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A synthetic crustacean bait to stem forage fish depletion

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ABSTRACT

Crustaceans, such as crab and lobster, comprise an important global food commodity. They are captured in traps using primarily forage fish (e.g. anchovies, herring, and menhaden), as bait. Approximately 18 million tons of these fish are used annually to bait traps, worldwide (U. Nations, 2014). In addition to natural predators dependent on forage fish (Pikitch et al., 2012), myriad other factors are further intensifying demand and collectively threatening stocks (*e.g.* Omega-3 supplements, pet food, livestock feed, – in addition to direct human consumption). Forage fish capture methods pose collateral environmental risks from by-catch (*e.g.* seals, dolphins, turtles) indiscriminately killed in nets. Sustainable alternatives to stem further depletion are desperately needed, and toward this end, a synthetic crustacean bait has been developed. The technology mimics molecules released from forage fish by employing a formulation that is dispersed at a controlled rate from a soluble matrix. The synthetic bait reliably caught stone crab, blue crab, and American lobster in field trials. This technology addresses major ecological threats, while providing economic and operational benefits to the crustacean fishing industry.

One Sentence Summary: A synthetic crustacean bait has been developed to obviate the need for forage fish capture and depletion.

1. Introduction

Rationale for forage fish conservation

The importance of forage fish in every ocean ecosystem is clear (Pikitch et al., 2012). As a critical link in the food chain, forage fish provide nutrition for marine and shore mammals, seabirds, and large fish species (Alder et al., 2008; Borrell, 2013; Cinner et al., 2013; Cury et al., 2011; Essington et al., 2015; Pennisi, 2010; Smith et al., 2011). In fact, pelagic fish and seabirds consume nearly 50% of forage fish every year (Pikitch et al., 2012). Forage fish provide a biological connection between the lower trophic-level planktonic species and upper trophic-level predators in the food web (Pikitch et al., 2014; Cury et al., 2000; Fréon et al., 2005). Their crucial role is most visible during periods in which their numbers collapse, as reflected in counts of deceased or distressed marine mammals, seabirds, and larger fish that depend on them as their primary source of nutrition (Pikitch et al., 2012; Cury et al., 2011; Smith et al., 2011). They are also vital for coral reef health, and studies have suggested that fishing restrictions have proven beneficial to various marine habitats (MacNeil et al., 2015; Edgar et al., 2014).

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Indeed, articles and reports cataloging the effects and trends of forage fish decline have continued to appear in various media with notable frequency (Essington et al., 2015; Dulvy and Kindsvater, 2015; Enticknap, 2014; Feltman, 2015; George, 2014; Pikitch, 2015; Sherwood, 2015; Welch, 2014).

Forage fish are also facing a myriad of industrial demands, which are intensifying pressures on their populations and providing the impetus for finding substitutes for their by-products (Lenihan-Geels et al., 2013; Salem and Eggersdorfer, 2015). One-third of the global wild fish catch is processed and fed to farm-raised fish (aquaculture) and livestock (pork and poultry industries) (Alder et al., 2008). National Oceanic and Atmospheric Administration (NOAA) and United States Department of Agriculture (USDA) data suggest an annual processing of 34 million pounds of forage fish for feed pellets, directly imperiling sustainability and raising the probability of sudden ecosystem collapse (Tacon and Metian, 2009). Yet local, regional and international governments and regulators continue to support these practices, possibly due to aquaculture's role in global food security. More protein for human nutrition is derived from farmed fish than from any other food source (including beef and poultry) (Larsen and Roney, 2013). Another significant demand is driven by fish oil dietary supplements, further impacting the ecosystems dependent on these species (Lenihan-Geels et al., 2013). Widely believed to be beneficial for human health, Omega-3 products, in particular, account for a rapidly growing, $25 billion industry with no sign of leveling off in the near term (Alder et al., 2008; Borrell, 2013; Pikitch et al., 2014). And, in addition to the ≈15%–20% required for direct human consumption, another 13% of the annual forage fish catch is used in domestic cat food production (Tacon and Metian, 2009).

In turn, using them as trap or "pot" bait, the crab and lobster fishing industries are among the largest end users of forage fish. The annual global market for crab and lobster has been estimated to be $66 billion dollars; this would equate to approximately six million metric tons of crustaceans caught for human consumption at average prices per pound (U. Nations, 2014). However, the global demand for forage fish to bait and trap them is difficult to estimate, given diverse methodologies among crab, lobster and regional fishing practices, as well as the species used and sold as bait. Variables also include fishing seasons, pot size, pots that fail to catch, trap deployment or "soak" durations, and bait quantities necessary to attract respective species. One field-based, conservative estimate suggests a 3:1 ratio (pounds) of bait fish to crustacean capture, or that approximately three tons of bait are required to harvest one ton of crustaceans. Therefore, it would take ≈18 million metric tons (≈40 billion pounds) of forage fish to yield the global crab and lobster catch. Based on United Nations estimates, this volume may actually be far greater due to underreporting in various regions by as much as 20%–50% (Mason, 2015). Absent a disruptive alternative, forage fish demands from natural ecosystems, emerging industries, and as crustacean bait would be projected to continue to intensify at an unsustainable rate.

2. Methods

Representative forage fish species (herring, mackerel, and menhaden) were incubated in water (salinity 35‰; parts per thousand) at 28 °C for 2-, 24-, 48-, 96-, and 192-h under agitation to replicate oceanic motion. Samples from each time point were collected and stored at −20 °C until thawed for analysis.

Amino acids and their by-products were identified using HPLC. Both water samples and known standards were prepared as described (Peng et al., 2003). Amines were isolated via benzoylation (Richard et al., 2008); diluted in mobile phase (Water:Acetonitrile; 58:42); and separated through C18 or C8 columns on a Varian 920LC system (Agilent Technologies, Santa Clara, CA). Benzoylation was initiated by the introduction of benzoyl ($C_6H_5CO^-$) by replacement of an H^- ion-attached amine ($-NH_2$) functional group of amino acids. In this reaction, the amine group of putrescine reacts with benzoyl chloride to form dibenzoylputrescine. Amino acids (and by-products) were identified using UV/Vis. Standard calibration curves of identified molecules were established by measurement of absorbance at 229 nm using commercially sourced, known chemicals.

Adult American Lobster (*Homarus americanus*) were used in the crustacean olfaction analysis. Groups of Olfactory Receptor Neurons (ORN) are arranged in clusters and housed in cuticular extensions, or aesthetascs, found on two, paired antennae (Michel et al., 1999; Tadesse et al., 2014). The lobster were housed individually in 40 L tanks with re-circulating artificial saltwater at 5 °C. The olfactory organs or "sensilla" located on the lateral branch of the first antenna were removed and cut into sections of single annuli (Michel et al., 1999; Tadesse et al., 2014). Antennule slices were then digested at room temperature with vigorous shaking using activated papain to remove impeding membranes and non-ORN material. Following digestion, the slices were washed with lobster saline, stained with a calcium sensitive dye [Oregon Green® 488 BAPTA-1 AM (OG 488)] and enclosed and vigorously shaken for 1 h to ensure proper ORN dye absorption (Derby et al., 1997; Schmidt and Mellon, 2011; Schmidt et al., 2011). Following dye loading, the ORN nuclei were then treated for 5 min with a nucleic acid stain (Hoechst 33324). The stained slices were washed and mounted onto coverslips for imaging.

Baseline fluorescence was measured for 100 s prior to the administration of each stimulant. Images of calcium release peaks were taken continuously for an additional 2 min following stimulant application. Fluorescence (measured in gray value) was measured within a predefined volume using confocal microscopy and evaluated using the manufacturer's optimized software (Zeiss AxioVision). In order to control for changes in fluorescence not attributed to calcium flux, the OG 488 signal was normalized against the nucleic acid stain, which does not vary in response to stimulant addition (Michel et al., 1999; Tadesse et al., 2014). Slices were analyzed in triplicate with either lobster saline (control) or respective molecules identified from decaying forage fish. The fluorescence signal was normalized to the level of baseline fluorescence measured at 100 time points prior to stimulant introduction. The change in fluorescence (ΔF) was determined by calculating the ratio of the measured fluorescence in the presence of the stimulant to the mean baseline fluorescence values.