EXAMINATION OF GEAR TYPE EFFICACY, TAGGING METHODOLOGY, AND

POPULATION STRUCTURE FOR ESTABLISHING A DIRECTED *ENTEROCTOPUS*

DOFLEINI FISHERY
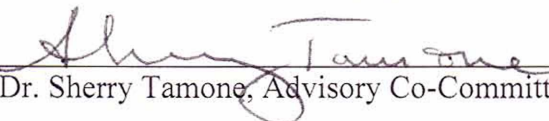
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[1]Barry, P. D., S. L. Tamone, and D. A. Tallmon. 2010. Evaluation of the capture efficiency and size selectivity of four pot types in the prospective fishery for North Pacific giant octopus (*Enteroctopus dofleini*). Fishery Bulletin. 108:39-44.

EXAMINATION OF GEAR TYPE EFFICACY, TAGGING METHODOLOGY, AND

POPULATION STRUCTURE FOR ESTABLISHING A DIRECTED *ENTEROCTOPUS*

DOFLEINI FISHERY

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

By

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[1]Barry, P. D., S. L. Tamone, and D. A. Tallmon. 2010. Evaluation of the capture efficiency and size selectivity of four pot types in the prospective fishery for North Pacific giant octopus (*Enteroctopus dofleini*). Fishery Bulletin. 108:39-44.

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Abstract

In recent years, there has been a renewed interest in a directed fishery for North Pacific giant octopus, *Enteroctopus dofleini*. This species continues to be managed as a bycatch-only species through the use of commisioner's permits primarily due to the lack of information on the basic ecology of *E. dofleini* and the logisitics of management. In the summer of 2007, we completed a survey in Kachemak Bay, Alaska, to determine the efficiency of different gear types for targeting *E. dofleini*, compared different methods of tagging individuals for movement and abundance estimates, and collected tissue samples for use in a genetic analysis of population structure. If a directed fishery develops in Alaska, our data suggest that unbaited lair pots may be the most effective means of capture while minimizing bycatch of other commercially important species. Most regions in Alaska lack sufficient data to estimate abundance and often estimates using catch-per-unit of effort can be inaccurate. If mark-recapture methods are used to estimate abundance of octopus populations, then results from our tagging indicate that visible implant elastomer may be the most effective means of marking individuals. Genetic analysis of *E. dofleini* populations revealed an enigmatic pattern of population structure with two haplotype lineages. The large amount of sequence divergence at the COI locus may indicate the presence of a cryptic species within the *E. dofleini* complex. It appears that North Pacific giant octopus will continue to be managed as a bycatch-only species for the near-term future. It is essential that management agencies resolve both the phylogenetic and population structure, as well as confidently estimate abundances of the stocks identified before a directed fishery is opened.

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General Introduction

The North Pacific giant octopus, *Enteroctopus dofleini* (Wülker 1910), is a long-lived benthic cephalopod. It is fished throughout its range, which extends from Baja California to the Aleutian Islands in Alaska and westward in the North Pacific to Japan. This species is a particularly important fishery resource in Japan and much of the research on *E. dofleini* has been published in non-peer reviewed, Japanese technical journals. Since the 1970s, emergent markets and high ex-vessel prices have encouraged several attempts at developing a directed octopus fishery in Alaska (Paust, 1989). While those attempts have shown that a directed fishery can be profitable (Paust, 1988), octopus continues to be managed as a bycatch-only species, managed using commissioner's permits. Renewed interest in a directed fishery for *E. dofleini* has raised both management and ecological questions about the abundance and population structure of this species.

In Alaska, a directed octopus fishery would be integrated as either an off-season or secondary fishery. Entrants into the developing fishery would experience high start-up costs and unproven returns. Previous studies investigating efficiency of gear types used to target *E. dofleini* have focused on the unbaited lair pots commonly used in the Japanese fishery (Paust, 1997). Start-up costs could be reduced by using gear from other fisheries in which the entrant may already be active. *E. dofleini* is caught as bycatch in shrimp, sablefish, and many crab fisheries, but because retention is limited to those boats holding commissioner's permits, no direct comparison of efficiency can be made among pot types

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using the present catch data. Given the desirability of data on the effectiveness of equipment used in other Alaskan fisheries to prospective entrants and regulatory agencies, we examined the efficiency of four pot types for their use in a directed octopus fishery.

In order to effectively manage a directed fishery, estimates of population size, reproductive biology, and information on movement would be critical. Often catch-per-unit-effort (CPUE) is used as a proxy to assess abundance (Hilborn and Walters, 1992) and is used to set harvest levels within the fishery. One of the major critiques of using CPUE for abundance estimates is that this estimate relies on the assumption that catchability over time remains constant (Pine et al., 2003). Despite declining population sizes, behaviors such as schooling can lead to high CPUE estimates (Hilborn and Walters, 1992) and lead to overestimates of stock sizes. Capture-recapture surveys with tagged individuals can be used to estimate both population size and probability of capture. In addition, this method is more sensitive to small changes in population abundance. This type of tagging study requires that individuals collected, tagged, and released be in good condition and be equally likely to be captured as unmarked individuals.

Many models also assume that marks are not lost or overlooked upon recapture. Peterson discs have previously been used to tag *E. dofleini*; however, the extent to which they are lost, irritate the skin, or alter behavior is debatable (Domain et al., 2000; Robinson and Hartwick, 1986). Peterson disc tags are inserted through the ventral side of the mantle and secured in place using two plastic screws. Any tag that pierces skin has

the potential to cause irritation and some studies have observed octopuses removing

external tags (Nagasawa et al., 1991). Dyes and pigments such as methylene blue, latex,

and silicone have been used successfully on other cephalopod species (Nagasawa et al.,

1991; Replinger and Wood, 2007) and these are attractive alternatives to Peterson disc

tags because of the relative ease in which they are applied and their inability to be

manipulated by the tagged animal. Visible implant elastomer (VIE), a silicone-based tag

which is injected as a liquid and subsequently hardens into a biocompatible solid, may

decrease tag loss and provide a more effective and efficient means of marking.

Effective fishery management requires knowledge of not only the abundance of

the species, but also information regarding the population structure of the species which

can be assessed in part through the analysis of genetic variation across space. While

ocean currents have the potential to carry planktonic individuals great distances, both

geological and oceanographic features can act as barriers to gene flow (Wing et al.,

1998a; Wing et al., 1998b). Movement as adults can also act to spread genes from nearby

locations. The extent to which adult *E. dofleini* move is poorly understood. Harvesting on

a scale larger than the population structure can lead to localized depletions, decreased

yield, and a decline in genetic diversity (heterozygosity and allelic diversity).

Heterozygosity, the possession of different alleles at one or more corresponding

chromosomal loci, has been positively correlated to fitness in many species (Koehn and

Galfney, 1984; Lundy et al., 1999; Mitton and Grant, 1984). As such, conservation

biologists and wildlife managers strive to maximize natural genetic variation within a

population. With this variation, species are more competent to adapt to the pressures of a changing environment.

The primary objectives of this project were to examine the catch rates for *E. dofleini* using different gear types, assess the abundance of *E. dofleini* in Kachemak Bay, compare two different methods to mark individuals, and investigate the demographic relationships among *E. dofleini* populations in Alaska.

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CHAPTER 1:

Evaluation of the capture efficiency and size selectivity of four pot types in the prospective fishery for North Pacific giant octopus (*Enteroctopus dofleini*)[1]

Abstract—Over 230 metric tons of octopus is harvested as bycatch annually in Alaskan trawl, long-line, and pot fisheries. An expanding market has fostered interest in the development of a directed fishery for North Pacific giant octopus (*Enteroctopus dofleini*). To investigate the potential for fishery development, we examined the efficacy of four different pot types for capture of this species. During two surveys in Kachemak Bay, Alaska, strings of 16–20 sablefish, Korean hair crab, shrimp, and Kodiak wooden lair pots were set at depths ranging between 62 and 390 meters. Catch-per-unit-of-effort estimates were highest for sablefish and lair pots. Sablefish pots caught significantly heavier North Pacific giant octopuses, but also produced the highest bycatch of commercially important species, such as halibut (*Hippoglossus stenolepis*), Pacific cod (*Gadus macrocephalus*), and Tanner crab (*Chionoecetes bairdi*).

Introduction

The North Pacific giant octopus (*Enteroctopus dofleini*) is a benthic cephalopod fished

throughout its range from Baja California to the Aleutian Islands in Alaska and westward

in the Pacific Ocean to Japan. Many artisanal fisheries use trailing hooks, longlines,

handlines, and spears as means of harvest. Since the 1970s, an increasing overseas food

market and local bait industries have triggered several attempts to develop a commercial

fishery for the North Pacific giant octopus (hereafter referred to as "giant octopus" in this

article) in Alaska (Paust, 1997), but little is known about the efficacy of different gear

types for the capture of this species in Alaska.

Currently, the Alaska Department of Fish and Game allows commercial harvest of

octopus within state waters only as incidental catch managed under state permits.

Retention levels as bycatch vary from about 5% in the pot gear fishery for shrimp in

southeast Alaska to about 20% in the state groundfish fisheries. Paust (1997) compared

four lair pot designs and found that because of low construction costs, reduced space

needed for storage, relative ease of handling, and a superior fishing performance over

other lair pot designs, the wooden Kodiak pot would be the best choice for fishery

development in Alaska. No baited pots were included in Paust's study design. Paust

(1997) assumed new entrants into a directed fishery for giant octopus will most likely

integrate directed fishing for the giant octopus as an off-season or secondary fishery.

Such a developing fishery would have high start-up costs and no proven returns, limiting

its participants to using fishing gear from other fisheries. Given the value of data on the

effectiveness at capturing giant octopus with equipment already used in Alaska fisheries,

we examined the efficiency and size selectivity of four pot types for their use in a directed fishery for this species.

Materials and methods

Two surveys were completed (2–13 October 2006, and 25 November to 6 December 2006) in Kachemak Bay, Alaska (Fig. 1). Four types of pots were used during the course of the study: lair, Korean hair crab, sablefish, and shrimp pots (Fig. 2). Lair pots were constructed of wood, measured 60.96 cm×30.48 cm×30.48 cm with a 15.42 cm×3 0.48 cm opening, and were left unbaited. Korean hair crab pots consisted of PVC piping and were 45 cm tall and had a 100-cm base diameter and a 26-cm plastic tunnel. Sablefish pots were made of 1.3-cm rebar and measured 147 cm tall and had a 122-cm bottom ring and a 71-cm top ring. The sock tunnel opening was 25 cm wide and located 76 cm from the bottom. The net used was 7-cm mesh black seine net. Korean hair crab and sablefish pots were baited with chopped herring. Both commercial and personal-use Ladner shrimp pots had three 7.62-cm tunnel openings and were baited with either herring or prawn pellets.

Each string of pots consisted of a single pot type to best replicate fishing practices and to make the process of setting and retrieving pots as safe as possible. Each string of pots consisted of 20 pots spaced nine meters apart and two marker buoys were attached to each end. Lair pot strings had only 16 pots. We removed the shrimp pots from the sampling design for the second survey because of their low catch per unit of effort (CPUE).

To facilitate handling, captured the giant octopuses were placed in mesh bags and kept in individual (53 liters/60.7×40.4×31cm) Rubbermaid® containers. Containers were filled with seawater and the water was exchanged every five minutes by using a pump placed approximately one meter below the surface of the water. The sex of each animal was determined by the presence (male) or absence (female) of the hectocotylized right third arm. If the animal had lost the tip of its right third arm, we could not determine sex. We recorded morphometric measurements including the interocular distance (IOD), mantle length (ML), and wet weight, as well as any identifying marks for each individual. Muscle tissue from the tip of the left third arm was clipped and preserved in 95% ethanol for future genetic analysis.

Descriptive summary statistics of individuals captured, capture rates of each pot type, and capture rates by survey were tabulated. A paired *t*-test was used to compare the weights of males (n=114) and females (n=128) captured. We could not determine sex for 8 individuals, and these octopuses were omitted from all analyses of differences between the sexes. Size selectivity was assessed by comparing mean weight of octopuses caught in each pot type. Because of heteroscedasticity in the data, we used a nonparametric Welch analysis of variance (ANOVA) to test for variation in the weight of octopuses by pot type, followed by Tukey's *post hoc* tests. In order to diminish the potential confounding effects of differences in depths at which pots were set, we reanalyzed weight by pot-type data using only North Pacific giant octopuses caught within Eldred Passage where depth did not differ significantly by pot type using an ANOVA and Fisher's least significant difference (LSD) test. Individual chi-square tests were used to determine

whether there was a disproportionate number of males or females caught in any one trap type.

No standardized method exists to measure octopus CPUE. In octopus fisheries around the world, CPUE is tailored to the type of fishery (Defeo and Castilla, 1998; Hernandez-Garcia et al., 1998; Sanchez et al., 2004). There are markets for both food and bait North Pacific giant octopuses. In Alaska, the fishery would not be size selective. In a full-time directed fishery where the ability to turn over gear is important, "the total catch in kilograms (kg) per days soaked" may be a most useful expression of effort. In contrast, kilograms per pot may be a more useful indicator of effort in a part-time fishery. The number of pots soaking may be more important if a vessel is employed to simultaneously take part in another fishery while its pots for giant octopus are soaking. Irrespective of the fishery structure, whether full-time or concurrent, both CPUE estimates provide valuable information for resource management. Both CPUE estimates were calculated for each individual survey and then averaged over surveys. No statistical tests were performed on CPUE data because the averages calculated over the two surveys are based on two estimates. Catch-per-unit-of-effort values for shrimp pots represent point estimates because shrimp pots were fished only in the first survey. We compared the average soak time of lair pots that caught giant octopuses to those that did not by using a *post hoc t*-test to investigate anecdotal evidence that would indicate that longer soak times increase the likelihood of capture.

Results

We captured a combined total of 254 giant octopuses, four of which were dead, and which were omitted from further analysis. The number of individuals captured was consistent between surveys; 122 and 128 individuals captured in the first and second surveys, respectively. Of the total 250 individuals captured, the numbers caught among the pot types was as follows: 107 in lair pots, 35 in Korean hair pots, 102 in sablefish pots, and 6 in shrimp pots.

Sablefish pots captured significantly heavier individuals than all other pot types (Fig. 3), but the weight of these giant octopuses captured in the other three pot types did not differ significantly from one another (Welch ANOVA, df=3,32.241, F=28.115, P<0.001 Tukey HSD). The depth at which pots were set, however, was not determined at random. Larger giant octopuses may be found in deeper water resulting in biased estimates. Although a regression of weight by depth showed a very weak correlation (coefficient of correlation (r^2)=0.0019, P=0.49), in order to mitigate the potential confounding effects of depth, we re-analyzed weight by pot type using only octopuses caught within Eldred Passage where depth did not differ significantly by pot type. Giant octopuses caught in sablefish pots remained significantly heavier than those caught in both lair and shrimp pots, but there was not a significant difference in mean weight between sablefish and Korean hair crab pots (ANOVA, df=3, F=10.599, P<0.001 Fisher's LSD). Among the 112 males and 126 females captured, males were heavier than females (t-test, df=1, F=7.166, P=0.008). However, this finding did not seem to be driving the observed difference in weight by pot type because for each pot type both

sexes were equally likely to be captured (lair: χ^2=0.154, P=0.695, Korean hair crab: χ^2=0.030, P=0.862, sablefish: χ^2=0.853, P=0.356, and shrimp: χ^2=0, P=1).

Capture efficiency varied both temporally and with the CPUE index (kg/days soaked vs. kg/pot set). Between the two surveys, the efficiency of both lair and Korean hair crab pots increased and the efficiency of sablefish pots decreased. The average CPUE for all pot types between surveys ranged from 5.5 kg/pot (±1.7 SD [standard deviation]) for the lair pots to 0.4 kg/pot for shrimp pots, and 17.6 kg/days soaked (±2.7 SD) for the sablefish pots to 2.1 kg/days soaked for shrimp pots (Fig. 4). Lair pots that caught giant octopuses were not soaked longer than pots that did not capture octopuses (*t*-test df=1, F=0.214, P=0.644).

Both sablefish and Korean hair crab pots caught large numbers of commercially important crab and fish species as bycatch. Korean hair crab pots caught Tanner crabs (*Chionoecetes bairdi*) and Pacific cod (*Gadus macrocephalus*; sizes ranging from 26- to 100-cm total length). Sablefish pots caught *C. bairdi*, *G. macrocephalus,* and halibut (*Hippoglossus stenolepis*; sizes ranging from 29- to 112-cm total length). Lair and shrimp pots did not contain bycatch of commercially important species. Other bycatch species, caught in all four pot types were lyre crabs (*Hyas lyratus*), decorator crabs (*Oregonia gracilis*), sunflower sea stars (*Pycnopodia helianthoides*), and Oregon hairy tritons (*Fusitriton oregonensis*).

Discussion

The internal volume of each pot may be a better indicator than the size of the pot entrance for the potential size of giant octopuses that will be caught. Although lair, Korean hair crab, and sablefish pots had similar size openings, the success of the sablefish pots may have been due to their volume being three times greater than that of the Korean hair crab pots and nearly twenty times that of the lair pots. The ability of giant octopuses to enter a trap is most likely determined by the size of its beak, the only hard part of its body.

Variation in the size of North Pacific giant octopuses caught by the different pot types may be influenced by differences in depth or substrate rather than by pot type alone. However, when pots laid at similar depths in Eldred Passage were analyzed, we observed similar trends in the data, indicating that sablefish pots have the potential to capture larger giant octopuses than the other pots.

Pot efficiency changed with different measurements of effort. Lair pots, because of their long soak times, may be more useful in a fishery where operators are fishing for giant octopus and another species concurrently. Anecdotal evidence from the use of lair pots indicates an optimal soak time of approximately seven days. These pots do not require baiting to be effective and therefore it is unlikely that prolonged soak times (longer than seven days) will decrease their effectiveness. We suspect that increasing the total volume of the pot and decreasing the size of the opening of lair pots may increase catch rates.

Sablefish pots proved to be the most efficient gear type tested in terms of kilograms per hours soaked, indicating that fewer pots could be fished with short soak

times. Although their efficiency in terms of kg/pot may be lower than that of lair pots, shorter soak times allow for increased pot turnover. The capital investment for fishermen already using pots in the sablefish fishery would be minimal and the season could begin after the sablefish season closes in November. Shrimp pots captured relatively few octopuses and those caught generally were smaller in size, possibly because of the small diameter of the pot entrance. Korean hair crab pots caught only slightly larger individuals and had a similarly low CPUE, despite having a much larger entrance.

The high incidence of bycatch of commercially important species by the sablefish pots may limit their usefulness in a directed fishery for North Pacific giant octopus. Vessels targeting the North Pacific giant octopus would not be allowed to target any other species and all bycatch would be limited to a small percentage of the total weight of the North Pacific giant octopus onboard. Bycatch reducing devices have been successful in reducing the bycatch of sea turtles (Fratto et al., 2008), crabs (Furevik et al., 2008), and birds (Butler and Heinrich, 2007) in multiple pot fisheries while maintaining high catch rates of their target species. Depending on our ability to minimize bycatch by sablefish pots, it may be a better strategy to use a pot type that is less efficient in terms of kilograms per hours soaked, but that has a lower bycatch rate.

Additional considerations to the season and type of pot used in a directed North Pacific giant octopus fishery must be made to ensure that fishermen are not disproportionately harvesting spawning North Pacific giant octopuses. During two captures in which we manipulated females, they extruded spermatophores. Although females after spawning survive to care for eggs, males die within a month of copulation

(Arnold et al., 1987; Hartwick, 1983) leading us to assume that the four octopuses captured dead were recently spawned males. Due to decomposition of the bodies, we could not confirm sex; however, other observations of deteriorating body conditions of males during the third survey support this conclusion. Lair pots, to be efficient, rely on the giant octopuses using them as a den for short periods of time. During the breeding season, females may use these pots to lay and brood their eggs. If females do use lair pots for denning and giant octopuses exhibit a migration to inshore waters to reproduce (Hartwick, 1983), care must be taken to ensure a large enough escapement to prevent localized depletions and ensure sustainable harvest.

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Figures
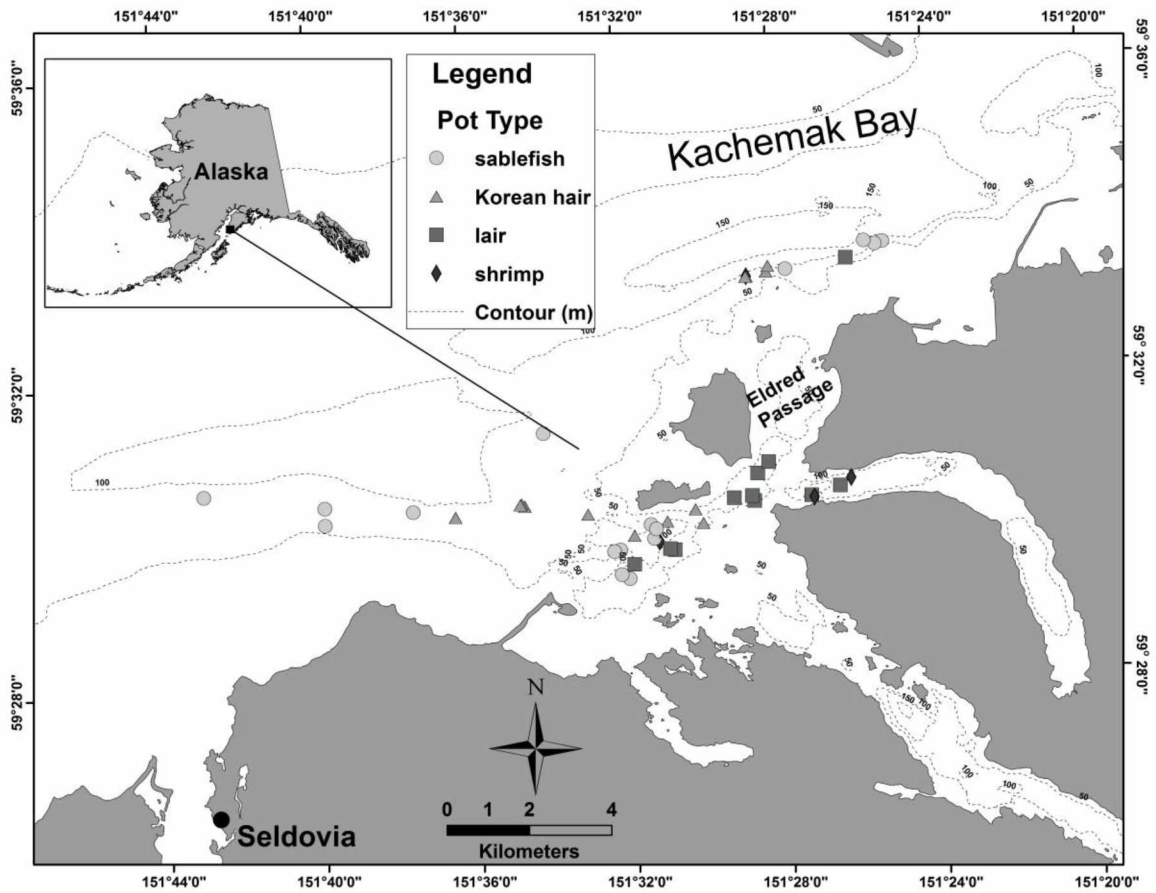
Figure 1: Map of Kachemak Bay, Alaska, and location of pots set to catch North Pacific giant octopus (*Enteroctopus dofleini*) to evaluate the efficiency of the pots for use in a directed North Pacific giant octopus fishery. Each marker represents the first marker buoy in a string of 16–20 pots, set during two surveys in 2006.
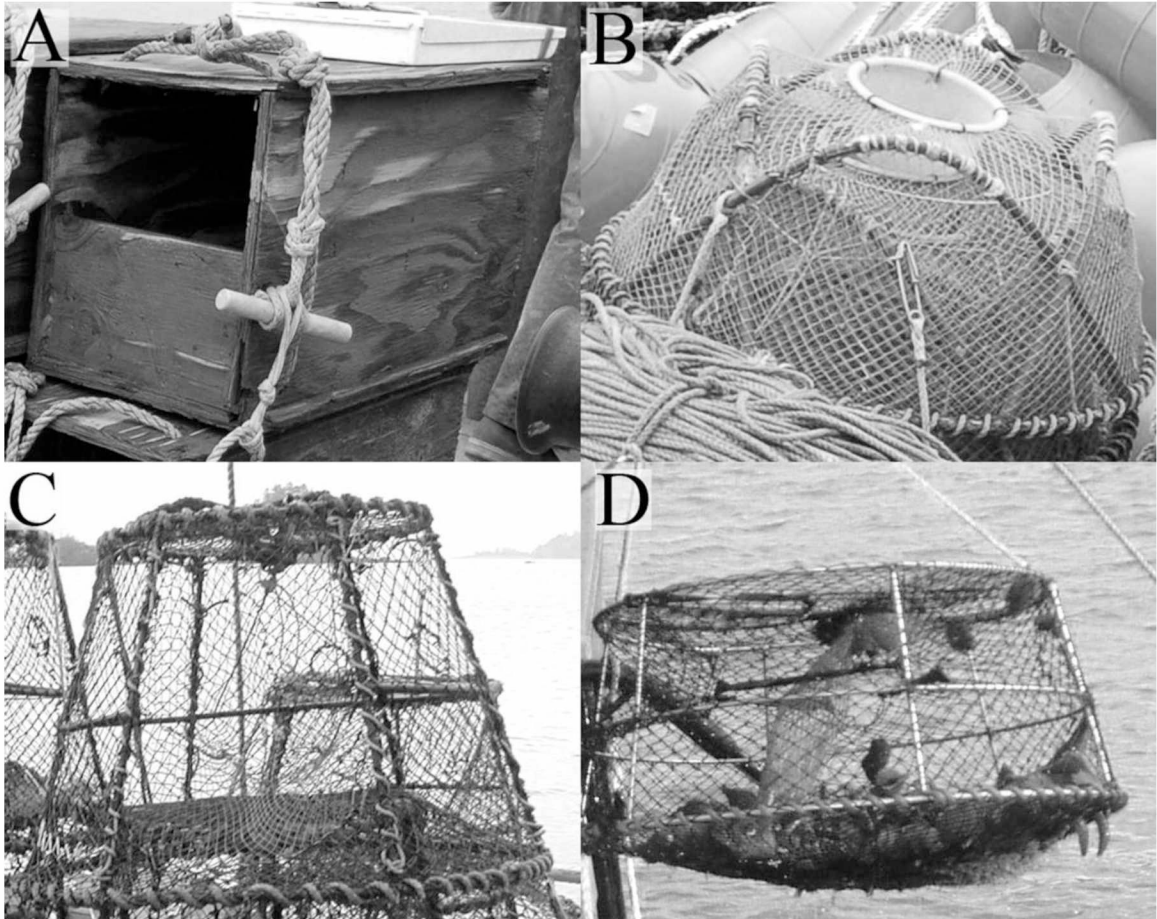
Figure 2: The four pot types we evaluated in Kachemak Bay, Alaska, in 2006 to determine their efficiency in capturing North Pacific giant octopus (*Enteroctopus dofleini*) for use in a directed fishery: (**A**) Lair pots; (**B**) Korean hair crab pots; (**C**) sablefish pots; and (**D**) shrimp pots.
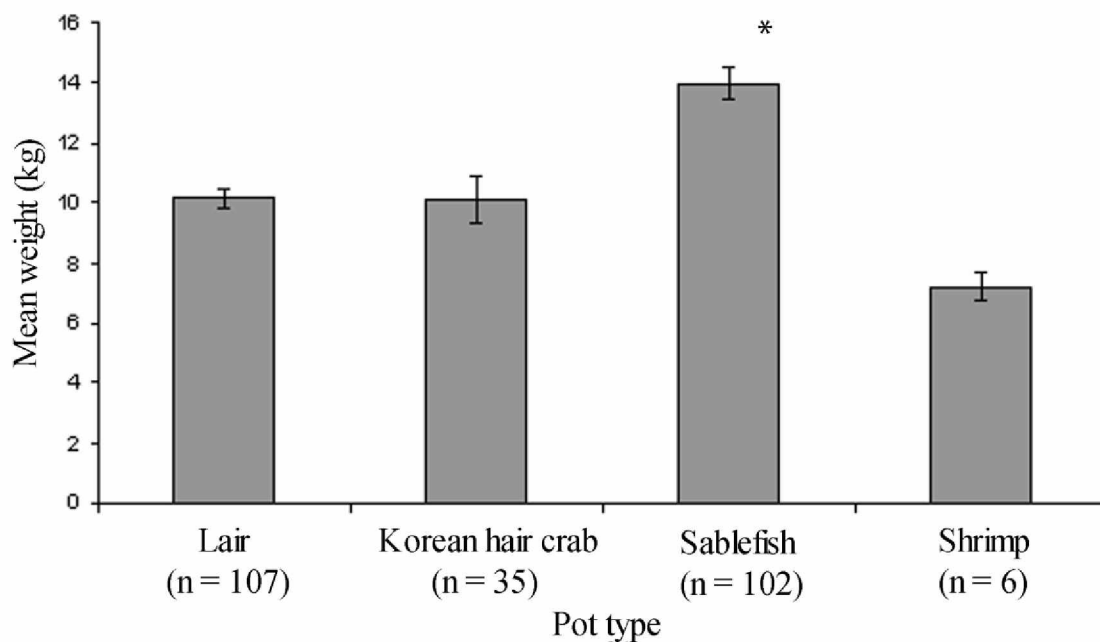
Figure 3: Mean weight (±standard error [SE]) in kilograms and numbers (in parentheses) of North Pacific giant octopus (*Enteroctopus dofleini*) caught in each of the four pot types, during two surveys in Kachemak Bay, Alaska, in 2006, evaluated for size selectivity for use in a directed North Pacific giant octopus fishery (Asterisk indicates a significant difference at $P<0.001$).
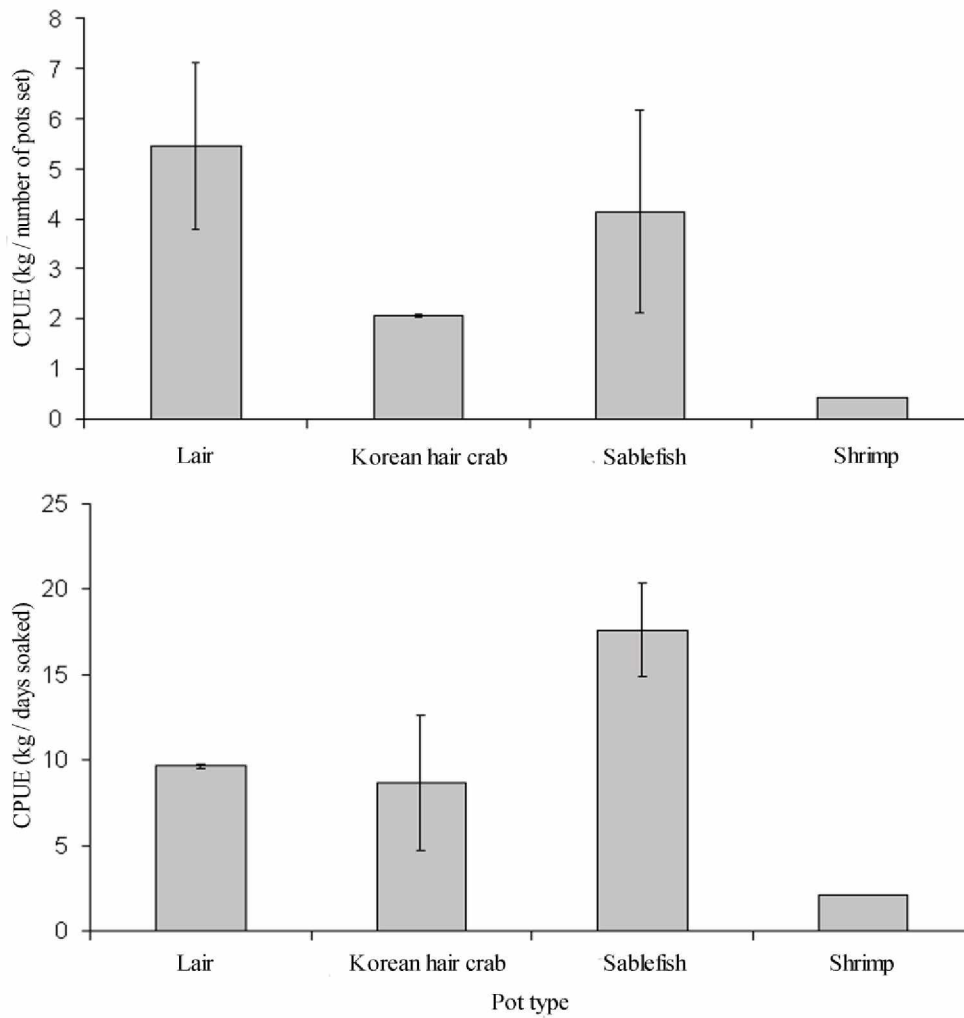
Figure 4: Mean catch per unit of effort (CPUE; ±standard deviation [SD]), measured as both kilograms per hours soaked and kilograms per number of pots set, for each of the four pot types evaluated for capture efficiency of North Pacific giant octopus (*Enteroctopus dofleini*) during two surveys in Kachemak Bay, Alaska, in 2006. Estimates of CPUE are based on a mean of two estimates; therefore no statistical tests were performed.

CHAPTER 2:

A COMPARISON OF TAGGING METHODOLOGY FOR NORTH PACIFIC

GIANT OCTOPUS *ENTEROCTOPUS DOFLEINI*[1]

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[1] Barry, P. D., S. L. Tamone, and D. A. Tallmon. A Comparison of tagging methodologies for North Pacific giant octopus *Enteroctopus dofleini*. Manuscript submitted to Fisheries Research 10 June 2010.

Abstract

There is no directed fishery for octopus in Alaska state waters, but trawl, long-line, and pot fisheries harvest over 230 metric tons of octopus as bycatch annually. An expanding market has fostered interest in the development of a directed fishery for North Pacific giant octopus *Enteroctopus dofleini* in state waters. However, our limited knowledge of octopus movement, age structure, and demography hamper our ability to successfully manage this potential fishery. Chief among these obstacles is the lack of abundance estimates on which to base harvest guidelines. While catch-per-unit effort estimates can be made from bycatch data and used as a proxy of abundance, these estimates can often be inaccurate. Mark-recapture estimates can be more accurate, but can also be expensive and require effective means of marking individuals. We compared two different types of tags in order to assess their effectiveness for use on octopuses and assessed some of the fundamental assumptions of mark-recapture on populations of *E. dofleini*. We tagged 97 octopuses with Peterson discs and visible implant elastomer tags in Kachemak Bay, Alaska. Only three octopuses were recaptured, making a statistically sound tag comparison difficult. Our data suggest that visible implant elastomer tags will be a more effective means of marking octopuses.

Key Words: *Enteroctopus dofleini*, tagging, mark-recapture, Peterson disc, visible implant elastomer

Introduction

External tags have been used to study aquatic animal behavior, movement and migration, and to provide information on age, growth, mortality, and abundance (McFarlane et al., 1990). Growing interest in a directed fishery for North Pacific giant octopus (*Enteroctopus dofleini*) has raised both management and ecological questions, including those concerned with octopus movement patterns and abundances. While early tagging studies looked at residence times (Hartwick et al., 1984; Mather et al., 1985), little is known about large-scale dispersal or abundance. Catch-per-unit-effort (CPUE) is often used as a proxy to assess abundance (Hilborn and Walters, 1992). One of the major critiques of using CPUE for abundance estimates is that it relies on the assumption that catchability over time and space remains constant (Pine et al., 2003). Behaviors such as schooling can lead to high CPUE despite declining population sizes (Hilborn and Walters, 1992). Capture-recapture methods with tagged individuals can estimate both population size and probability of capture and are more sensitive to small changes in population abundance.

The types of tags used to study cephalopods can be grouped into five categories: natural, chemical, electronic, external, and tattooing/branding. Although natural tags (both parasites and genetic markers) have the potential to aid in understanding migration of *E. dofleini*, neither has been used widely. Little is known about the geographic distribution of parasites and few genetic markers are reliably amplified. Chemical tags

such as strontium:calcium (Sr:Ca) ratios have been use to study migratory behavior of the common squid *Todarodes pacificus* (Ikeda et al., 2003) and while Ikeda et al. (1999) showed the potential usefulness of this type of tag with *E. dofleini*, no studies have used this method of tagging. Electronic tags including PIT and acoustic tags have seen limited application (Anderson and Babcock, 1999; Mather et al., 1985) because of the cost associated with tagging many animals. External tags such as Atkins and Petersen tags are the most commonly used tag to study *E. dofleini* (Hartwick et al., 1984; Nagasawa et al., 1991; Robinson and Hartwick, 1986). Tattoo and branding, particularly visible implant elastomer tags (VIE; Northwest Marine Technology, Shaw, Washington) have been used successfully on the squid *Sepioteuthis sepioidea* to investigate growth rates (Replinger and Wood, 2007). However, size of individuals may limit their usefulness. When used on the cuttlefish *Sepia (Mesembrisepia) latimanus*, this method produced significant wounds and mortalities (Nagasawa et al., 1991).

While Peterson tags are the most commonly used tag, the extent to which they affect animals is debated. Robinson and Hartwick (1986) observed no ulcerations around tags and octopuses did not seem to be adversely affected by tagging. Domain et al. (2000) compared spaghetti and Petersen disc tags, and determined that Petersen disc tags stayed on longer. However, the author did not attribute observed tag loss to necrosis of surrounding tissue or manipulation of the tag by octopuses. In either case, VIE tags may decrease tag loss and provide a more effective and efficient means of marking. The goal of this study was to compare two different marking techniques and assess some of the fundamental assumptions of mark-recapture on *E. dofleini*.

Materials and Methods

We caught *E. dofleini* during three surveys (29 May to 18 June 2006, 2-13 October 2006, and 25 November to 6 December 2006) in Kachemak Bay, Alaska. During each survey four different types of pots were used; Korean hair crab pots, both commercial and personal use Ladner shrimp pots, black cod pots, and lair pots.

After capture, each octopus was placed in a mesh bag and kept in individual (53 liters / 60.7 x 40.4 x 31cm [centimeters]) Rubbermaid® containers until it could be measured, weighed, tagged, and its sex determined. Containers were filled with seawater (34 ppt [parts per thousand]) and the water exchanged every 5 minutes using a pump placed approximately one meter (m) below the surface of the water. The sex of each animal was determined by the presence (male) or absence (female) of the hectocotylized right third arm. If the animal had lost the tip of its right third arm, we could not determine sex. We recorded morphometric measurements including the interocular distance (IOD), mantle length (ML), and weight, as well as any identifying marks. Before tagging, each octopus was transferred to water cooled to approximately 1°C to anesthetize them.

Due to the difficulty in tagging larger octopuses (>11Kg [kilograms]) with the Petersen discs available, and to minimize the effect of handling time on the health of each animal when many octopuses were caught in a string of pots, 28 octopuses were tagged using only the VIE tags. Modified Petersen discs were inserted through the ventral side of

the mantle, secured with plastic nuts, and the excess screw length cut flat using scissors (Fig. 1). We injected the VIE tag subcutaneously on the ventral side of the mantle where the pigmentation is lightest (Fig. 1). Each elastomer tag consisted of four injected dye spots, occupied by any of four colors (red, orange, yellow and/or green) to individually identify each animal. Tags were injected using a tuberculin syringe fitted with a 20-gauge needle. Marked animals were held onboard in a bucket of seawater until respiration and activity levels returned to normal. They were then released within 10 meters of the GPS coordinates from which they were captured. During the third survey, animals were not tagged with either Petersen or VIE tags. To identify any recaptures within the third survey approximately 2.5cm from the tip of the right third arm was amputated.

Results

During the first and second surveys, 3 and 94 *E. dofleini* were tagged, respectively, using Petersen discs and VIE tags to allow for their direct comparison. Of the three recaptures that occurred between surveys, two animals were marked with both tags while the other received only the VIE tag. We observed from one individual that the Petersen discs can irritate the tissue surrounding the tag resulting in necrosis (Fig. 2). Of the two individuals recaptured that were initially tagged with both methods, one had lost its Petersen tag while all retained the VIE tag. Upon examination of the VIE tags, no noticeable tissue damage was caused at the site of injection. None of the colors became dull or difficult to distinguish, and there was no noticeable difference in size of the marks between mark and recapture.

Discussion

Though hampered by a small sample size, our results indicate that VIE tags may be a more reliable tagging method than the commonly used Petersen tag and that animals may exhibit a strong trap response. Low recapture rates make direct comparison of tagging methods difficult; however, our efforts provide some important insights into methods for marking individuals and further our ability to successfully implement mark-recapture studies. While our results have implications for tagging studies for studying growth, movement, and survival, we focus here on the implementation of mark-recapture on estimates of abundance.

Results of recaptures suggest that VIE tags may provide a more durable method of marking octopuses than Petersen tags. Petersen tags were obvious upon recapture and were easily felt and seen when handling recaptured octopuses, but produced significant tissue damage in two individuals, which may lead to tag loss. Because they are external tags, octopus may be able to remove the plastic nuts which secure both plates in the mantle. The VIE tags produced no signs of irritation, but are not immediately obvious upon recapture without manipulation of the posterior mantle. Also, consecutively injected elastomer colors can be embedded in the epidermis such that the colored dots move with respect to each other when the mantle tissue is manipulated and may lead to the misidentification of recaptures. Of the three recaptures, the one individual that had lost the Petersen tag showed significant tissue damage around the site where the tag had been inserted. Although brown trout, *Salmo trutta,* have been observed to absorb the VIE during growth or for layers of musculature or epidermis to cover the tag (Olsen and

Vollestad, 2001), we would still be able to identify those individuals by their Petersen tag. Consequently, low recapture rates in the third survey are not likely due to tag loss.

Capture probability may depend on previous capture experience. Octopuses are capable of complex learning (Boal et al., 2000; Hvorecny et al., 2007; Moriyama and Gunji, 1997), processing information, and forming both long-term and short-term memory in the same way as vertebrates (Hochner et al., 2003; Shomrat et al., 2008). It is possible that the stress incurred by capture and tagging produce memories that might affect recapture. The plastic screws of the Petersen disc had a tendency to bend when pushed through the thick mantle of larger octopuses. Unsuccessful attempts at puncturing the mantle can make the application of this method particularly stressful for the animal. The VIE tag appears less invasive and is quicker and more efficient to apply.

Capture probability is generally estimated from the recapture of tagged individuals and should be as high as possible for reliable estimates of population parameters (Pine et al., 2003). While our recapture rate was low (2.5%), previous studies on octopus have had recapture rates between 19% and 70% (Hartwick et al., 1984; Lang, 1997; Robinson and Hartwick, 1986). Any number of factors (e.g., trapping method, spatial/temporal variation) could account for these differences.

Acknowledgements

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Figures
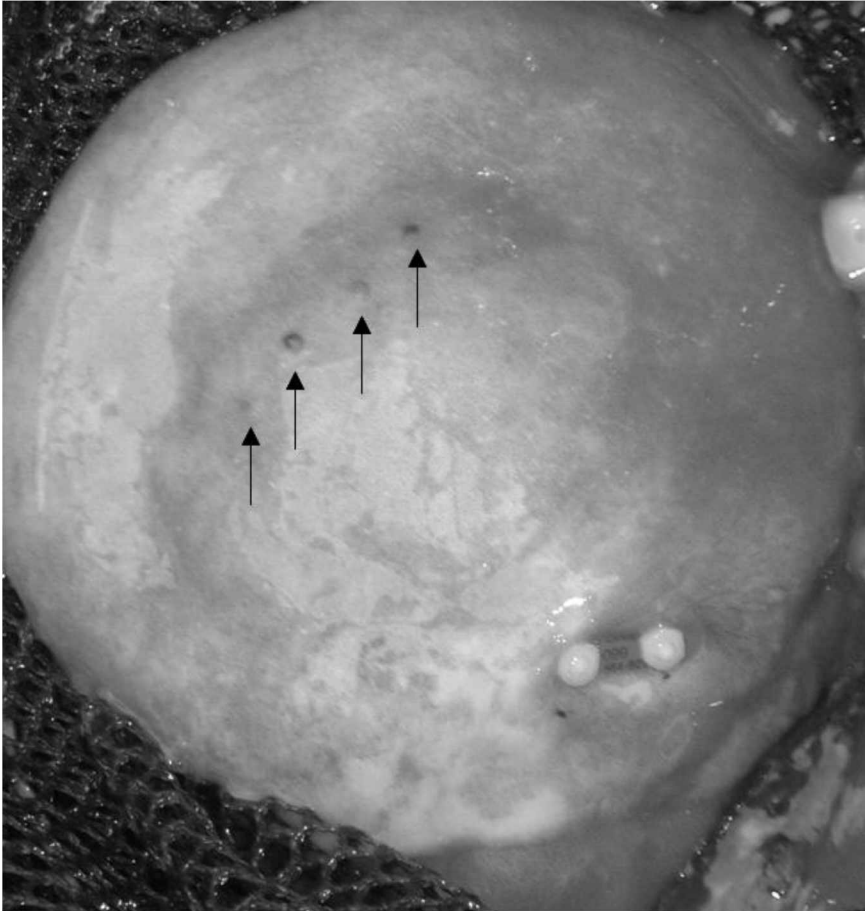
Figure 1: North Pacific giant octopuses (*Enteroctopus dofleini*) were tagged using two different marks: Petersen disc tags and visible implant elastomer (VIE) tags. This photo shows the location of both tags on the ventral side of the mantle. Arrows indicate where each VIE dye was injected. Injected as a liquid, the elastomer forms a pliable biocompatible solid. The Peterson discs were inserted through the opening in the mantle on the ventral surface, secured by puncturing the mantle with the two plastic screws, and securing a plate with two plastic nuts.

Figure 2: Piercing the mantle of the North Pacific giant octopus (*Enteroctopus dofleini*) may cause significant wounds and result in necrosis and tag loss. Left- This photo shows part of the ventral mantle where the tag has been successfully inserted immediately after application of the mark. Tissue was healthy and the tag was clearly visible. Right- This photo shows the ventral side of the mantle where a Petersen tag was beginning to cause necrosis after approximately 54 days.

CHAPTER 3:

A COMPLEX PATTERN OF POPULATION STRUCTURE IN THE NORTH PACIFIC GIANT OCTOPUS *ENTEROCTOPUS DOFLEINI*[1]

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[1] Barry, P. D., D. A. Tallmon, and S. L. Tamone. A complex pattern of population structure in the North Pacific giant octopus *Enteroctopus dofleini*. For submission to: Fisheries Research.

Abstract

We investigated the population structure of the North Pacific giant octopus, *Enteroctopus dofleini*, within Alaska. Octopuses were collected from four locations (Kachemak Bay [KB; *n*=45], Prince William Sound [PWS; *n*=18], Glacier Bay [GB; *n*=33], and Southeast Alaska [SE; *n*=39]) and sequenced at the cytochrome oxidase I and III (COI and COIII) loci of the mitochondrial genome. We identified two major mitochondrial DNA (mtDNA) haplogroups. Sequence divergence ranged from 0.2 – 3.1%. Haplotypes were not distributed evenly among the populations, resulting in an enigmatic pattern of population structure. We observed no genetic differentiation between KB and GB nor between PWS and SE. F_{ST} was extremely high for all other pairwise comparisons, ranging from 0.869-0.947. The COI subunit was markedly more variable than the COIII subunit; two mutations within the COIII sequence accounted for two singleton haplotypes (2 and 5) occurring in GB and SE. We did not observe an isolation-by-distance pattern or a strong clinal gradient often detected in other marine species. Strong genetic drift, serial bottlenecks, or sweepstakes events may contribute to the pattern observed. The high level of sequence divergence observed at the COI locus may also be due to cryptic species within the *E. dofleini* complex with limited exchange of populations. Ecological data has been used to corroborate cryptic species identified with genetic markers; however, this is difficult within the ocean.

Introduction

The North Pacific giant octopus (*Enteroctopus dofleini*) is a charismatic cephalopod distributed from Baja California to the Aleutian Islands in Alaska and westward in the North Pacific to Japan and it is important both ecologically and economically. An important component of Steller sea lion, *Eumetopias jubatus*, diets in the Bering Sea, fluctuations in the abundance of *E. dofleini* may impact resident harbor seals (*Phoca vitulina richardsi*), Pacific halibut (*Hippoglossus stenolepis*), Pacific cod (*Gadus macrocephalus*), and snow crab (*Chionoecetes sp.*) populations (Conners and Jorgensen 2005). Octopuses are harvested commercially in Japan, and interest in a directed fishery *for E. dofleini* in Alaska has grown in recent years (Paust 1988, 1997). While research has focused on the basic ecology (Scheel 2002, Hartwick et al. 1984a, 1984b), reproductive biology (Gabe 1975) and development (Hartwick et al. 1981), the existence of any population structure and what factors might be driving that structure are unknown.

The biological and ecological factors that lead to the genetic differentiation of populations are difficult to observe and measure in the ocean. Both planktonic dispersal of juveniles and large scale movements by adult *E. dofleini* have the potential to influence the dynamics and structure of their populations. Currents and active movement have the ability to carry marine propagules from a few kilometers to greater than 400 km (Shanks et al. 2003). No scientific evidence of seasonal migration by *E. dofleini* is evident in Alaskan waters, and while small-scale movements have been tracked using acoustic tags in British Columbia (Mather et al. 1985), the extent to which individuals

move during the course of their lives is unknown. With little known about dispersal

mechanisms and oceanographic features that may lead to population structure, crashes in

population size due to stochastic events or overharvest could lead to a reduction in

heterozygosity or the localized depletion of a population. With limited migration from

surrounding subpopulations, recolonization of that area may not occur for generations,

having wide-ranging ecological and economical ramifications.

The identification of discrete populations or stocks is of particular importance in

implementing successful management strategies (Begg et al. 1999). Management on a

scale larger than the population structure can lead to the erosion of spawning components

with potentially wide ranging ecological consequences (Stephenson 1999). Recent

advances in molecular genetic techniques have resolved problems with population

discrimination for many species including European hake (*Merluccius merluccius*), chum

salmon (*Oncorhynchus keta*), and Atlantic cod (*Gadus morhua;* Altukhov 1981; Lundy et

al. 1999; Ruzzante et al. 2000). Few population genetic studies have been conducted on

octopus; instead, genetic studies with octopus have mainly focused on using

mitochondrial DNA (mtDNA) to resolve phylogeny. Such studies have relied heavily on

the cytochrome oxidase I and III (COI and COIII) subunits to infer the phylogenetic

relationship of species (Sosa et al. 1995) and divergence at these loci has suggested

cryptic species complexes for some taxa (Soller et al. 2000). Many phylogenetic analyses

compare few sequences within a species to sequences from other species using

interspecies sequence divergence to infer relationships; intraspecies sequence divergence

is typically poorly documented. The phylogenetic relationship of *E. dofleini* has been

well characterized above the family level (Sosa et al. 1995, Strugnell et al. 2005, Takumiya et al. 2005); however, our understanding of population structure is completely absent. Here, we examine the population structure and phylogenetics of *E. dofleini* within Alaska using the COI and COIII mtDNA subunits.

Methods

Sample collection

We collected *E. dofleini* from May 2006 – July 2009 using commercial and personal use fishing pots in Kachemak Bay (KB; n = 45) and Southeast Alaska (SE; n = 39) and large net trawls in Prince William Sound (PWS; n = 18). From August-October 2008, planktonic *E. dofleini* were collected from Glacier Bay (GB; n = 33) using light traps. Approximately 2-4 cm of muscle tissue was collected from the left third arm of each octopus. Muscle samples were then preserved in 95% ethanol, and no voucher specimens were collected.

DNA extraction, PCR, and Sequencing

We extracted DNA from muscle samples using a protein kinase K and ammonium acetate procedure (Puregene DNA^{TM} isolation protocol - Gentra Systems, Minneapolis, MN). Extracted DNA was hydrated in Tris-EDTA solution and stored in 1.5 ml tubes at 3°C until we completed our analyses, and samples were permanently stored at -20°C.

We amplified a 710 base pair (bp) fragment of the COI gene using primers described by Folmer et al. (1994). A 380 bp of the COIII gene was amplified using primers designed by Oosthuizen et al. (2004). The final concentration for each reagent in a 10µl PCR reaction were: 0.75 mM each primer, 2.5 mM MgCl$_2$, 1mg/ml BSA, 0.2 M

deoxyribonucleic triphosphates, 2 µl of 1:50 (DNA:MiliQ H_2O) diluted DNA template,

1x PCR buffer (Promega, San Luis Obispo, CA) and 1 unit of Taq polymerase. We tested

for successful amplification of both fragments on 1.5% agrose gel stained with GelredTM

(Biotium Inc., Hayward, CA.). We successfully amplified both fragments; however, the

COIII primers, originally designed for *Octopus vulgaris,* amplified a nonspecific

fragment in *E. dofleini.* We used a TA cloning kit (Invitrogen, Carlsbad, California) to

redesign primers specific to *E. dofleini.* A 112 bp fragment was amplified using the

following primers: mtDNA forward 5'-gaacattccaaggatttcacgcta-3' and reverse 5'-

ccagaagatagaaggatagcggta-3'. Amplified gene products were sequenced at the High

Throughput Genomics Unit at the University of Washington (Seattle, Washington).

Data Analysis

Forward and reverse sequences were aligned, examined for congruence, and quality

trimmed using the program Geneious Pro (Drummond et al. 2007). In order to avoid

nucleotide base-scoring mistakes that would introduce unique haplotypes, we were

conservative in trimming sequences. The COI sequences were trimmed to 517bp, while

the COIII sequences were trimmed to 70 bp. Sequences from both loci were concatenated

for all analyses. High quality forward sequences were aligned and saved in FASTA

format. We ran an analysis of molecular variance (AMOVA) in Arlequin (Excoffier et al.

2005) to partition genetic variance within and among populations. To test for genetic

differentiation among populations, we calculated pairwise F_{ST} values and ran a Fisher's

exact test in Arlequin (Excoffier et al. 2005). We used MODELTEST (Posada and

Crandall 1998) and used the Akaike information criterion (AIC) to identify the best-fit

model of nucleotide evolution. In PAUP (Swofford 1993), using the TIM+I model of nucleotide evolution, we constructed both a bootstrapped consensus tree using individual sequences and a maximum likelihood tree using unique haplotypes which were determined from the 587 bp concatenated sequences using the program DNAcollapser (Villesen 2007). The alignment was reduced to only variable sites, and the median-joining network algorithm (Bandelt et al. 1999) in the program Network with default settings (weights = 10 and $\varepsilon = 0$) was used to create a minimum spanning network (MSN) to investigate relationships between haplotypes.

Results

Eighteen mutations - 17 transitions and 1 transversion - occurred along the 587 bp concatenated COI and COIII fragments producing 5 haplotypes among the 135 octopuses sampled. There were no indels and 2 haplotypes occurred as singletons. Sequence divergence ranged from 0.2-3.1% between haplotypes.

We observed strong genetic differentiation among populations; however, standard population genetic analysis did not reveal a clear pattern. Mean F_{ST} was extremely high (0.89) and the exact G test for differentiation was significant ($P = 0.000 \pm 0.000$). Pairwise F_{ST} values ranged from 0.010 – 0.947. Four pairwise values were significant; GB and KB compared to both SE and PWS (Table 1). The majority of the genetic variance in our samples was observed among populations (88.76%) rather than within populations (11.24%).

The MSN showed two major haplogroups separated by 15 mutations. Haplogroup 1 was composed of haplotypes 1 and 2 while haplogroup 2 was composed of haplotypes

3, 4, and 5 (Fig. 1). Haplotypes were represented disproportionally among sampling locations. Haplotype 1 was the only haplotype represented in each of the four populations sampled; however, both PWS and SE only had 3 and 2 individuals respectively with this haplotype. All individuals from KB had haplotype 1, as did the majority of the octopuses sampled from GB. Haplotype 2, a singleton in GB, had one mutation that distinguished it from haplotype 1. Similarly, haplotypes 4 and 5 both had one mutation that distinguished them from haplotype 3. Octopuses from SE made up the majority of individuals with haplotype 3 as did most of the octopuses from PWS. Haplotypes 4 and 5 consisted only of individuals from SE.

The patterns we observed in both the maximum likelihood tree of haplotypes and a bootstrapped consensus tree of individual sequences mirrored the frequency distribution of sampling locations by haplotype observed in the MSN. In the maximum likelihood tree we observed two clusters of haplotypes representing each of the two haplogroups identified in the MSN (Fig 2). Support for the clustering of the two haplogroups with individual sequences in the consensus tree was high, 99/100 bootstrap replicates (data not shown).

Discussion

We observed multiple genetically differentiated populations along the coast of Alaska with enigmatic haplotype frequencies. We did not observe a clinal distribution typical of isolation by distance, nor did we observe a break in haplotypes that might suggest recolonization from glacial refugia (Grant et al. 2006, Grant and Utter 1984). The observed frequency distribution may be the result of genetic drift acting on small isolated

populations, the occurrence of repeated bottlenecks, or a sweepstakes effect. Genetic drift

has the ability to drastically influence allele frequencies when population sizes are small.

As a bycatch species, no estimates of biomass exist and little effort has been made to

quantify abundance within Alaska. *E. dofleini* have been observed to occupy a small

home range for periods of time (Mather et al. 1985) and move large distances quickly

(Grund and Scheel 2007). The low overlap in haplotypes within our populations may

suggest that, while *E. dofleini* has the potential for large-scale movements, these do not

occur on the Alaska coast. Repeated bottlenecks or a sweepstakes may also drive

haplotype distributions. There exists anecdotal evidence for 8 year cycles in abundance of

E. dofleini populations (Hartwick 1983); however, the cause of these fluctuations is

unknown. Females lay 18,000-100,000 eggs (Gabe 1975) and the survival of an octopus

from egg to 10 mm mantle length is ~1% (Mottet 1975). It is not implausible that the

offspring of one individual could be disproportionately represented in a cohort due to

favorable breeding location, access to food, or decreased predation pressure. All samples

in this study, except for those in GB, represent multiple cohorts. Those individuals

sampled from GB were collected over multiple months and are presumably not all

siblings. Both of these hypotheses either rely on limited dispersal of *E. dofleini* as adults

or philopatry.

Mitochondrial DNA analysis indicates that two distinct lineages of *E. dofleini*

exist in Alaska. The taxonomic classification of *E. dofleini* has undergone many revisions

since its description by Wülker (1910). Originally placed in the genus *Octopus*, Pickford

(1964) proposed the existence of three subspecies: *O.d.dofleini*, *O.d.apollyon*, and

O.d.martini. Hochberg (1998) later placed *O. dofleini* in *Enteroctopus* and rejected all subspecies classifications, describing *E. dofleini* as a species 'complex'. Recently sequence divergence at the COI locus (termed DNA barcoding) has been suggested as a tool for the identification of specimens (Hebert et al. 2004b) and holds potential to aid in the identification of new species (DeSalle et al. 2005). Kaneko and Kubodera (*in press*) characterized the amount of intra- and interspecies divergence within and among species in the family Octopodidae providing rough guidelines for the amount of sequence divergence expected within a species. The majority of the sequence divergence we observed was due to mutations in the COI locus. The level of divergence observed between individuals of the two haplogroups in this study is substantially larger than the intraspecific sequence divergence observed by Kaneko and Kubodera (*in press*). While our data suggest that the two distinct mitochondrial lineages may represent cryptic subspecies, morphological and ecological data must be used to corroborate this assertion. Hebert et al. (2004a) identified 10 cryptic species of the skipper butterfly, *Astraptes fulgerator*, based on sequence divergence at the COI locus which was supported by ecological data. This may not be a trivial task in the marine environment. Morphometric data and nuclear markers (microsatellites or single nucleotide polymorphisms) may help in resolving the observed patterns with mtDNA data. Irrespective of the taxonomic status of the two mtDNA lineages, and in light of the ecological and economic importance of *E. dofleini*, efforts should be made to disentangle the complex pattern of population structure observed.

Acknowledgements

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Table 1. Pairwise F_{ST} values between populations of Enteroctopus dofleini for COI and COIII.

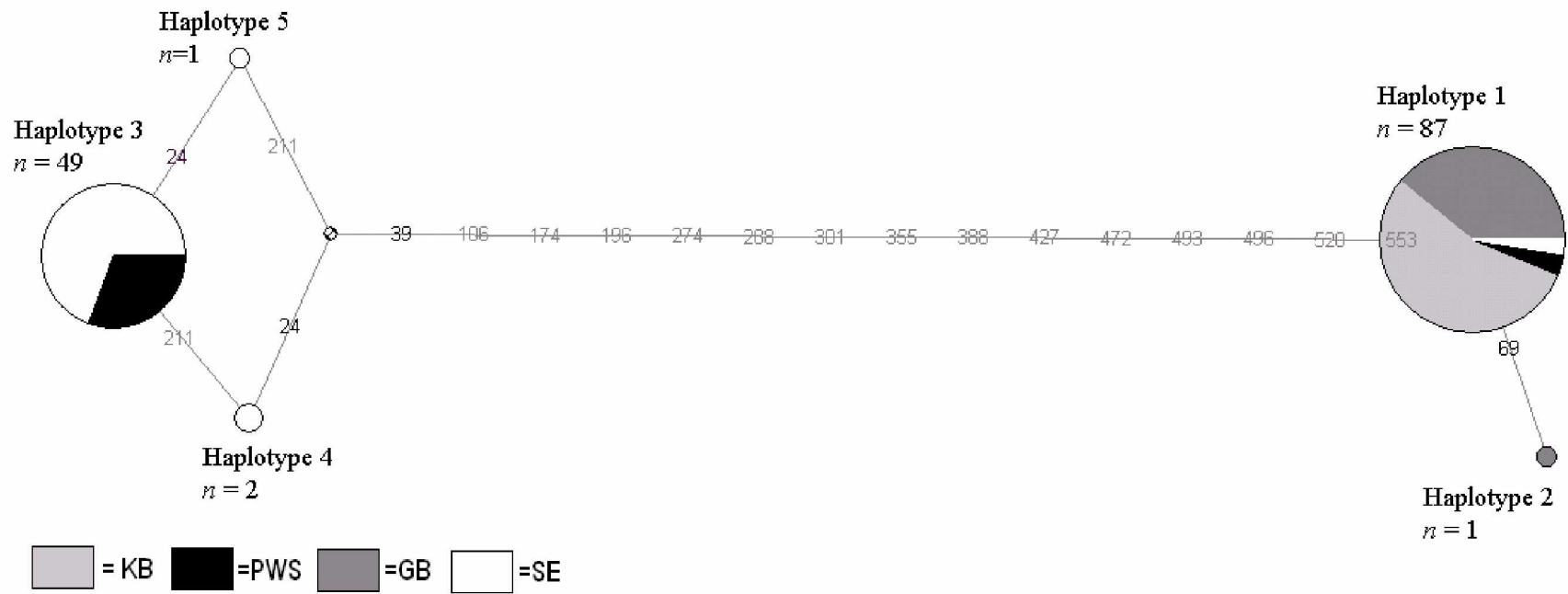|	GB	KB	PWS	SE
GB	0			
KB	0.010 (0.426±0.016)	0		
PWS	0.869 (0.000±0.000)	0.894 (0.000±0.000)	0	
SE	0.937(0.000±0.000)	0.947 (0.000±0.000)	0.034 (0.325±0.017)	0

Fig. 1. Minimum spanning tree network for mtDNA haplotypes created using the median-joining algorithm in the program Network (Fluxus Engineering, fluxus-engineering.com). Each haplotype is represented by a circle, the diameter of which is proportional to the haplotype frequency that occurred for all samples. Each branch is labeled with the position of the mutation within the sequence which separates the haplotypes.
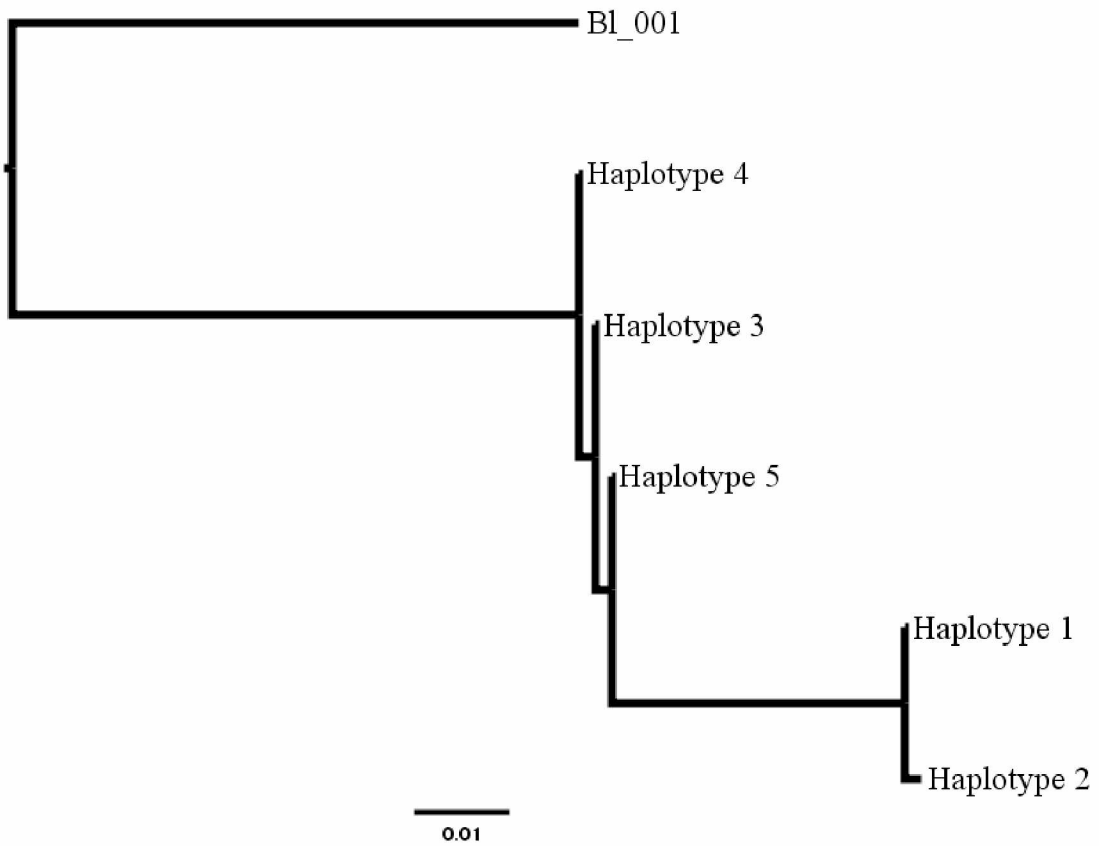
Fig. 2. Maximum likelihood tree constructed using the TIM+I model of nucleotide evolution from mtDNA COI and COIII sequence data. *Benthoctopus leioderma* was used as an outgroup.

General Conclusions

It has been demonstrated that directed harvest of *E. dofleini* can be profitable (Paust 1988) and, while early studies investigated the efficiency of unbaited lair pots to capture octopus, the efficacy of fishing gear used in pot fisheries in Alaska was unknown. Paust (1988) assumed that a directed octopus fishery would be integrated with a primary fishery; however, without proven returns these fishermen may rely on gear currently using in other Alaska fisheries. Our data suggest that while sablefish pots may catch larger octopus and have higher CPUE in terms of kilograms per days soaked, their high bycatch rates of other commercially important species may limit their usefulness in a directed fishery. Lair pots had high capture rates and resulted in no bycatch of other commercial species. Despite increasing ex-vessel prices and interest in directed harvest of *E. dofleini*, octopus will continue to be harvested as bycatch in Alaska unless management agencies can confidently estimate abundance and both the phylogenetics and the population genetic structure can be resolved.

Visible implant elastomer appeared to be a more effective means of tagging octopus than the commonly used Petersen disc tags. With only three recaptures it is difficult to make inferences about effects of tagging and retention rates; however upon recapturing the two octopuses marked with Petersen disc tags, one octopus sustained substantial tissue damage while the other lost its Petersen disc tag. All three recaptured octopuses retained their VIE tag with no visible trama. The difficulty in tagging octopus with the Petersen disc tags may also influence recapture probabilities in octopus. VIE were applied quicker and the decreased handling time could potentially affect octopus

behavior less. Tagging studies can also provide some insight into the connectedness of populations adding in the resolution of population structure.

Traditional population genetic analysis did not reveal any clear trends among the four populations of *E. dofleini* sampled in Alaska. We observed significant genetic differentiation among populations and an enigmatic pattern of haplotypes not characteristic of an isolation by distance pattern or secondary contact from two glacial refugia. Strong genetic drift due to limited migration and small population sizes, or a sweepstakes effect may be responsible for the observed pattern; however, nuclear genetic markers are needed to resolve these patterns. The large amount of sequence divergence observed at the COI subunit suggests the presence of cryptic species within the *E. dofleini* complex. If there are many reproductively isolated populations, or if two cryptic subspecies exist along the coast of Alaska it is important to characterize the genetic structure before harvest occurs. With limited dispersal, directed harvest on a large scale could lead to localized depletions or the potential loss of an entire subspecies.

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