Journal of Experimental Marine Biology and Ecology 463 (2015) 115–124

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Grooming behavior by elongated third maxillipeds of phyllosoma larvae of the smooth fan lobster riding on jellyfishes

Michiya Kamio [a,*], Dai Furukawa [a], Kaori Wakabayashi [a], Kaori Hiei [b], Hirona Yano [a], Hiroshi Sato [a], Yumiko Yoshie-Stark [c], Tatsuro Akiba [d], Yuji Tanaka [a]

[a] Graduate School of Marine Sciences, Tokyo University of Marine Science and Technology, Tokyo, Japan
[b] Department of Ocean Sciences, Tokyo University of Marine Science and Technology, Tokyo, Japan
[c] Faculty of Life Sciences, Toyo University, Oura-gun, Japan
[d] Biomedical Research Institute, National Institute of Advanced and Industrial Science and Technology, Tsukuba, Japan

ARTICLE INFO

Article history:
Received 24 August 2014
Received in revised form 20 November 2014
Accepted 21 November 2014
Available online 5 December 2014

Keywords:
Antifouling
Chemoreception
Glycine
Plankton
Programmed behavior
Quantitative NMR
Scyphozoa

ABSTRACT

Animals groom their body surface to prevent or remove parasites and non-biological fouling that can negatively impact their mobility, appearance, and health. The jellyfish riders, phyllosoma larvae of the smooth fan lobster *Ibacus novemdentatus* Gibbes which spend their planktonic larval period riding on and eating live jellyfish, have a characteristic body shape, a leaf-like flat body without gills and a pair of elongated third maxillipeds. Although the third maxillipeds lack exopods and are very long compared with that of other types of decapod crustacean adults and larvae, their function is not known. In the present study, we hypothesized that the elongated third maxillipeds of *I. novemdentatus* phyllosoma larvae constitute a grooming organ specialized to remove fouling from their body surface and/or an organ to manipulate their food, and tested the hypothesis through morphological and behavioral experiments. Our morphological observations show that the third maxillipeds are longer than the larva's body and that the tip of the maxillipeds has a comb-like structure. Our behavioral experiments show that the larvae spent about 50% of their time grooming with their third maxillipeds but did not use these maxillipeds to manipulate jellyfish for eating. The maxillipeds removed an experimentally-applied foulant from most of their body parts, suggesting that this artificial foulant stimulated grooming on that location. Thus, the grooming has characteristics of both programmed and stimulus-driven behaviors. Inhibition of this behavior enhanced growth of microbes on the body surface of that phyllosoma fed with jellyfish. Glycine was identified as major free amino acid in jellyfish tissue using amino acid analysis and as a major metabolite by nuclear magnetic resonance (NMR) spectroscopy. Glycine induced appetitive and grooming behaviors in the larvae. Thus, we conclude that the phyllosoma larvae use the elongated third maxillipeds for cleaning their entire body surface and that the maxillipeds are functionally specialized for this cleaning behavior. This frequent whole body cleaning behavior with specialized appendages might be adaptive to their lifestyle of riding on jellyfish, where they are exposed to large amounts of mucus released from the jellyfish which could otherwise cause growth of microbes on their body surface.

1. Introduction

Grooming, involving all forms of body surface care, is important for animals to avoid biological and non-biological fouling, which can negatively affect their mobility, appearance, and health. In terrestrial animals, self-grooming to remove ectoparasites or pathogens is known for diverse groups of animals (Hart, 2011). Impala remove ticks with their mouth and by scratching using their hind hooves (Mooring et al., 1996), rock pigeons remove flies using their bill (Waite et al., 2012), and ants remove fungal contamination using their mandibles and legs (Reber et al., 2011). These grooming activities have been shaped by the evolutionary process. For example, ungulates living in habitats with more parasites groom more than those in habitat with less (Mooring et al., 2004). These grooming behaviors are controlled by two means: programmed regulation, which is driven by an internal timing mechanism that periodically evokes a bout of grooming independent of peripheral stimulation from fouling; and stimulus-driven regulation, which is driven by cutaneous stimulation from fouling such as bites by ticks. Programmed regulation is an effective defense against parasites because it can remove parasites before they damage the skin of the host animal, but it has a cost of affecting the production of other behavioral abilities of the host animal because when an animal is grooming the animal has to stop or decrease the rate of other behaviors (Mooring and Samuel, 1998).

* Corresponding author.
E-mail address: mkamio@kaiyodai.ac.jp (M. Kamio).

http://dx.doi.org/10.1016/j.jembe.2014.11.008
0022-0981/

Marine crustaceans live under constant exposure to a wide variety of microbial (bacteria fungi, sessile protists) and macroscopic fouling organisms (bryozoan, hydroids, barnacles, ectoparasites) and inorganic particulate matter (sediment and debris) in the water column (Bauer, 1989, 2013). These foulants may cover surfaces of respiratory organs, sensory organs, and other body parts including appendages, and consequently negatively affect their functions. Although all crustaceans renew their body surface upon molting, fouling occurring between the molts has to be removed by grooming (Bauer, 1989) and other antifouling behaviors such as burying in the sediment, hiding in rock crevices or below stones, night-time activity, and exposure to air (Becker and Wahl, 1996). Grooming of gills and antennules by the third maxillipeds and grooming of general body surfaces by walking legs are common in adult decapod crustaceans (Bauer, 2013). The sensory mechanism underlying grooming is well studied in antennular grooming. Food-derived molecules, such as glutamate (Barbato and Daniel, 1997), are detected by specific non-olfactory chemoreceptors on the antennules (i.e. asymmetric sensilla) to elicit antennular grooming in Caribbean spiny lobsters *Panulirus argus* (Schmidt and Derby, 2005). The chemical stimuli that stimulate grooming are different among species (Cericola and Daniel, 2010) and narrowly tuned in some species: L-glutamate for *Panulirus guttatus*, *Panulirus interruptus*, and *Homarus americanus* (Daniel et al., 2001) and glycine for *Scyllarides* spp. (Cericola and Daniel, 2010). L-Glutamate (Barbato and Daniel, 1997), glycine, L-alanine, and trimethylamine-*N*-oxide elicit grooming in *P. argus* (Schmidt et al., 2012). Although much is known about grooming by adult decapod crustaceans, behaviors including grooming of their larvae are not well described because rearing of planktonic larvae of decapod crustaceans is difficult, due mainly to a lack of knowledge about feeding, tank design, and disease control (Matsuda and Takenouchi, 2007). An alternative to rearing larvae is to collect them and test them immediately; however, obtaining enough healthy larvae appropriate for behavioral experiments is difficult.

Phyllosoma larvae of the smooth fan lobster *Ibacus novemdentatus* Gibbes (Decapoda: Palinura: Scyllaridae) are known as jellyfish riders, since they spend part of their planktonic life on jellyfish (Shojima, 1963; Wakabayashi et al., 2012a), as reported in other scyllarid lobsters (Herrnkind et al., 1976; Thomas, 1963). A culturing method in a closed recirculating water system for these larvae was established recently (Wakabayashi et al., 2012b). Although there are no data showing how long the larvae spend time on the jellyfish in the field, there are reports indicating that they are closely associated with jellyfish. Phyllosoma of this species riding on jellyfish have been observed in the field (Shojima, 1973) and in the laboratory by cultured phyllosoma riding on multiple species of jellyfish (Wakabayashi et al., 2012a, 2012b). Although jellyfish have tentacles that are equipped with numerous nematocysts (Tardent, 1995) containing proteinaceous venoms (Nagai, 2012) to capture their prey including planktonic crustaceans (Hamner et al., 1995; Ishii and Tanaka, 2001), phyllosoma larvae of *I. novemdentatus* ride on jellyfish that are larger than their body size and consume the jellyfish as a food. Phyllosoma larvae in the laboratory can complete their planktonic stages by exclusively consuming jellyfish and then settling to the bottom of the tank to start benthic juvenile phase of their life (Wakabayashi et al., 2012b). Predatory behavior of the phyllosoma larvae is adaptive. When the phyllosoma larvae consume jellyfish, they ride on to the jellyfish and first eat the dangerous venomous tentacles. After eliminating these tentacles, they consume the other, safer body parts (Wakabayashi et al., 2012a).

Phyllosoma is a characteristic larva of palinurid and scyllarid crustaceans (Scholtz and Richter, 1995). “Phyllo” means leaf in Greek and derives from the fact that the phyllosoma has a dorsoventrally flattened body and carapace (Gurney, 1941). This flattened carapace is called a cephalic shield (Sekiguchi et al., 2007). Because of this flattened cephalic shield, the phyllosoma does not have a branchial chamber with gills throughout its planktonic larval life (Kittaka et al., 1997; Marinovic et al., 1994; Mikami and Greenwood, 1997; Robertson, 1968). Lacking gills, the dorsal side of cephalic shield and legs function as respiratory organs and the ventral side of cephalic shield likely mediates ion exchange due to its high ion permeability and thick mitochondria-rich epithelium (Haond et al., 2001). Thus the body surface of phyllosoma has important life-sustaining functions.

Elongated mouthparts – the third maxillipeds – are another obvious morphological character in phyllosoma. In general, maxillipeds of decapod crustaceans are literally mouth parts that are located around the mouth and functionally specialized for food handling (Garm, 2004a, 2004b) and grooming of mouth region and pereiopod (Bauer, 1989, 2013). Grooming of antennules (Schmidt and Derby, 2005) which are located above the maxillipeds at the anterior end of the cephalic region, is an important behavior to maintain its chemosensory function. In this case, the decapod lowers its antennules down into mouth area, where they are cleaned by the third maxillipeds. The third maxillipeds of phyllosoma of scyllarids (Palero et al., 2011; Webber and Booth, 2001), including *I. novemdentatus* (Wakabayashi and Tanaka, 2012), are as long as their pereiopods. The relative length of the third maxillipeds in these species is much greater for the phyllosoma than for their benthic juvenile and adult stages (Sekiguchi et al., 2007) and long enough to touch other body parts in addition to the mouthparts and antennules. Behaviors using these elongated maxillipeds have not been described well in scyllarid phyllosoma. In two related species, the palinurid spiny lobsters *Sagmariasus verreauxi* and *Jasus edwardsii*, manipulation of food and grooming their body and other appendages using the third maxillipeds were observed in feeding experiments (Cox and Bruce, 2003; Cox and Johnston, 2003), though the function of grooming was not explored. Grooming of body surfaces is important because most of the external exoskeleton covering the body of phyllosoma functions in respiration and ion exchange (Haond et al., 2001). By riding on and eating jellyfish, the phyllosoma is exposed to frequent fouling of mucus secreted from jellyfish (Niggl et al., 2010) as a chemical defense (Shanks and Graham, 1988) and for capturing food (Orton, 1922; Southward, 1955). Feeding by phyllosoma damages jellyfish and releases pieces of tissue and various macromolecules such as proteins from the body of jellyfish. These tissue and macromolecules can be foulants on the phyllosoma. This fouling from jellyfish will be the starting point of a biofouling process that starts with organic compounds that cause microfouling by bacteria, diatoms, spores of microalgae, and protozoans, then continues with macrofouling by multicellular sessile organisms (Abarzua and Jakubowski, 1995). Extensive biofouling may inhibit transport of oxygen and ions across the body surface of phyllosoma, which is necessary to maintain their life. According to this scenario, we hypothesized that the elongated third maxillipeds of *I. novemdentatus* phyllosoma larvae constitute a grooming organ specialized to remove fouling from their body surface and/or an organ to manipulate their food.

To test this hypothesis, we asked the following questions and designed experiments to answer the following question: (1) Do the third maxillipeds of phyllosoma larvae have reasonable morphological characters for grooming or food manipulation? This question was examined by quantifying the relative length of the maxillipeds and observing the structure of their distal tip. (2) Do phyllosoma larvae use their maxillipeds to manipulate their food? We observed the motion of the maxillipeds while phyllosoma were feeding. (3) Do phyllosoma use their maxillipeds for grooming the body? We made observations on behavior to determine if the maxillipeds were involved in grooming. (4) Is grooming controlled by programed or stimulus-driven process? This question was tested by comparing the frequency of grooming with or without artificial fouling stimuli. (5) Does inhibition of grooming behavior enhance fouling of their body surface? Grooming of phyllosoma larvae was inhibited and the impact on the extent of fouling was tested. (6) Do phyllosoma larvae groom in response to jellyfish odors? To answer this question, water soluble, small molecules in jellyfish odor were identified using nuclear magnetic resonance