

The effects of AVT on other behavioral indicators of territoriality were also status dependent. The numbers of inspections and feeding bites were unaffected by AVT treatment in T-TP males ( $P > 0.1$ ,  $P > 0.1$ ; Fig. 3B). In contrast, AVT treatment increased the fre-

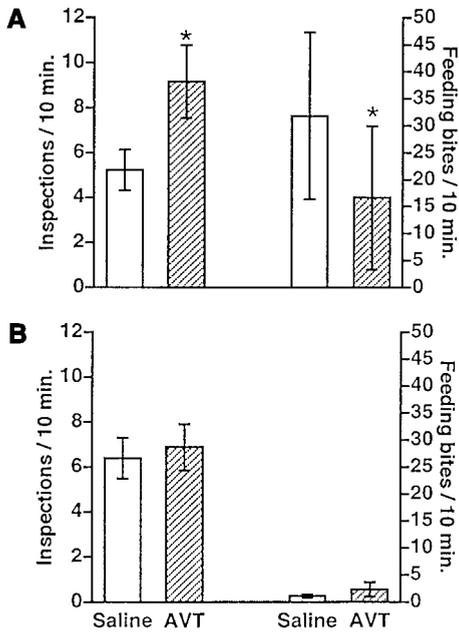
quency of inspections and decreased the frequency of feeding bites in NT-TP males ( $P < 0.01$ ,  $P < 0.01$ ; Fig. 3A). Additionally, NT-TP males stopped moving around the reef after receiving AVT. Each of the NT-TPs in the study was seen to stay over a small area of reef near the other spawning sites on the days of AVT treatment but not on saline treatment days. These "sites" were the same size as traditional territories, although they were not currently being used as a spawning site by other males.



**FIG. 1.** AVT effects on courtship behavior in (A) NT-TP males ( $n = 9$ ) and (B) T-TP males ( $n = 11$ ). Data shown are means  $\pm$  SEM; note the differences in axes scales.



**FIG. 2.** AVT effects on aggressive behavior toward IP individuals in (A) NT-TP males ( $n = 9$ ) and (B) T-TP males ( $n = 11$ ). Data shown are means  $\pm$  SEM; note the differences in axes scales.



**FIG. 3.** AVT effects on inspection and feeding bite frequencies in (A) NT-TP males ( $n = 9$ ) and (B) T-TP males ( $n = 11$ ). Data shown are means  $\pm$  SEM.

### AVP Receptor Antagonist (Manning Compound) Administration

The administration of Manning compound to 11 T-TP males significantly inhibited behaviors influenced by AVT in the experiments described above. Specifically, Manning treatment significantly decreased both the frequency and summed duration of courtship ( $P = 0.01$ ,  $P < 0.01$ ; Fig. 4A) as well as decreasing the average duration of individual courtships ( $P = 0.01$ ). In addition, the less time a male spent courting on the day of saline treatment, the greater the decrease observed in time spent courting with Manning treatment ( $r = 0.73$ ;  $P < 0.05$ ). Accompanying the decrease in courtship, Manning treatment significantly decreased the frequency of inspections ( $P = 0.01$ ; Fig. 4B) and there was a nonsignificant trend to decrease spawning frequency ( $P < 0.1$ ). There was no difference in feeding frequency ( $P > 0.1$ ; Fig. 4B). Since only 2 of the 11 males displayed looping behavior, we did not analyze this behavior for Manning treatment.

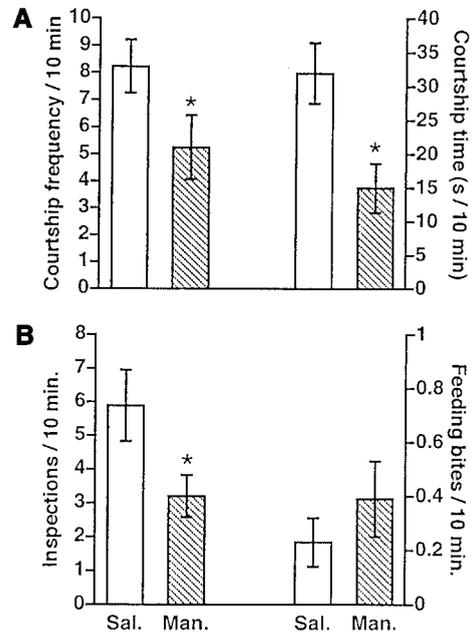
Manning treatment significantly decreased aggression toward IPs by decreasing the frequency, summed duration, and average individual duration of chases ( $P < 0.01$ ,  $P = 0.01$ ,  $P < 0.01$ ; Fig. 5A). As with courtship, the less the time spent chasing IPs on the day of saline treatment, the greater the decrease in

time spent chasing IPs with Manning treatment ( $r = 0.92$ ;  $P < 0.05$ ). The effect of Manning treatment on aggression toward TPs showed trends in the opposite direction. T-TP males showed nonsignificant trends toward increasing both the frequency and summed duration of chases toward other TP males with Manning treatment ( $P = 0.06$ ,  $P = 0.06$ ; Fig. 5B). Five of the eleven T-TP males given Manning compound were observed leaving their spawning site for at least half of the spawning period (compared to 0/11 of these males with saline treatment;  $\chi^2$  test of equal proportions,  $P < 0.05$ ).

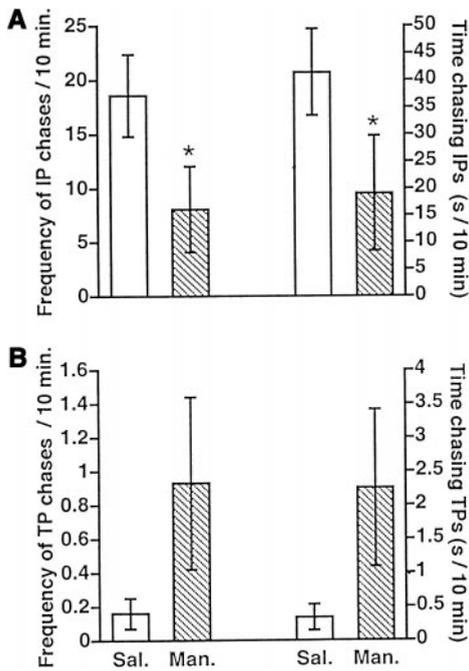
We found no effects of the order in which males received injections between control saline and experimental AVT or Manning treatments (all  $P$  values  $> 0.10$ ). There were also no correlations between the size of the male and the behavioral response to hormone treatment (magnitude of change between saline and hormone treatment) in any of the behavioral measures across experiments (all comparisons,  $P > 0.10$ ).

## DISCUSSION

AVT and AVP are key regulators of sociosexual behaviors in vertebrates. Accumulating evidence suggests that these peptides mediate differences between



**FIG. 4.** AVP receptor antagonist (Manning compound) effects on (A) courtship and (B) inspections and feeding bites in T-TP males ( $n = 11$ ). Data shown are means  $\pm$  SEM.



**FIG. 5.** AVP receptor antagonist (Manning compound) effects on aggression in T-TP males ( $n = 11$ ) toward (A) IP individuals and (B) TP males. Data shown are means  $\pm$  SEM.

species (Young, 1999; Goodson 1998a,b) and between discrete alternate male phenotypes within species (e.g., Goodson and Bass, 2000a,b). This study extends these findings by indicating that AVT can also regulate behavioral differences among individuals of the same phenotype. In bluehead wrasses, manipulations of the AVT system can shift the behavior individual males between behavior typical of the territorial and nonterritorial social classes. Since among-species variation in social behavior and social systems is likely to arise as among-individual variation within species, this is an important connection.

### AVT Effects on Sexual Behavior

Both social classes of TP males, NT-TP males and T-TP males, responded to exogenous AVT by increasing at least one measure of courtship behavior, while T-TP males responded to an AVP antagonist by decreasing courtship behavior. This result mirrors the effects of AVT in many other species representing all other vertebrate classes (see reviews in Moore, 1992; DeVries, 1995). Specifically, NT-TP males increased courtship to levels near that of T-TP males on less successful sites which show low to moderate levels of

control courtship behavior. T-TP males increased the amount of time they spent courting with AVT treatment. However, they showed no change in the average duration of courtship. Although this suggests that T-TP males are increasing the amount of time spent courting by increasing the frequency of courtship, we only observed a trend for increased courtship frequency. The increase in time spent courting by T-TP males in response to AVT may be explained by additive effects of non-significant changes in both courtship frequency and average duration.

AVT effects on courtship were dependent on baseline levels of behavior. NT-TP males with baseline courtship levels near zero exhibited strong increases in courtship behavior with AVT treatment. Also, T-TP males with low baseline courtship levels exhibit the same trend to increase courtship with AVT treatment. However, T-TP males with high baseline courtship levels did not increase courtship in response to AVT. Although this is a significant “ceiling” effect, it is not clear whether this limitation is physiological or behavioral. It could represent a physiological ceiling in which T-TP males with active sites already have such high levels of AVT (Grammer, 1998) that exogenous administration cannot further influence the neural substrates of this behavior. An alternative, but not mutually exclusive, hypothesis is that this effect represents a behavioral ceiling. Active males may be time and/or female-constrained such that there is not the time and/or additional females to increase courtship regardless of possible effects on motivation.

These increases in aspects of courtship behavior of T-TP males and NT-TP males appear most likely due to effects on male motivation rather than the attraction of a greater number of females to their spawning site. Females are highly site faithful and usually return to the same site every time they spawn (Warner, 1987, 1988, 1998). Female site fidelity may explain why the increases in courtship found here did not result in an increased rate of spawning. The NT-TP males who exhibited territorial behavior typically did so on locations that were not traditionally used as spawning sites on these reefs. Therefore, these males courted females who were en route to established spawning sites. It would be necessary to use extended treatments of AVT to test whether females would change preferred spawning sites in response to altered male behavior, perhaps resulting in increased reproductive success for males with high AVT levels (see Warner and Schultz, 1992).

### AVT Effects on Aggression

Unlike courtship behavior, where the effects of AVT were dependent on baseline behavioral level rather than on status, the effects of AVT on aggression toward subordinates were status dependent. NT-TP males do not normally defend sites and therefore have very low levels of aggression toward IPs. AVT increased aggression toward IPs, suggesting that AVT acts to induce territorial behavior. This agrees well with the effects of AVT on courtship since the primary reproductive tactic of TP males is to defend a spawning site which females visit to spawn (Warner, 1984). Conversely, T-TP males tended to decrease the amount of time they spent chasing IPs without chasing less often. Although our data show only a trend for T-TP males to decrease aggression toward IPs, they also show a strong relationship between baseline aggression and response to AVT. Males who were most aggressive with saline treatment showed the strongest decrease in aggression in response to AVT. Whether these results represent a trade-off between courtship and aggression is unclear.

Administration of AVP antagonist also resulted in decreased aggression toward IPs and a trend for increased aggression toward TP males. Although these results appear to be in the same direction as AVT effects, they appear to result from the temporary abandonment of the territory in response to Manning compound followed by a subsequent contest to regain the site. These contests are artifacts of having abandoned these sites in the first place. Empty territories are occupied by other males very quickly in bluehead wrasses (often within minutes, personal observation). Supporting this interpretation is the observation that T-TP males who exhibited high levels of aggression toward other TP males were the same males that had left their territories for part of the spawning period. These males then returned to find another male had occupied their territory and had to aggressively expel this intruder.

Although AVP has been shown to reverse dominance relationships in other species (Ferris, 1992; Ferris, Meenan, Axelson, and Albers, 1986), we did not observe this in bluehead wrasses. Neither T-TP nor NT-TP males increased the frequency of chases toward other TP males. This may indicate a permissive effect of AVT on aggression in bluehead wrasses since NT-TP males increase aggression directed toward individuals to which they are normally socially dominant, but not toward individuals to whom they are subordinate. For example, Sapolsky (1986) suggests

that in primates, testosterone may modulate existing patterns of aggression rather than induce novel aggression. Alternatively, in our study, the AVT dose we used may not have been sufficient to induce dominance reversals. Future studies using intracerebroventricular injections will help address this issue.

While AVT does not appear to alter dominance roles, it does alter social status by making NT-TP males act more like T-TP males, while an AVP antagonist does the opposite. Both normally and following saline treatment, NT-TP males move continuously over the reef during the spawning period, continuing to feed and rarely inspecting or courting females or chasing other individuals. However, when they were given AVT, NT-TP males decreased feeding and increased almost all behaviors typical of territorial males (inspections, courtship, and aggression). Although NT-TP males did not attempt to take over an established territory, they did appear to attempt to establish a novel territory on the reef. Observers, while still unaware of the treatment received by a focal male, reported that these males stayed over a specific area of the reef. These areas had never been known to be spawning sites or were marginal sites not currently occupied. These "sites" were always on the down current side of the reef where other spawning sites are located. Since bluehead wrasses exhibit strong site fidelity and the same sites remain in use for generations (Warner, 1988), we expected that if AVT induced territorial behavior, males would challenge a territorial male on an established territory. Instead, they used novel sites and displayed levels of courtship and aggressive behavior comparable to those of T-TP males on less successful spawning sites. These lower behavioral frequencies appear to be due to the presence of fewer females and IP males at these sites, reducing the opportunity for courtship and aggression, rather than an inadequate dose of AVT. In contrast, T-TP males given AVP antagonist often abandoned their sites, resulting in decreases in the suite of territorial behaviors.

### Potential Mechanisms of Action

It has been previously suggested that the AVT/AVP system may modulate social behaviors at generalized motivational levels rather than through the direct activation of specific behaviors (Tito, Hoover, Mingo, and Boyd, 1999). In contrast, Thompson and Moore (2000) suggest that AVT affects courtship in rough-skinned newts (*Taricha granulosa*) through effects on specific sensorimotor pathways. A third possibility is

that AVT has nonspecific effects on arousal or attention (see Thompson and Moore, 2000, for further discussion of these alternatives). In bluehead wrasses, the changes induced by AVT differed across behaviors yet were consistent with the behavioral phenotypes displayed by males of different social status. These observations argue against a nonspecific effect on behavior such as general arousal. We cannot distinguish between generalized motivation effects and effects on responses to specific sensory modalities since all males in this experiment were exposed to a full range of natural sensory cues. However, we suggest that the complexity of the behavioral effects argues that AVT acts to alter motivation in the bluehead wrasse.

The best evidence for generalized motivational influence of AVT in bluehead wrasses is its effect on habitat use. When NT-TP males take up novel territories in response to AVT, they do so in the absence of any obvious releasing stimulus. Likewise, in T-TP males, putative inhibition of endogenous AVT activity with an AVP antagonist often leads to the abandonment of territories. These shifts in social status are accompanied by changes in a suite of behaviors associated with territoriality, including courtship, aggression, inspection, and feeding. For bluehead wrasses, territoriality allows TP males to court and spawn with females. Therefore, it follows that if increases in territorial behavior are due to increases in sexual motivation, there should be increases in not only aggression, but also courtship and inspection, as well as decreases in feeding.

Perhaps additional evidence for an effect on motivation is the effect of AVT on inspection behavior. This behavior naturally takes place both on and off territories and appears to be involved in assessing the sexual identity of IP individuals and female readiness to spawn. The fact that NT-TP males increased the display of this behavior following AVT treatment may indicate an increase in sexual motivation. AVT treatment did not affect inspections in T-TP males who are already sexually motivated, but Manning treatment decreased inspections. Similarly, AVP increases affiliative behaviors in nonterritorial prairie voles but not in territorial montane voles while increases in AVP receptors in territorial mice lead to increased affiliative behavior (Young *et al.*, 1999). While motivation is difficult to assess, affiliative behaviors such as inspection may be good indicators of motivational states.

The diversity and complexity of the behavioral effects observed in these experiments suggest that the peripherally injected AVT and AVP antagonist are acting centrally to influence sexual and territorial be-

havior. AVT receptors are located in regions of the teleost brain that mediate male sexual behavior (Demski, 1983; Moons, Cambre, Batten, and Vandesande, 1989). If AVT or AVP antagonist were acting at peripheral locations, it is unlikely that they would affect such a wide range of specific behaviors. Additionally, in one measure of potential peripheral effects of the dose of AVT used here, measures of plasma osmolality 1 h following administration of the AVT dose used here do not differ from those of saline controls (data not shown). Also, in white perch (Salek *et al.*, submitted for publication), ICV injections of AVT are effective in altering sexual behavior at doses at least 1500-fold lower than the intraperitoneal injections used here. We are currently examining the question of where AVT acts more directly by administering ICV injections and analyzing AVT receptor distributions in bluehead wrasses.

### *Comparisons across Species, Morphs, and Status*

Status-dependent effects of AVT on aggression toward subordinate individuals in bluehead wrasses may correspond to variation in the effects of exogenous AVT/AVP among species that vary in aggressiveness and territoriality. For example, in species of anurans that defend calling sites, AVT causes advertisement calls to become more like those of less aggressive males (e.g., *Acris crepitans*, Marler *et al.*, 1995). In birds, AVT increases aggression in colonial, nonterritorial species, such as the zebra finch (Goodson and Adkins-Regan, 1999), yet decreases aggression in territorial species such as the closely related violet-eared waxbill (Goodson, 1998b) and the field sparrow (Goodson, 1998a). Similarly, in mammals, AVP increases aggression in nonterritorial prairie voles, but not in congeneric territorial montane voles (Young *et al.*, 1997). Even within a single species, alternate male phenotypes of the plainfin midshipman have different endogenous AVT neural phenotypes (Foran and Bass, 1998) and the neurons subserving vocal behavior respond differently to AVT treatment in the two morphs (Goodson and Bass, 2000a,b). Our results suggest that even within a single phenotype of one species, AVT modulates aggression differently depending on the territorial status of the individual, serving to increase aggression in nonterritorial males while decreasing aggression in territorial males.

Ethologically, the critical difference in many of the interspecific comparisons of aggression in territorial and colonial species may be whether males tolerate the close proximity of other males. The definition of

close proximity would clearly vary depending on the animal considered. T-TP male bluehead wrasses do not tolerate the close proximity of either other TP males or IP males during the spawning period. In contrast, NT-TP males rarely exhibit aggression toward either TP or IP males and will occasionally participate in group spawns where they are within one body length of several IP males or other NT-TP males. This difference in tolerance of the proximity of other males mirrors the differences among species examined in other studies (e.g., Goodson 1998a,b). More generally, it may provide an operational distinction useful in assessing the detailed neural bases of such behavioral differences.

### Conclusion

AVT induces behaviors typical of the dominant individuals in bluehead wrasses, and manipulations of the AVT system can shift behavior between territorial and nonterritorial social behaviors within a single phenotype. These behavioral alterations in response to AVT and an antagonist occur in the entire suite of complex behaviors related to the defense and use of a spawning site. The varying response of male bluehead wrasses of different social status may mirror a general pattern in AVT mediation of behavior across diverse social systems.

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### REFERENCES

- Boyd, S. K. (1994). Arginine vasotocin facilitation of advertisement calling and call phonotaxis in bullfrogs. *Horm. Behav.* **28**, 232–240.
- Cardwell, J. R., and Liley, N. R. (1991). Androgen control of social status in males of a wild population of stoplight parrotfish, *Sparisoma viride* (Scaridae). *Horm. Behav.* **25**, 1–18.
- DeVries, G. J. (1995). Studying neurotransmitter systems to understand the development and function of sex differences in the brain: the case of vasopressin. In P. E. Micevych and R. P. Hammer (Eds.), *Neurobiological Effects of Sex Steroid Hormones*, pp. 254–280. Cambridge Univ. Press, New York.
- Demski, L. S. (1983). Behavioral effects of electrical stimulation of the brain. In R. E. Davis and R. G. Northcutt (Eds.), *Fish Neurobiology*, pp. 317–369. Univ. of Michigan Press, Ann Arbor.
- Fernald, R. D., and Hirata, N. R. (1977). Field study of *Haplochromis burtoni*: Quantitative behavioural observations. *Anim. Behav.* **25**, 964–975.
- Ferris, G. M. (1992). Role of vasopressin in aggressive and dominant/subordinate behaviors. *Ann. N.Y. Acad. Sci.* **653**, 212–226.
- Ferris, C. F., Meenan, D. M., Axelson, J. F., and Albers, H. E. (1986). A vasopressin antagonist can reverse dominant/subordinate behavior in hamsters. *Physiol. Behav.* **38**, 135–138.
- Foran, C. M., and Bass, A. H. (1998). Preoptic AVT immunoreactive neurons of a teleost fish with alternative reproductive tactics. *Gen. Comp. Endocrinol.* **111**, 271–282.
- Francis, R. C., Soma, K., and Fernald, R. D. (1993). Social regulation of the brain-pituitary-gonadal axis. *Proc. Natl. Acad. Sci. USA* **90**, 7794–7798.
- Gladfelter, W. B., and Gladfelter, E. H. (1987). Fish community structure as a function of habitat structure on West Indian patch reefs. *Rev. Biol. Trop.* **26**, 65–84.
- Godwin, J., Sawby, R., Warner, R. R., Crews, D., and Grober, M. S. (2000). Hypothalamic arginine vasotocin mRNA abundance variation across sexes and with sex change in a coral reef fish. *Brain Behav. Evol.* **55**(2), 77–84.
- Goodson, J. L. (1998a). Territorial aggression and dawn song are modulated by septal vasotocin and vasoactive intestinal polypeptide in male field sparrows (*Spizella pusilla*). *Horm. Behav.* **34**, 67–77.
- Goodson, J. L. (1998b). Vasotocin and vasoactive intestinal polypeptide modulate aggression in a territorial songbird, the violet-eared waxbill (Estrildidae: *Uraeginthus granatina*). *Gen. Comp. Endocrinol.* **111**, 233–244.
- Goodson, J. L., and Adkins-Regan, E. (1999). Effect of intraseptal vasotocin and vasoactive intestinal polypeptide infusions on courtship song and aggression in the male zebra finch (*Taeniopygia guttata*). *J. Neuroendocrinol.* **11**, 19–25.
- Goodson, J. L., and Bass, A. H. (2000a). Forebrain peptides modulate sexually polymorphic vocal circuitry. *Nature* **403**, 769–772.
- Goodson, J. L., and Bass, A. H. (2000b). Vasotocin innervation and modulation of vocal-acoustic circuitry in the teleost *Porichthys notatus*. *J. Comp. Neurol.* **422**, 363–379.
- Grammer, M. (1998). *The Neurohormonal Basis of Individual Behavioral Variation in the Bluehead Wrasse, Thalassoma bifasciatum*. M.S. thesis, North Carolina State University.
- Mahlmann, S., Meyerhof, W., Hausmann, H., Heierhorst, J., Schonrock, C., Zwiers, H., Lederis, K., and Richter, D. (1994). Structure, function, and phylogeny of [arg<sup>8</sup>] vasotocin receptors from teleost fish and toad. *Proc. Natl. Acad. Sci. USA* **91**, 1342–1345.
- Marler, C. A., Chu, J., and Wilczynski, W. (1995). Arginine vasotocin injection increases the probability of calling in cricket frogs, but causes call changes characteristic of less aggressive males. *Horm. Behav.* **29**, 554–570.
- Moons, I., Cambre, M., Batten, T. F. C., and Vandesande, F. (1989). Autoradiographic localization of binding sites for vasotocin in the brain and pituitary of the sea bass (*Dicentrarchus labrax*). *Neurosci. Lett.* **100**, 11–16.
- Moore, F. L. (1992). Evolutionary precedents for behavioral actions of oxytocin and vasopressin. *Ann. N.Y. Acad. Sci.* **652**, 156–165.
- Moore, F. L., and Zoeller, R. T. (1979). Endocrine control of amphib-

- ian sexual behavior: Evidence for a neurohormone-androgen interaction. *Horm. Behav.* **13**, 207–213.
- Pickford, G. E., and Strecker, E. L. (1977). The spawning reflex response of the killifish, *Fundulus heteroclitus*: Isotocin is relatively inactive in comparison with arginine vasotocin. *Gen. Comp. Endocrinol.* **32**, 132–137.
- Propper, C. R., and Dixon, T. B. (1997). Differential effects of arginine vasotocin and gonadotropin-releasing hormone on sexual behaviors in an anuran amphibian. *Horm. Behav.* **32**, 99–104.
- Sapolsky, R. M. (1986). Endocrine and behavioral correlates of drought in wild olive baboons (*Papio anubis*). *Am. J. Primatol.* **11**(3), 217–228.
- Semsar, K., Klomberg, K. F., and Marler, C. (1998). Arginine vasotocin increases calling-site acquisition by nonresident male grey treefrogs. *Anim. Behav.* **56**, 983–987.
- Thompson, R. R., and Moore, F. L. (2000). Vasotocin stimulates appetitive responses to the visual and pheromonal stimuli used by male roughskin newts during courtship. *Horm. Behav.* **38**, 75–85.
- Tito, M. B., Hoover, M. A., Mingo, A. M., and Boyd, S. K. (1999). Vasotocin maintains multiple call types in the gray treefrog, *Hyla versicolor*. *Horm. Behav.* **36**, 166–175.
- Voorhuis, T. A. M., De Kloet, E. R., and De Wied, D. (1991). Effect of a vasotocin analog on singing behavior in the canary. *Horm. Behav.* **25**, 549–559.
- Warner, R. R. (1984). Mating systems and hermaphroditism in coral reef fish. *Am. Sci.* **72**, 128–136.
- Warner, R. R. (1987). Female choice of sites versus mates in a coral reef fish, *Thalassoma bifasciatum*. *Anim. Behav.* **35**, 1470–1478.
- Warner, R. R. (1988). Traditionality of mating-site preference in a coral reef fish. *Nature* **335**, 719–721.
- Warner, R. R. (1998). The role of extreme iteroparity and risk avoidance in the evolution of mating systems. *J. Fish Biol.* **53**, 82–93.
- Warner, R. R., and Schultz, E. T. (1992). Sexual selection and male characteristics in the bluehead wrasse, *Thalassoma bifasciatum*: Mating site acquisition, mating site defense, and female choice. *Evolution* **46**(5), 1421–1442.
- Warner, R. R., and Swearer, S. E. (1991). Social control of sex change in the bluehead wrasse. *Biol. Bull.* **181**, 199–204.
- Young, L. J. (1999). Oxytocin and vasopressin receptors and species-typical social behaviors. *Horm. Behav.* **36**, 212–221.
- Young, L. J., Nilsen, R., Waymire, K. G., MacGregor, G. R., and Insel, T. R. (1999). Increased affiliative response to vasopressin in mice expressing the V1a receptor from a monogamous vole. *Nature* **400**, 766–768.
- Young, L. J., Winslow, J. T., Nilsen, R., and Insel, T. R. (1997). Species differences in V1a receptor gene expression in monogamous and non-monogamous voles: Behavioral consequences. *Behav. Neurosci.* **111**, 599–605.