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FULL LENGTH ARTICLE

Antifouling activity of crude extracts isolated from two Red Sea puffer fishes

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Abstract Crude extracts were isolated from the ovary and mucus of the puffer fish *Amblyrhynchotes hypselogenion* and *Lagocephalus sceleratus*. The crude toxin extracts (from the ovaries of *L. sceleratus* puffer fish (4.5, 9.0, and 18.0 g crude extract/100 g paint) and only one concentration (3.0 g crude extract/100 g paint) from the skin mucus of *A. hypselogenion* puffer fish were mixed) with an inert simple paint formulation (consists of (vinyl chloride–vinyl acetate) copolymer using tricresyl phosphate as plasticizer. The viscosity of paints was adjusted using a blend of solvents consisting of methyl isobutyl ketone and toluene) applied to poly vinyl chloride (PVC) plates and exposed to Suez Gulf water for about 24 weeks. Percentage covers of fouling organisms on plates were estimated for coated panels over six weeks, and the wet weight (gram/plate) of fouling organisms on experimental plates was recorded till about 24 weeks. The percentage of plates coated by formulation with (2007 for 24 weeks) the crude toxic extracts from the ovaries of *L. sceleratus* and the mucus of *A. hypselogenion* ranged from 22% to 24% and 11% to 12%, respectively. The wet weights of the plates covered by formulation with the same crude toxic extracts of the previous two species were 124–147 g and 82–93 g, respectively. Antifouling properties were observed for the extracts of the two species under investigation, however, extracts from *A. hypselogenion* showed better properties.

Introduction

Biofouling is the settlement and subsequent growth of marine organisms on submerged surfaces. Because these surfaces are in many instances artificial, e.g., boat hulls, oil and gas platforms, aquaculture nets and enclosures, fouling organisms present a significant problem to human activities in the marine environment. The formation of fouling communities on boats increases drag; reducing ship speed while increasing fuel costs and dry dock time (Champ, 2000). Fouling of semi-permanent

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offshore structures increases structural loading and corrosion, and fouling of nets and enclosures adds significant costs to the maintenance of aquaculture systems. The increased fuel usage, dry-docking, structural inspection, maintenance, etc. resulting from biofouling have been estimated to cost the world's marine industries over $3.5 billion dollars per year (Wolfram et al., 1993).

Fouling is primarily combated by the use of antifouling coatings which are applied to the surfaces of boats or other marine structures. These coatings rely almost exclusively on heavy metals particularly copper and tin based compounds for their active ingredients. These compounds leach into the water from the coating, killing fouling organisms. Unfortunately, these compounds also kill non-target organisms in the environment at large, and the general environmental effects of these compounds are of worldwide concern (Dalley, 1989). Tin based coatings are particularly toxic, and such coatings are now partially (restricted to vessels > 25 m in length in Europe and parts of the USA) or totally banned (Japan) in the world's waterways. It is generally accepted that the use of tin (specifically tributyl tin) based coatings will be completely banned in most countries within the next 5–10 years (Yebra et al., 2004).

The demise of heavy-metal based antifouling coatings has prompted the search for more environmentally benign antifoulants, and one of the most promising alternative technologies is the use of naturally occurring antifouling compounds derived from natural metabolites from marine organisms (Holmstrom and Kjelleberg, 1994).

Marine organisms such as seaweeds, sponges, etc. are also submerged surfaces, and they must deter fouling of their bodies to avoid increases in drag (and being pulled off the bottom) or being smothered. It is now abundantly clear that one of the ways in which marine organisms defend against fouling is via the production of natural antifoulants. Because these compounds already occur in nature, it is thought that these natural metabolites will be less environmentally damaging than the current heavy-metal based paints. A number of metabolites or extracts from marine organisms with antifouling activities typically tested against barnacle larvae have now been identified (Holmstrom and Kjelleberg 1994; Targett et al., 1983; Standing et al., 1984; Rittschof et al., 1985; Gerhardt et al., 1988; Keifer et al., 1986; Mary et al., 1993; Maki et al., 1989; Holmstrom et al., 1992; Todd et al., 1993).

One of the promised as antifouling activity may be the puffer fishes crude extracts. Puffer fish are a part of a group called tetrodotoxic fishes. The ancient Chinese and Japanese were aware of the toxin contained in puffer fish and used the non-poisonous parts of the fish as a general health tonic. Japanese researchers tested the crude puffer fish extract to treat migraines and menstrual cramps. It is mainly found in the ovaries, liver, and intestines of various species of puffer fish, lesser amounts were found in the skin (Kao, 1966 and Blankenship, 1976). The tetrodotoxin compound has been obtained from an extract of puffer fish viscera in the form of colorless crystal that are slightly soluble in water. The toxin is unstable at pH levels above 8.5 and below 3 (Schantz, 1973 and Scheuer, 1977).

The effects of tetrodotoxin, have been tested experimentally on a large variety of animal species such as crap, rat, Frog, Rabbit, Dog and Cat (These effects involve primarily the peripheral neuromuscular system, which is paralyzed to different extents because of interference with generation and conduction of electrical impulses (Russell and Dart, 1991). Tetrodotoxin is a highly potent emetic agent (Atwell and Stutchbury, 1978; Mohamed, 2003). Therefore the objective of this study is to explore in the presence of antifouling activity the crude extracts of two Red Sea puffer fish species, *Lagocephalus sceleratus* and *Amblyrhynchotes hypselogenion*.

Figure 1 The puffer fish, *A. hypselogenion*.

Materials and methods

Puffer fish sampling and extraction procedure

Two species of Red Sea puffer fishes *L. sceleratus* Fig. 1 and *A. hypselogenion* Fig. 2, were chosen for the present study. The crude bioactive materials from their ovaries and skin mucus were isolated and their antifouling property was tested. The samples were collected from the Red Sea fishing ports and were taken to the laboratory of NIOF (National Institute of Oceanography and Fisheries) in Suez for immediate processing. The ovaries of *L. sceleratus* were homogenized with 1% acetic acid. The obtained homogenate was centrifuged at 2700 rm. Then the pellet re-extracted twice with the same volume of 1% acetic acid solution. The supernatant was concentrated under reduced pressure and then defatted by shaking gently with an equal volume of dichloromethane three times. The aqueous layer was collected and concentrated under reduced pressure to remove residual dichloromethane. The resulting crude extracts were stored in a freezer till later testing.

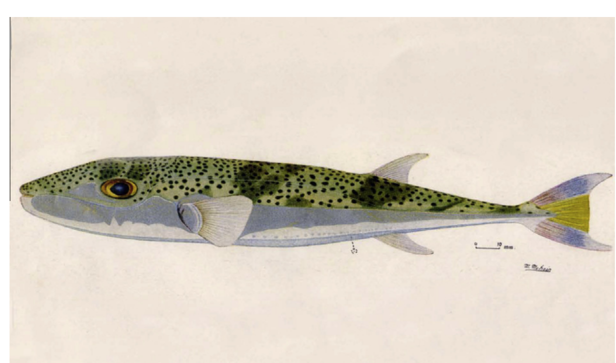

Figure 2 The puffer fish, *L. sceleratus*.