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The importance of chemical environmental cues for juvenile *Lethrinus nebulosus* Forsskål (Lethrinidae, Teleostei) when settling into their first benthic habitat

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Abstract

A commercially important coral-reef fish, the spangled emperor *Lethrinus nebulosus*, settles into seagrass beds at the end of its pelagic larval phase, but the mechanism for locating these beds is unknown. To investigate this mechanism we first used a wide-choice, *ex situ* setup to examine the ability of captivity-reared naïve *L. nebulosus* settlers to select their first benthic habitat by reference to chemical cues. Second, we examined the morphology and ultrastructure of the nasal olfactory organ in settling *L. nebulosus* juveniles. We obtained the first evidence of a tropical seagrass-settling coral reef fish that can use chemical environmental cues in selecting its first benthic habitat at ranges up to at least 2 m. The *L. nebulosus* settlers exhibited a well developed pair of nasal olfactory organs, positioned in nares on the dorsal side of the head. These organs were elliptical radial rosettes, one in each of the olfactory chambers, and each comprised 12 lamellae, six on each side of a midline raphe, which were totally covered with sensory and non-sensory cilia, except for the margins. This type of cilia distribution is thought to indicate an acute sense of smell. The olfactory epithelium contained mature and immature ciliated receptor neurons bearing three to five cilia, and a second type of receptor neuron bearing six to eight microvilli.

Keywords: Chemical senses; Commercial importance; Coral reef fish; Habitat detection; Habitat selection; Seagrass bed habitat

1. Introduction

The movement of individuals between populations in numbers large enough to be demographically significant, i.e., to provide demographic connectivity, is a largely unanswered question in marine ecology (Leis, in press). Demographic connectivity is a crucial parameter for the understanding and modelling of marine population dynamics, and, therefore, in the management of fisheries and marine parks (Kingsford et al., 2002; Leis and McCormick, 2002; Leis, in press).

Recent studies of larvae and juveniles of tropical shallow-water coral-reef fishes have revealed highly developed swimming abilities and the use of sensory modalities when settling into their first benthic habitat (recently reviewed by, e.g., Kingsford et al., 2002; Leis and McCormick, 2002; Leis, in press), and have stimulated a re-examination of the larval dispersal models (Leis and McCormick, 2002). Recognition of suitable settling sites has been hypothesized to be based on acoustic, chemical, solar direction, rheotactic, magnetic, wave motion or thermal cues. However, only the visual, olfactory and auditory

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senses are known to be functional in reef fishes when they settle into their first benthic habitat, and the last two of these in only a few species (Myrberg and Fuiman, 2002; Leis, in press). Vision may be significant over short ranges, up to 15 m (Leis and McCormick, 2002). Sound is omnidirectional, and pervasive on coral reefs, as a result of bioactivity (Cato, 1978), and recent studies have demonstrated its importance for several species of tropical marine fishes as they settle into their first benthic habitat (e.g., Tolimieri et al., 2000; Wright et al., 2005). Several species of damselfishes (Family Pomacentridae) can use chemical cues related to the habitat or to conspecifics when settling to their first benthic habitat (e.g., Sweatman, 1985; Murata et al., 1986; Miyagawa, 1989; Elliot et al., 1995; Arvedlund and Nielsen, 1996; Arvedlund et al., 1999; Lecchini et al., 2005; Wright et al., 2005; in the latter study, a species of damselfish (*Pomacentrus nagasakiensis*) at the setting stage could smell habitat cues, but it was unclear if it could use this ability for habitat detection). Some species of cardinalfish can use chemical habitat cues for orientation, when settling at the end of their pelagic larval phase (Family Apogonidae: Atema et al., 2002). In addition, adult members of at least two species from the last-named family can use chemical cues from conspecifics when homing (Døving et al., 2006). Adults of the tropical pearl-fish *Carapus bermudensis* can use chemical cues to locate their sea cucumber host *Actinopyga agassizi* (Van Meter and Ache, 1974). Last, but not least, embryos of the anemonefishes *Amphiprion ocellaris* and *A. melanopus* can memorise chemical cues secreted by their sea anemone host, and later use these chemical cues for orientation over ranges of a few metres when settling into their first benthic habitat (Arvedlund and Nielsen, 1996; Arvedlund et al., 1999, 2000a,b).

Seagrass beds are important shallow coastal-water nurseries for the growth and survival of many marine fishes (Jackson et al., 2001; Nagelkerken et al., 2002). In the shallow coastal waters around Okinawa, southern Japan, juveniles of the spangled emperor *Lethrinus nebulosus* settle into seagrass beds, with a peak season around June (Ebisawa, 1990; Kanashiro, 1998). The emperors (Lethrinidae, Teleostei) are prominent percoid fishes, found principally as adults along shallow coral reef habitats of the subtropical and tropical Indo-Pacific region (Wilson, 1998; Grandcourt et al., 2006), and are an important component of reef-based fisheries throughout their range. Their diet mainly comprises molluscs, crustaceans, polychaete worms, and echinoderms (Fischer and Bianchi, 1984). *L. nebulosus* is a protogynous hermaphrodite (Ebisawa, 1990) with a prolonged nocturnal spawning season that extends from spring to at least early autumn, with peaks in spring and autumn (Johannes, 1981). Data on how long *L. nebulosus* juveniles remain in seagrass beds are sparse; Wilson (1998) observed juvenile emperors of 11 species, including *L. nebulosus*, at Magnetic, Green and Lizard islands on the northern part of the Great Barrier Reef, Australia, where they stayed almost exclusively over shallow-water (≤ 7 m) seagrass areas until "at least 80 mm".

The mechanism(s) by which *L. nebulosus* juveniles settling into seagrass beds select their habitats is/are not known. However, Forward et al. (2003) found that chemical cues from seagrass beds could provide nursery habitat orientation for premoult megalopae of the blue crab *Callinectes sapidus* and this finding, together with the past observations of coral reef fishes using chemical habitat cues when settling into a reef, may indicate a type of habitat cue used by settling *L. nebulosus* juveniles.

Olfaction, rather than other chemically based senses, is normally involved in remote chemoreception tasks (Basil et al., 2000; Atema et al., 2002). However, although the nasal olfactory organs of some fish taxa have been studied for more than 100 years, there have been very few studies of their embryogenesis and of their larval and juvenile stages in most taxa, and of all stages of tropical shallow-water coral reef fishes (Leis and McCormick, 2002). No studies have been published of the morphology of the olfactory organ in *L. nebulosus*.

The objective of the present study was to examine the hypothesis that *L. nebulosus* settlers are able to select their first benthic habitat by using chemical cues emitted from a seagrass bed habitat. Firstly, naïve *L. nebulosus* settlers that had been reared in captivity were exposed, in *ex situ* choice test environments, to chemical or visual cues from a seagrass bed habitat, or to chemical cues from rocks. Secondly, the morphology and the ultrastructure of the nasal olfactory organ in naïve, captivity-reared *L. nebulosus* settlers was examined by means of light and electron microscopy.

2. Methods and materials

2.1. Acquisition of animals

Lethrinus nebulosus were reared at the Prefectural Sea Farming Centre, Motobu, Okinawa, southern Japan. A group of 36 locally caught, sexually mature *L. nebulosus* adults, in a male:female ratio of 1:2, kept in a 50-tonne concrete tank, spawned on two nights every month from May to August 2004. The tank was supplied with fresh sea water from an open sand-filtered system. The floating, fertilized eggs were collected by siphoning, and