



Original research article

A synthetic crustacean bait to stem forage fish depletion

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ARTICLE INFO

Article history:

Received 6 May 2016

Received in revised form 5 July 2016

Accepted 5 July 2016

Keywords:

Crustaceans

Bait

Ocean conservation

Sustainability

Forage fish

Aquaculture

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.

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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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<http://dx.doi.org/10.1016/j.gecco.2016.07.001>

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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 $\approx 15\%$ – 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 benzylation (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). Benzylation 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 papsin 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.

3. Bait synthesis and field testing

The chemotactic chemicals not only needed to mimic the forage fish emissions but also to emanate likewise from a focal point within each pot in order to entice the crab and lobster to actually *enter* the trap. Thus, identification of these molecules alone was not enough to define a crustacean bait alternative; these attractants would clearly dissipate long before reaching the ocean floor unless contained in a manner that would allow their gradual release from within the traps subjected to a myriad of marine variables. Numerous materials and various formulations were tested in order to develop a dissolvable matrix that would not only mimic the olfactory stimulants, but also the gradual decay of the forage fish. Each preparation was 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. The most consistent recipe employed the hemihydrate form of calcium sulfate [(CaSO₄)_{1/2} · H₂O].

Preparation of the dissolvable matrix includes: (1) mixing at high speed (manually or by rotary mixer) approximately one part of calcium sulfate; approximately one part by weight of solvent (water); and sodium benzoate (hygroscopic preservative) until the mixture is sufficiently viscous to allow for mold formation; (2) blending a second, attractant-containing solution into the mixture; (3) pouring the combined ingredients into a mold; (4) allowing the resulting mixture to exothermically set in the mold until semi-solid; (5) removing the soft “cake” from the mold and allowing it to solidify completely. Differing concentrations of the hemihydrate and solvent were tested to achieve various dissolution rates. Such calibration of the dissolution rates will be optimized to adjust for specific depth, temperature, and water current (friction) that characterize geographic and regional variables in the final embodiment of the bait.

Synthetic bait evaluations with captive lobsters maintained under tank conditions can be problematic and produce irregular and/or erroneous results. Lobster held under typically intensive communal aquaculture (or tank environments) often exhibit an array of aberrant and aggressive behaviors not observed in the wild (e.g. cowering, isolating themselves without eating, or fighting to establish and maintain dominance hierarchies) (Gherardi et al., 2010; Karavanich, 1998; Kravitz, 2000). The risk of failing to study “normal” crustacean behavior was compounded by the need to evaluate dissolution and potential attraction in open “real world” waters far beyond what might be emulated with any number of laboratory mixers, water heaters, or tank dimensions. Therefore, several formulations were field tested by loading individual traps with one synthetic bait (Fig. 3). Crustacean fishing industry collaborators in Florida, North Carolina, California, and the British West Indies evaluated the synthetic baits. Optimized for each field test location, at least 30 traps loaded with the respective synthetic bait matrix were directly compared to 30 traps employing traditional flesh bait. To further assess actual performance in the field, the collaborators were asked only to substitute the synthetic bait, using it exactly as they would use the forage fish in the context of their established territory and species-specific fishing protocols. In general, traps were deployed between 4 and 6 days for blue crabs, 8–12 days for spiny lobsters, and 12–16 days for stone crab. After trap retrieval, the lobsters or crabs were counted and reported. Field evaluations were tested in a myriad of methodologies: alternating, linked, and isolated. For alternating patterns, each pot line was baited with alternating synthetic and traditional baits. The linked pattern consisted of a string with three pots in a row deployed with synthetic bait followed by three traps baited with fish. When possible, and to remove competitive nature between the two bait sources, either synthetic or traditional baits were evaluated isolated from other bait sources. In isolated experiments a specific location would be baited with 30 synthetic traps for the desired fishing duration and immediately following trap pulling the same traps in the same location were loaded with traditional bait.

4. Results

Characterization of chemotactic cues

To conceive such a disruptive technology, studying the dynamics of how forage fish lure crustaceans into traps provided the framework for developing an alternative, synthetic bait. Crab and lobster species have been shown to respond to scent cues released from dead and decaying flesh. That is, by identifying the molecules released from forage fish, these chemicals were hypothesized to be capable of mimicking the attraction from decaying fish when used to capture crustaceans, without the costly and environmentally destructive use of any fish or fish by-products. Fig. 1 depicts high performance liquid chromatography (HPLC) characterization of the chemotactic molecules emanating from forage fish when used in traditional bait methods (i.e., herring, mackerel, and menhaden).

When analyzed, every species revealed production and release of foul smelling, organic polyamines, shaping the theory that these pungent compounds were critical in attracting crustaceans. Numerous molecules released from various forage fish were identified including: biogenic polyamines (putrescine, cadaverine, spermidine); amino acids (taurine, arginine, ornithine, lysine, glycine); enzymatic proteins (adolase and trypsinogen); fatty acids (isovaleric and butyric acid); and organic acids (creatine and acetic acid). Of note, each forage fish species released large quantities of biogenic polyamines consistent with the catabolism of amino acids by microorganisms present in dead and decaying flesh. Significant peaks for several polyamines were detected at a minimum of 24 h in a saline preparation to emulate seawater. Samples taken at 48-h showed the highest release for all species tested.

Crustacean olfaction relies on groups of olfactory receptor neurons (ORN) arranged in clusters and housed in the aesthetasc sensilla located on two paired antennae; these cuticular extensions are comprised of chemosensory hairs used to determine the concentration and direction of a scent (Michel et al., 1999; Tadesse et al., 2014; Derby et al., 1997; Schmidt and Mellon, 2011; Schmidt et al., 2011). It has been previously shown that the magnitude of stimulation of these neurons

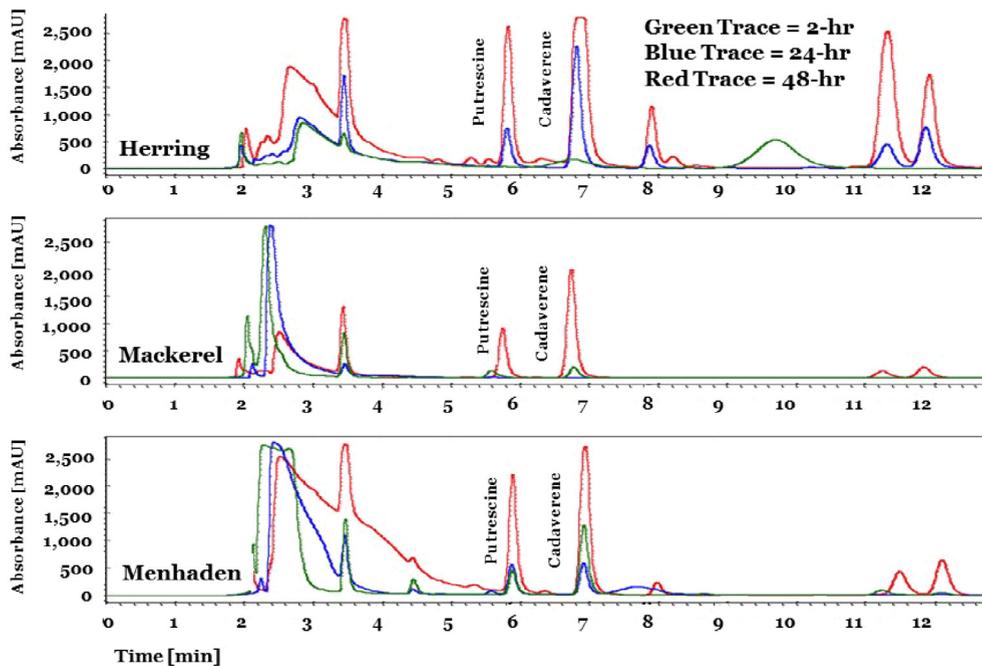


Fig. 1. Identification of molecules released from decaying flesh of various forage fish using HPLC. Herring, mackerel, and menhaden (≈ 4 g) were incubated in simulated ocean conditions (15 ml) for 2 (green), 24 (blue), and 48-h (red). At each time point, the water was removed and replaced with fresh water. Peaks were confirmed using known concentrations and standards. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

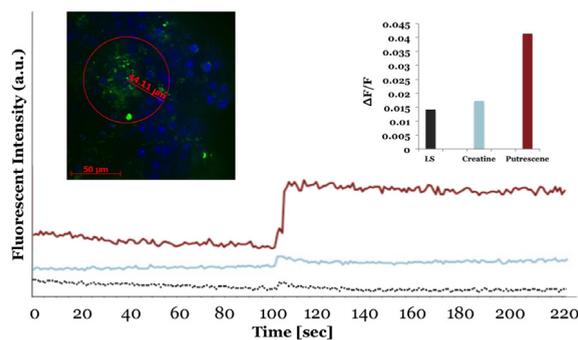


Fig. 2. *Homarus americanus* lobster ORN stimulation. Fluctuations in relative fluorescent intensity of calcium sensitive dye (OG 488, inset image green) normalized to nuclear stain (Hoechst 33342, inset image blue) after exposure to a positive chemotactic odorant, putrescine (red); compared to creatine (blue), and lobster saline (dashed black, control). Inset depicts a representative confocal image of ORN cells loaded with calcium binding OG 488 dye (green) or DAPI (blue) prior to exposure to stimulation with putrescine. Bar graph represents the change in fluorescent intensity after exposure to chemotactic solution or control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in response to stimuli is indicative of the attractive or repulsive nature of the stimulus. This earlier work also demonstrated that only molecules smaller than 8.5 kDa are able to cross the aesthetascs cuticle to reach the ORN. These studies provided the basis for a panel of appropriately sized molecules discovered through HPLC to be analyzed for ORN stimulation (Derby et al., 1997). ORN in antennae of *Homarus americanus* were challenged with various molecules, and the intracellular release of calcium was measured to determine the rates and intensities of action potentials (Michel et al., 1999; Tadesse et al., 2014). The intensity of ORN response to the forage-fish-derived molecules varied greatly. A subset of biogenic polyamines, amino acids, and organic acids that were discovered through HPLC analysis were evaluated for ORN stimulating. As seen in Fig. 2, one of the HPLC-detected biogenic polyamines, 1,4 diaminobutane (also known as putrescine), elicited a substantial ORN response in the *Homarus americanus* species of lobsters when compared to another forage-fish emitted molecule, creatine, and to the control, lobster saline. Biologically, 1,4 diaminobutane is the direct result of catabolism of amino acids (arginine, agmatine, ornithine, and lysine).

Given the preliminary ORN *in vitro* screening assays that detected robust stimulatory responses, specific biogenic polyamines were selected for testing the attraction of crustaceans under typical fishing conditions. (See Fig. 3.) Using



Fig. 3. Synthetic bait comprised of chemo-attractant formulation in calcium-sulfate soluble matrix.

Table 1
Comparative evaluation of a synthetic bait alternative.

	Traditional bait	Synthetic bait
Consistently low priced		✓
Constant availability		✓
Sustainable		✓
Consistently high performance		✓
No spoilage or refrigeration		✓

commercially available, raw materials, a synthetic chemo-attractant bait formulation was engineered so as to mimic the forage fish derived molecules when released from a dissolvable matrix for use in field testing (Fig. 2).

5. Proof of principle

Experiments in Florida and in North Carolina were conducted to determine whether the new bait could attract and catch *Menippe mercenaria* (Florida stone crab) and *Callinectes sapidus* (blue crab), respectively. Tests in California and the British West Indies evaluated performance in attracting and capturing *Panulirus argus* and *Panulirus interruptus* (Caribbean and California spiny lobster, respectively). In these field tests, the synthetic bait results matched or exceeded established forage fish and barnyard flesh methods when averaged across three diverse ecosystems capturing three types of crustaceans (Fig. 4(A)). At least 30 traps of synthetic bait were compared to 30 traps of traditional bait following the established practices and bait methodologies for each species and ecosystem: spiny lobster (Fig. 4(B)), blue crab (Fig. 4(C)), and stone crab (Fig. 4(D)). The bait also performed equally, or better, in field testing for *Homarus americanus* with a similar protocol, collectively achieving a proof of principle. In addition to the aforementioned environmental aspects and the performance capabilities illustrated in Fig. 4, the synthetic bait provided several key advantages over traditional bait (Table 1).

6. Conclusion

A disruptive alternative

During the field tests, it was also noted that the synthetic bait would save the time and energy costs associated with forage fish harvesting, handling, and frozen storage requirements—in addition to offsetting dramatic direct and societal challenges of over-utilization for crustacean fishers, environmentalists, and regulators. The overwhelming stressors on forage fish populations have also reverberated inside the crustacean fishing industry, resulting in erratic availability due to competing demands that are fueling higher bait prices; as well as catch restrictions from evolving regulations; and metastatic damage to the crustacean habitats supporting the fishing industry's livelihood. Further, environmentalists have continued to fight to ban the methods used to capture forage fish that result in by-catch (e.g. seals, dolphins, turtles, and other non-target species)

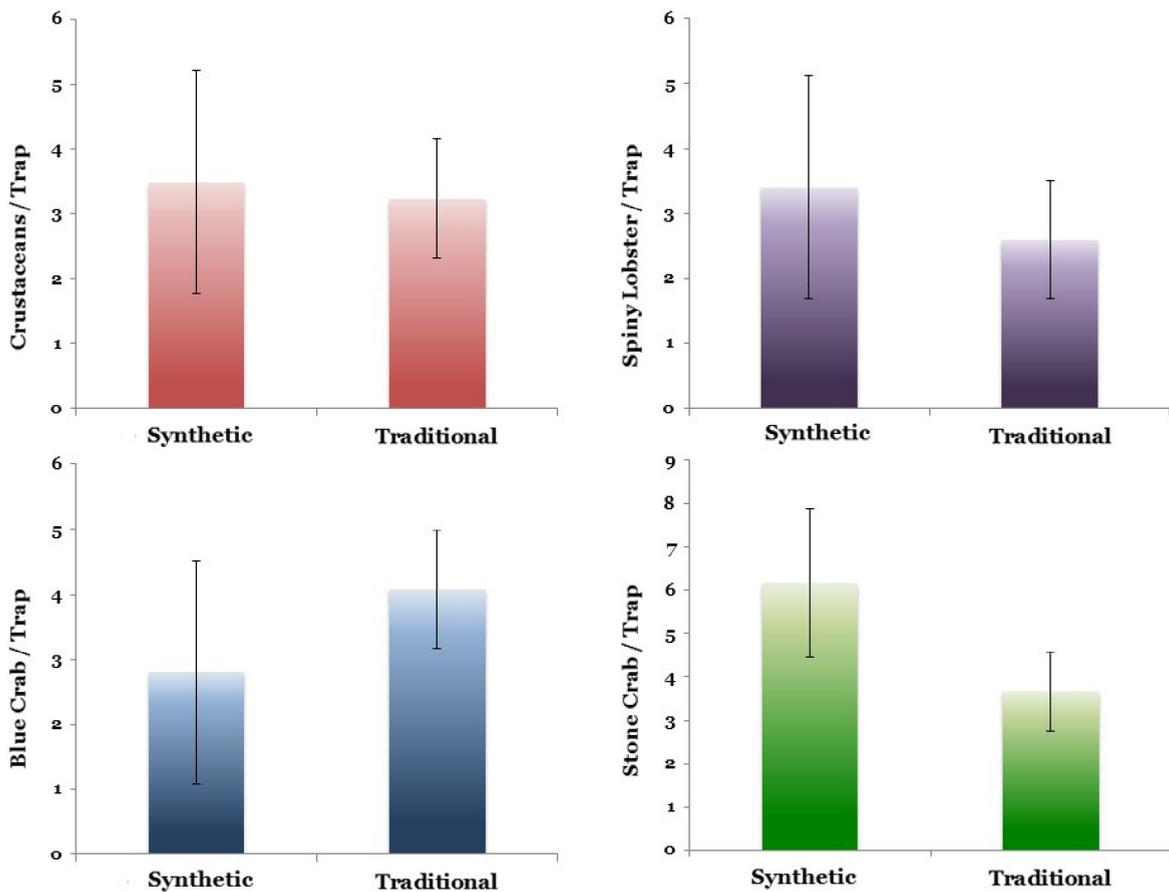


Fig. 4. Synthetic bait attraction and capture results. Average number of all crustaceans (A) captured per trap using synthetic and traditional forage fish bait across three species: (B) spiny lobster; (C) blue crab; and (D) stone crab. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

perishing in forage fish nets. In turn, the decline in forage fish stocks has forced Federal and state agencies across many regions to petition the courts to impose fishing restrictions (Dept. of Conservation, 2014; Wilson et al., 2007; Pauly et al., 1998; Oceana, 2014; Bakun et al., 2009). Helping to mitigate oceanic ecosystem collapse due to the overfishing of forage fish is also a societal imperative with direct benefits to wildlife and the subsequent sustainability of the fishing and nutritional resources on which humans also depend. Ultimately, with the potential to eliminate the crustacean fishing industry demand for up to 18 of 47.4 million metric tons of forage fish, this synthetic bait could help to conserve and replenish nearly 40% of the global consumption of these vital species each year (U. Nations, 2014; Alder et al., 2008; Larsen and Roney, 2013; Molyneux, 2001).

Author contributions

A.L.D., C.L.K. and T.B. designed all experiments. A.L.D., J.P., and B.D. performed all experiments and analyzed the data for the figures. C.L.K., A.L.D. and T.B. drafted the paper.

Competing financial interests

The authors have competing interests as defined by Nature Publishing Group, or other interests that might be perceived to influence the results and/or discussion reported in this article. Authors: A.L.D. is an employee of K.B.I., Greensboro, N.C and C.L.K., A.L.D., and T.B. are stakeholders in K.B.I., Greensboro N.C.

Acknowledgments

The authors thank their colleagues and collaborators who assisted in the research and field work. Support was provided by two National Science Foundation (NSF) Grants (IIP1447431 and IIP1542757). The authors would like to thank Melinda K.M. Goddard and Dr. Kristen Bowey for editorial comments on the manuscript. The group acknowledges patent pending numbers #14/659,710 and #PCT/US2015/2086 related to the synthetic bait.

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