Morphological and Developmental Differences in Three Species of the Snapping Shrimp Genus *Alpheus* (Crustacea, Decapoda)

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Abstract - Living, freshly collected individuals of three species of snapping shrimps were studied to determine any differing morphological, developmental, and ecological features: *Alpheus heterochaelis*, collected from Beaufort, NC; *A. angulosus*, found mainly in Jacksonville, FL, but also at one site in Beaufort; and *A. estuariensis*, collected at another Jacksonville site. Structural characteristics of these superficially similar species are summarized, with particular attention to coloration. Adult *A. angulosus* individuals have blue-green 2nd antennal flagella (vs. tan in the other two species) that are significantly shorter than those of *A. heterochaelis*. *Alpheus angulosus* and *A. estuariensis* bear smaller eggs (<1 mm, regardless of embryonic stage) than *A. heterochaelis* (>1 mm), and the former species displays the zoea larval form typical of alpheids (vs. abbreviated larval development in *A. heterochaelis*).

Introduction

Perhaps the most persistent and widespread noise in shallow-water tropical and subtropical seas is that produced by "snapping shrimps," decapod crustaceans belonging to the family Alpheidae. In most alpheids, the chelate first pereiopods ("claws") are asymmetrical, one member of the pair (the major chela) being much larger and more heavily calcified than the other (Fig. 1). The noise is produced by vast numbers of individuals, each one rapidly closing the dactylus, or movable finger, of its major claw onto the pollex, or immovable finger, of the propodus, in the manner of a pistol hammer (hence the alternative name "pistol shrimp") (Knowlton and Moulton 1963, Versluis et al. 2000).

Many alpheid species are morphologically very similar and consequently "difficult to recognize using traditional systematic methods" (Knowlton 1986). Preserved specimens have been relied upon in the process of defining species, but some distinguishing characteristics, such as color, cannot be considered as they are lost in the process of preservation (Knowlton 1986). Alternative methods are required to discern some species.

A number of morphologically similar species from the so-called *Alpheus edwardsii* group are found along coastlines of the southeastern United States. *Alpheus heterochaelis* Say (big-claw snapping shrimp) was first described in 1818, but since then new species have been delineated among specimens originally thought to be *A. heterochaelis*. One of these

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is *Alpheus estuariensis* Christoffersen (1984); another is *Alpheus angulosus* McClure (2002), initially named *Alpheus angulatus* (McClure 1995). Although the *A. heterochaelis* neotype is from northern Florida (Amelia Island), *A. angulosus* is actually the most common species collected in Florida locations, and *A. heterochaelis* is more commonly found in North Carolina (McClure 1995).

The morphological differences recognized among these three species are very subtle, and verbal descriptions, especially color, can be vague. Field identification is important because the species are sympatric, yet some of the most significant morphological differences documented are not useful distinguishing features in identifying live shrimps. For example, the angular carapace characteristic of *A. angulosus* (McClure 1995) is obscured by the pereiopods.

Developmental differences between *A. heterochaelis*, *A. angulosus*, and *A. estuariensis* have not yet been established. Knowlton (1970) described the developmental pattern of *A. heterochaelis* from North Carolina and postulated that then-assumed *A. heterochaelis* from Florida (now known to be *A. angulosus*) was a different species based on observations of smaller eggs in the Florida shrimps.

Snapping shrimps are characterized by unique behaviors and interesting interactions with other species and abiotic aspects of the environment. Aspects of behavioral-ecological investigation have included mechanics of the snap (e.g., Versluis et al. 2000), sociality (Duffy et al. 2002), aggression (Knowlton and Keller 1982), chemical and visual signaling (Hughes 1996), and habitat selection (Corfield and Alexander 1995). Animals thought to be *A. heterochaelis* have been and continue to be used as subjects of many investigations, making it all the more important to facilitate correct identification of this and its "look-alike" species.

The purpose of our research project was to discern and describe developmental, ecological, and additional morphological differences between *Alpheus* populations from two geographically separated areas: southern North Carolina and northern Florida. These lines of investigation, centered around the examination of live specimens, together can provide a further and clearer basis for distinguishing the three species.

Figure 1. Dorsal view of male *A. heterochaelis*, showing "balaeniceps-type" minor chela.

Field-site Descriptions

Shrimp were collected at two sites in each state. Both North Carolina sites were located at the mouth of the Newport River (estuary), Beaufort, approximately 0.4 km apart and on nearly opposite sides of Beaufort Inlet. The primary collection location was near the Duke University Marine Laboratory (DUML), on the western side ("Research Cove") of Pivers Island Road. The other North Carolina site was "Duncan's Green" (DG), at the west end of Front Street in Beaufort. The collection locations in Florida, about 10 km apart, were Fort George Inlet (FGI), and Round Marsh (RM), both along the shore of the St. Johns River (estuary), Jacksonville. The FGI collection site was near the mouth of the river, in Huguenot Memorial Park, near the south side of the bridge (FL Route A1A) linking Fort George and Little Talbot Islands. The RM site was near a salt marsh in the backwaters of the St. Johns River, near an observation platform in the Fort Caroline portion of the Timucuan Preserve.

Substrata at the collection sites consisted of a mixture of sand and mud in different proportions (of sand/silt/clay), and always included, to various degrees, *Crassostrea virginica* (Gmelin) (oyster) shells and some live oysters. At DUML, shell clumps were sitting on a muddier substrate, sloping toward the water, than the other locations. The FGI site was very flat, with a sandier substrate supporting rocks (mainly concrete slabs and other rubble from reconstruction of the bridge) encrusted with oysters. At RM, the oyster shells were comparatively more plentiful but generally looser (i.e., not in clumps). The salinity at RM was lower (25 ppt) than at the other sites (35 ppt).

Methods

Collection and maintenance

Shrimp were collected manually during low tide, at DUML, FGI, and RM, during two different seasons: July 1–5, 2004 (hereinafter designated "summer"), and November 15–17 and December 4–5, 2004 ("fall"); on March 14, 2005, a brief collection was made at DG. Animals were mostly found either by turning over shell clumps or rocks located around the mean low-water mark or pushing a hand-held dip net through loose shells. On site, each collected shrimp was placed into a plastic bag half-filled with seawater from the site, along with 1–2 oyster shells to provide a shelter for the shrimp. Multiple animals found under the same rock were usually two in number and of opposite sex, presumed to be a mating pair, thus kept in the same bag.

The bags and their contents were transported by plane to the laboratory at George Washington University, where they were catalogued (organized based on location, each animal assigned a number) and placed in 20-cm-diameter glass bowls individually (except for summer-collected paired animals; these were placed together in plastic 20-cm x 12-cm aquaria). Collection water was replaced with a solution of artificial sea salt mix made to the salinity of the water in which the shrimp were collected. Oyster shells

that were free of macroscopic encrusting organisms (to reduce the risk of bacterial infiltration) were positioned in each tank. Each animal was sized (carapace length measured) and characterized in terms of sex, "handedness" (side bearing the major chela), and any unusual features.

Temperature, salinity, and pH were kept within normal ranges while the animals were maintained in the laboratory. Water was aerated with pumps and air stones, supplemented with potassium iodide to facilitate molting, and changed approximately every 3 days. Laboratory lights were turned on and off in concert with the natural photoperiod to the extent possible. Shrimp were fed TetraMin tropical fish flakes or shrimp pellets every few days, generally preceding water changes to minimize fouling of the tank water. Shrimp that died were fixed using 4% formalin and preserved in 70% ethyl alcohol for future reference and morphological study. Voucher specimens were deposited into the US National Museum of Natural History (USNM), Washington, DC, as follows: *A. angulosus*—two specimens (mating pair), USNM 1098194, Fort George Inlet of St. Johns River, Jacksonville, FL, coll. R.E. and M.K. Knowlton, 3 July 2004 (died in lab 12 July 2004); *A. estuariensis*—one specimen, USNM 1098195, Round Marsh of St. Johns River (Timucuan Preserve: Fort Caroline), Jacksonville, FL, coll. R.E. Knowlton, 2 July 2004 (died in lab 7 September 2004); *A. heterochaelis*—two specimens (mating pair), USNM 1098193, "Research Cove" near Duke University Marine Laboratory, Beaufort, NC, coll. H. Spence, 1 July 2004 (died in lab 20 July 2004).

Morphology

Morphological features of adult shrimp and developmental stages were determined through observation and photography of our collected living material, supplemented by examination of all available preserved specimens of the three species stored in the USNM.

Digital photographs, made using an MTI 3CCD camera and FlashPoint FPG 3.10 program through a Leica MZ12 microscope and analyzed with program ImageJ 1.20s, were taken as quickly as possible after collection to document natural coloration. We found that using a bowl of about the same diameter as the animal, combined with drawing off some of the sea water in the bowl to about the animal's height, was reasonably successful in immobilizing a shrimp long enough to photograph it without desiccating it. Ventral views could be obtained by inverting the animal contained within a covered Petri dish. Turning off or dimming the lights between taking photographs also helped the shrimp stay still, as did the use of backlighting.

Development

Reproductive activity, such as the presence of eggs on pleopods (swimmerets) of females, or ripe ovaries, was noted at time of collection. Embryos of *A. heterochaelis* and *A. angulosus* in various stages of development were examined and photographed (as above), referenced with preserved specimens in USNM collections and Knowlton's (1973) description of *A. heterochaelis* development. Egg characteristics, such as

approximate number, size, shape, color, stage of embryonic development (indicated by percentage of egg area occupied by yolk and appearance of compound eyes), were recorded upon arrival at the laboratory and tracked subsequently until hatching or loss. Early larval characteristics were noted for a single live specimen.

Results

Collections

Overall, 77 individuals were collected. The summer collection yielded a total of 51 shrimps: 24 from Florida (19 *A. angulosus* from FGI, 5 *A. estuariensis* from RM) and 27 *A. heterochaelis* from North Carolina (DUML). Included among them were 12 ovigerous females: 5 *A. angulosus* and 7 *A. heterochaelis*. The fall collection yielded a total of 22 specimens: 15 from Florida (11 *A. angulosus* from FGI, 4 *A. estuariensis* from RM) and 7 *A. heterochaelis* from North Carolina (DUML); of these, 5 *A. angulosus* females bore eggs. In March, 4 shrimps were found from DG, the only site where both species were collected: 3 individuals of *A. angulosus* (including a mating pair) and one large female *A. heterochaelis*. The latter was found closer to the water and deeper into the mud than the former, which were, as in Florida (FGI), under more shallowly situated rocks.

Morphology

All alpheids collected from DUML clearly matched species descriptions for *A. heterochaelis* (e.g., McClure 1995, Williams 1984), while those from FGI and RM generally matched the species descriptions for *A. angulosus* and *A. estuariensis*, respectively (e.g., McClure 1995, 2002). Individuals of *A. heterochaelis* (in summer collection) were generally larger (mean carapace length ± standard deviation = 10.1 ± 2.5 mm; number of individuals = 24) than *A. angulosus* (8.2 ± 1.1 mm, n = 19), although there was some overlap; those of *A. estuariensis* were consistently smaller (7.1 ± 0.5 mm, n = 4). The difference in means between *A. heterochaelis* and each of the other two species was significant (vs. *A. angulosus*: $t = 3.06$, $df = 41$, $P < .01$; vs. *A. estuariensis*: $t = 2.33$, $df = 26$, $P < .05$), but between *A. angulosus* and *A. estuariensis*, it was not ($t = 1.85$, $df = 21$, $P > .05$).

At DG, where *A. heterochaelis* or *A. angulosus* were sympatric, an overall color difference between these species was discernable. While some *A. angulosus* individuals (collected at FG) and *A. estuariensis* (from RM) were seen to have diffuse blue pigment on their uropods (Fig. 2a), bright blue spots, with orange on anterior margins, were found to be a major distinguishing feature of *A. heterochaelis* (from DUML) (Fig. 2b). Also, there is a flattened triangular area of the carapace at the base of the *A. angulosus* rostrum (Fig. 3a), but not in the other two species (Fig. 3b). The minor chela of *A. angulosus* is visibly broader than that of *A. heterochaelis* (Figs. 4a, b), and it does not bear the row of long setae ("balaeniceps-type" claw) characteristic of *A. heterochaelis* males (Fig. 1; also noted and illustrated in McClure 1995, McClure and

Wicksten 1997). The long and slender minor chela of *A. estuariensis* (Christoffersen 1984, McClure 1995) is distinguishable (Fig. 4c). Another important feature of *A. estuariensis* is its diffusely banded color pattern (Christoffersen 1984); on the dorsal side of each abdominal segment, the anterior margin is lighter than the posterior one (Fig. 5).

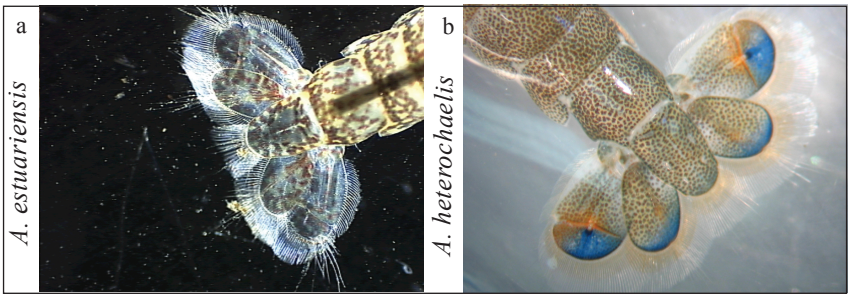

Figure 2. a. Tan to pale blue tail fan of *A. estuariensis* (also characteristic of *A. angulosus*). b. Tail fan of *A. heterochaelis*, with characteristic bright blue spots on uropods.

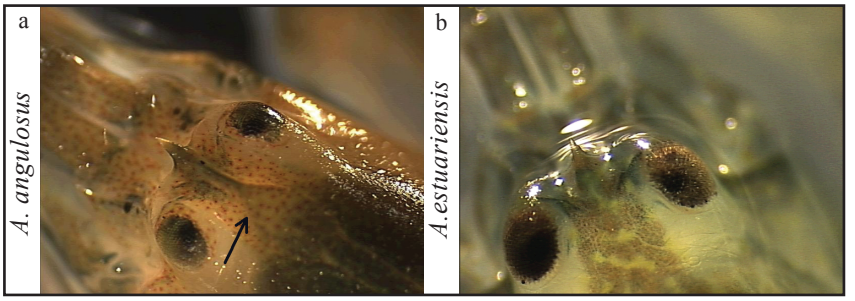

Figure 3. a. Rostrum of *A. angulosus*, exhibiting triangular base (indicated by arrow) and flanked by eyes. b. Rostrum of *A. estuariensis*, which lacks triangular base (as does the rostrum of *A. heterochaelis*).

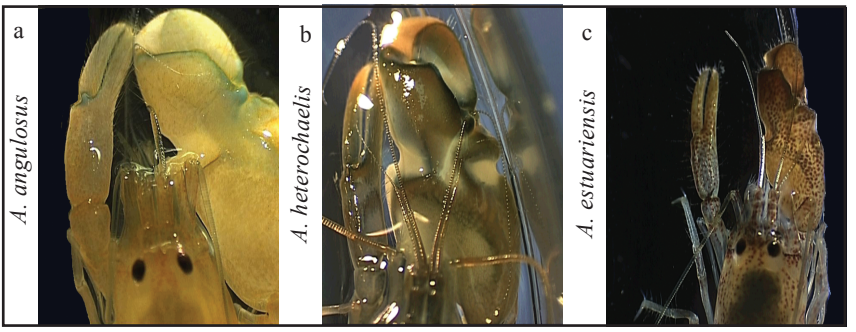

Figure 4. a. Anterior region of *A. angulosus* exhibiting relatively wide minor chela and paler coloration after being kept in the laboratory. b. Anterior region of female *A. heterochaelis*, showing thinner (vs. *A. angulosus*) minor chela and tan antennal flagella. c. Anterior region of *A. estuariensis*, exhibiting characteristic slender minor chela, tan antennae, and angular dactylus of major chela.