

# Evaluation and application of BIOPOLE, a biopsy device for *in situ* non-lethal tissue extraction for fishes

Joshua G. Smith<sup>1,\*</sup>, Dan Malone<sup>1</sup>, Diana S. Baetscher<sup>2,3</sup>, Mark H. Carr<sup>1</sup>

<sup>1</sup>University of California, Santa Cruz, Department of Ecology and Evolutionary Biology, Santa Cruz, CA 95060, USA

<sup>2</sup>University of California, Santa Cruz, Department of Ocean Sciences, Santa Cruz, CA 95060, USA

<sup>3</sup>Southwest Fisheries Science Center, National Marine Fisheries Service, Santa Cruz, CA 95060, USA

**ABSTRACT:** With the rapidly expanding use of genetics for basic research, conservation, and management purposes, efficient *in situ*, non-lethal tissue sampling methods have wide application. We designed, applied, and evaluated a device designed to collect non-lethal tissue samples *in situ* from nearshore marine fishes. Using SCUBA, tissue samples were collected in the field with a biopsy pole (BIOPOLE) from a total of 1591 adults of 4 demersal (*Scorpaenichthys marmoratus*, *Hexagrammos decagrammus*, *Sebastes chrysomelas*, *Ophiodon elongatus*) and 1 mid-water (*Sebastes atrovirens*) fish species that ranged in total length from 15 to 75 cm. DNA concentration, samples per unit effort (SPUE; mean no. of ind. collected d<sup>-1</sup>), and extended monitoring of lethality and sub-lethal effects (i.e. infection) of the BIOPOLE were compared against the commonly employed fin-clip technique for fishes captured using hook-and-line fishing. Mean DNA concentration of a subset of 20 tissue samples collected with the BIOPOLE (11.53 µg ml<sup>-1</sup>) was less than fin-clipped individuals (62.50 µg ml<sup>-1</sup>), but nonetheless produced high-quality genotypes using a PCR-based protocol. BIOPOLE was more successful in obtaining tissue samples from target species (SPUE: 21.37) over hook-and-line fishing (SPUE: 8.19). Extended monitoring in a laboratory open seawater system showed no lethal or sub-lethal effects post-biopsy. BIOPOLE presents an efficient non-lethal device for obtaining tissue samples from species accessible by SCUBA or snorkeling when genetic analyses are of interest. Furthermore, the utility of BIOPOLE is highly desirable when target species are difficult to collect with more conventional methods, or when management or conservation concerns prohibit bycatch or the lethal take of samples.

**KEY WORDS:** Genetic sampling · Tissue collection · Biopsy probe · Fishes · Non-lethal sampling · SCUBA

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## INTRODUCTION

The non-lethal collection of tissue samples from organisms in their natural environment is necessary for a variety of basic research applications, including genetic, ecological, and evolutionary studies, as well as applied conservation and management research. This is especially true for species of concern (i.e. threatened or endangered) and species residing in highly protected areas (terrestrial nature preserves or no-take marine reserves). For teleost fishes, DNA

samples are typically collected via fin-clipping, which is normally conducted in association with mark-recapture studies using hook-and-line fishing (Shiozawa et al. 1992, Lucentini et al. 2006). Incidental bycatch is often associated with this indiscriminant sampling technique, resulting in potential negative effects on non-target species (Hall et al. 2000). Hook-and-line fishing and other capture methods (e.g. traps, trawls) are expensive, time-intensive, and require bringing fish to the surface (Bartholomew & Bohnsack 2005, Campbell et al. 2009). These tech-

niques all impose significant risks of mortality due to adverse effects including barotrauma, hook punctures, infection, and physiological stress (Jarvis & Lowe 2008, Campbell et al. 2009). Studies that require large numbers of genetic samples depend upon a technique that efficiently collects non-lethal tissue samples of targeted species.

Non-lethal genetic biopsy probes have been used extensively for collecting tissue samples in cetaceans and elasmobranchs (Reeb & Best 2006, Robbins 2006, Daly & Smale 2013). Barbed biopsy probes are commonly fired from crossbows, harpoons, rifles, and spear guns to obtain non-lethal tissue samples. Several studies have described how biopsy sampling can be used to effectively obtain mega-faunal DNA while minimizing lethal and sub-lethal (e.g. infection, stress) effects (Amos & Hoelzel 1990, Robbins 2006, Daly & Smale 2013). However, the application of *in situ* biopsy probes in studies of marine and freshwater teleost fishes is limited. An extensive literature review found only 1 application of a speargun-fired biopsy probe for collecting teleost fish tissue samples (Evans 2008). Therefore, the development and empirical evaluation of the efficiency (i.e. large number of samples collected per collector per unit time and effort) and lethality of an *in situ* biopsy device was needed.

To effectively collect large numbers of tissue samples in marine fishes, we developed a biopsy pole (hereafter, BIOPOLE) specifically designed for underwater use by SCUBA divers and snorkelers targeting teleost fishes. The BIOPOLE is particularly useful in structurally complex underwater environments (e.g. kelp forests, coral reefs, streams, caves), where other techniques (e.g. fishing) risk entanglement, or where conservation or management concerns prohibit lethal sampling or harm to non-target species. The device can also be used from vessels for sampling surface-schooling fishes and in freshwater systems to sample fish in shallow streams from streambanks. The objective of this study was to design, apply, and experimentally evaluate the performance of a standardized biopsy probe for collection of genetic samples in small to medium-sized demersal (i.e. bottom-dwelling) marine fishes. Although the BIOPOLE is not the first diver-operated genetic biopsy device, our review of the liter-

ature did not find a thorough description of the design, construction, use and relative efficiency of other commonly employed non-lethal genetic biopsy methods (e.g. hook-and-line catch and release). Here we describe the design, implementation, and effectiveness of the BIOPOLE.

## MATERIALS AND METHODS

### Study system

*In situ* trials of the BIOPOLE were conducted along the Monterey Peninsula (36° 37' 37.5" N, 121° 54' 33.7" W) and in Carmel Bay, CA, USA (36° 33' 29.05" N, 121° 56' 24.7" W). SCUBA divers used the BIOPOLE to collect tissue samples from 5 species of kelp forest fishes in 5–20 m of water. The sampled assemblage included adults of 4 demersal (*Scorpaenichthys marmoratus*, *Hexagrammos decagrammus*, *Sebastes chrysomelas*, *Ophiodon elongatus*) and 1 mid-water (*Sebastes atrovirens*) fish species that ranged in total length (TL) from 15 to 75 cm. The lethal and sub-lethal effects of the device were tested at the Long Marine Laboratory, University of California, Santa Cruz, CA, USA.

### BIOPOLE design and construction

The basic design of the BIOPOLE is an interchangeable tissue extraction probe attached to a fiberglass rod (Fig. 1A), which is powered by a band

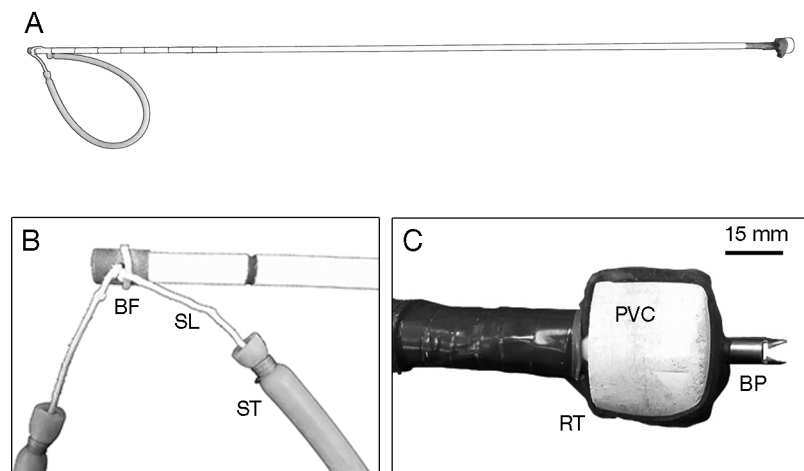


Fig. 1. (A) Genetic biopsy pole (BIOPOLE). (B) Band of elastic surgical tubing (ST) secured using spectra line (SL) and a brass fitting (BF) to prevent fracturing. (C) Interchangeable biopsy probe (BP) housed in a PVC fitting and secured using rubber tubing (RT)

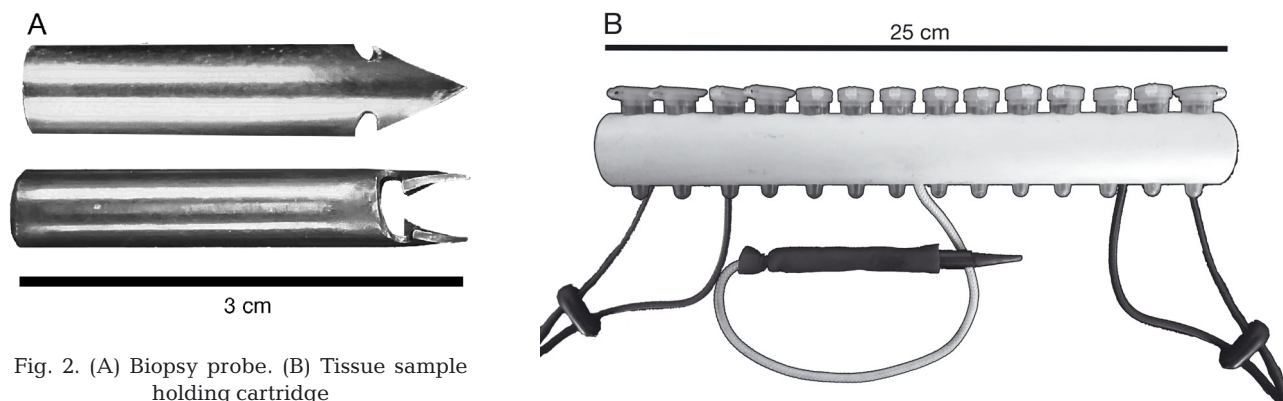


Fig. 2. (A) Biopsy probe. (B) Tissue sample holding cartridge

of elastic surgical tubing (Fig. 1B). To use the BIOPOLE, the operator places the band between the thumb and forefinger and grasps far up on the fiberglass rod, then releases the rod to fire. The purpose of this design is to extract a relatively small epidermal tissue sample from a target fish while minimizing bycatch and incidental lethal and sub-lethal effects. It is designed for use in a wide variety of natural environments (e.g. kelp forests, coral reefs, streams, caves) for a wide range of target species sizes (10–100 cm). Additionally, the interchangeable tissue extraction probe design (Fig. 1C) allows the operator to efficiently extract samples from dozens of individuals during a single dive.

Tissue extraction probes (Fig. 1C) were constructed akin to the design of those employed in elasmobranch tagging studies (Robbins 2006, Daly & Smale 2013), but redesigned specifically for targeting finfishes while using SCUBA. The tissue extraction probes were constructed using stainless steel hypodermic tubing (5 mm diameter) cut into 3 cm lengths. A triangle-shaped tissue-extraction end was carved into one end of each unit using an electric table grinder (Fig. 2A). The angle of the triangle probe is critical for extracting sufficient tissue to perform genetic analyses while avoiding incidental trauma to a target species. An optimal 30° angle was chosen based on field trials for extracting a sufficient tissue sample for DNA extraction (i.e. a minimum of 1 scale with connective tissue), while minimizing trauma and potential infection to the study species. A single tissue extraction probe was affixed to a fiberglass pole and held in place using rubber tubing with a central bifurcation that was placed around the probe, which provided sufficient resistance to hold each probe in place and allowed for rapid interchangeability underwater.

The frame of the BIOPOLE was constructed from a cylindrical fiberglass rod (2 cm diameter, 1.5 m long). A 2 × 3 cm PVC end cap (1.27 cm schedule 40) was fixed to the end of each fiberglass rod with epoxy

glue and drilled at the center with a 5 mm (wide) × 15 mm (deep) hole to house the steel tissue extraction probe. The base of each rod was fitted with a band of elastic surgical tubing (1 cm diameter × 60 cm length) secured using 2 mm spectra line and a brass fitting to prevent the fiberglass from fracturing. Standard measuring units were marked in 5 cm increments from the base of the elastic band along the fiberglass rod for operators to adjust the force based on target size and distance (Fig. 1A).

We also developed a method of storing collected tissue samples that greatly enhanced the sampling rate (samples collected dive<sup>-1</sup>) of the BIOPOLE. Tissue housing cartridges were constructed for divers to carry and collect multiple samples during a single dive. These cartridges were built using sections of 25 cm PVC pipe. Vertical holes (1 cm diameter) were drilled along each PVC pipe to secure 14 Eppendorf centrifuge tubes (1.5 ml) that were used to house the tissue extraction probes with genetic samples underwater (Fig. 2). Two additional horizontal holes (0.5 m diameter) were drilled at each end of the PVC cartridges to attach elastic armbands for carrying the cartridges underwater.

### ***In situ* operation and application**

SCUBA divers used the BIOPOLE to collect tissue samples from a total of 1591 adult *S. marmoratus* (Cottidae), *H. decagrammus* (Hexagrammidae), *O. elongatus* (Hexagrammidae), and 2 species of rockfishes (Scorpaenidae) from the genus *Sebastes* (*S. atrovirens*, *S. chrysomelas*) from 2013–2016 (Fig. 3, Table 1). Based on *in situ* visual estimates by divers, sampled individuals ranged in TL from 15 to 75 cm. Tissue samples were collected from the upper caudal-peduncle region to avoid damage to vital organs. The amount of force used to obtain samples varied by fish size and distance, but divers generally applied

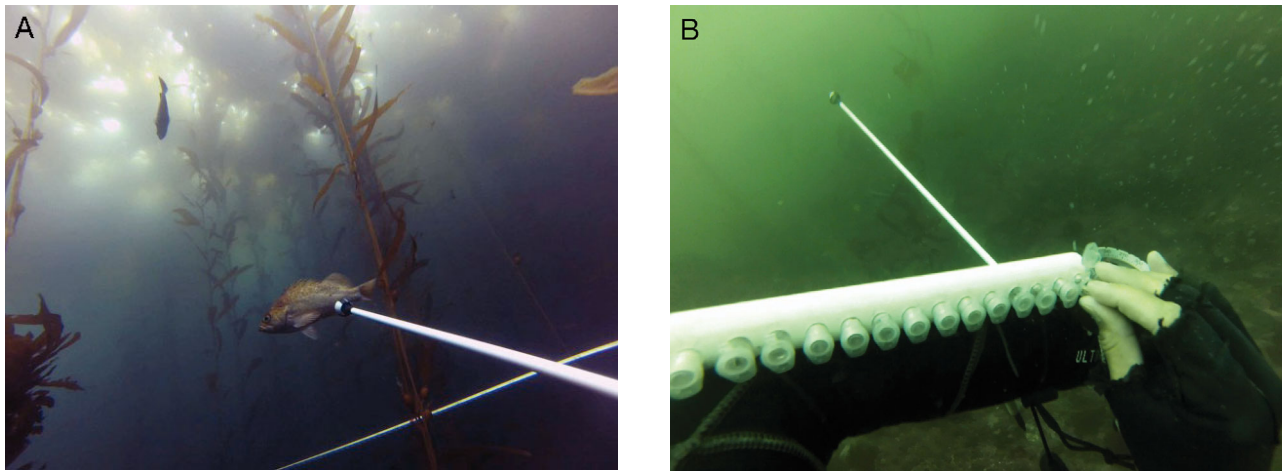


Fig. 3. (A) Diver preparing to extract a genetic sample from a kelp rockfish *Sebastes atrovirens* using the BIOPOLE. (B) Diver storing a genetic sample in a cartridge

20–30 cm of tension on the elastic band and were within 1 m of the target. This diver-controlled elastic design allowed the BIOPOLE operator to control the amount of force applied. Although the precise amounts of force were not measured, the elastic band and calibration marks (5 cm increments) on the fiberglass pole provided an estimate of standardized force. Probes with retained tissue samples were removed and placed in an Eppendorf tube (Figs. 2B & 3B). Fish species and size were recorded with pencil on the PVC cartridge below the tube containing the genetic sample. Divers typically carried 2 cartridges dive<sup>-1</sup> capable of holding 28 individual genetic samples. After each dive, cartridges were placed on ice to preserve DNA integrity during transit to the lab.

### Sub-lethal effects of BIOPOLE and fin-clipping

Laboratory experiments were conducted to evaluate the lethal and sub-lethal effects of the BIOPOLE, and to compare mortality rates, condition, and infection prevalence with the commonly used fin-clipping technique. Two species were selected for this experiment: kelp rockfish *S. atrovirens* and black-and-yellow rockfish *S. chrysomelas*.

The 66 experimental fish were captured using hook-and-line fishing from nearby kelp forests in Santa Cruz and Monterey, CA. Fishes were transported to tanks in an open sea water flow through system at the Long Marine Laboratory. Individuals were

Table 1. Species, sample numbers, and size of fish collected in the field using BIOPOLE

Family	Species	Samples (n)	Body length (mean ± SD, cm)
Sebastidae	<i>Sebastes atrovirens</i>	1368	28 ± 5.75
	<i>Sebastes chrysomelas</i>	165	26 ± 4.68
Hexagrammidae	<i>Hexagrammos decagrammus</i>	34	34 ± 7.45
	<i>Ophiodon elongatus</i>	5	59 ± 22.7
Cottidae	<i>Scorpaenichthys marmoratus</i>	19	44 ± 8.14

allowed to acclimate to the 3000 l experimental tanks for 7 d and fed once daily. After acclimatization, all fishes were temporarily placed in individual 20 l tanks where they were weighed (g), measured (TL and girth) to the nearest half centimeter, and randomly assigned a treatment (non-sampled control, BIOPOLE, or fin-clipped). The distribution of sizes ranged from 22 to 34 cm, with 50% of fish between 26 and 31 cm (mean ± SE: 29 ± 0.11 cm). Fishes assigned a BIOPOLE treatment were probed underwater and inside of the 20 l aquaria using a standardized force of 20 cm on the device, which had been determined in field trials as adequate to extract tissue for genetic analyses. For fin-clipped fishes, a 1 cm segment of the third dorsal spine was partially removed using shears. This mimicked our hook-and-line field sampling protocol, which simultaneously collects sufficient tissue for DNA extraction and permanently marks the individual as previously sampled. Non-sampled fish of each species were used as controls. Trials lasted 14 d and fishes were fed once a week. After each trial, all individuals were measured, weighed, and observed for signs of infection.

To test the hypotheses that mortality rate, condition factor, or infection prevalence of either species differed between the 2 sampling methods and non-sampled controls, 33 individuals of each species were randomly allocated to each of the 3 treatments equally (11 ind. treatment<sup>-1</sup>). The experiment was conducted in 11 tanks in an outside tank field. Each 3000 l tank included 1 individual of each species across 3 treatment levels (6 individuals total), in a randomized block design.

To estimate change in condition, we used Fulton's condition factor ( $K$ ; Le Cren 1951, Froese 2006) defined as:

$$K = \frac{W \times 100}{L^3} \quad (1)$$

where  $K$  = condition,  $W$  = weight of fish (g), and  $L$  = TL (cm). A 2-way analysis of variance (ANOVA) was used to determine whether changes in condition factor ( $K$ ) differed between the 3 treatments (control, BIOPOLE, fin-clipped), and whether this effect differed between species (*S. chrysomelas*, *S. atrovirens*) between the onset and end of the experiment.

A multivariate mixed model was used for determining differences in fish weight, girth, and length within and between species as a result of treatment. The model was fit using a single response (i.e. change in weight, girth, or length) across all independent and interactive treatment combinations (control, clipped, biopsy) and species. Lower-order terms were sequentially removed using Akaike's information criterion to fit the best model (Bozdogan 1987).

Past experience (authors' pers. obs.) with these 2 species under these same laboratory conditions have revealed that injured individuals are highly susceptible to rapid spread of infection (e.g. lesions, raised or missing scales, clouded eyes). Each individual was visually inspected for external symptoms of infection at the onset and end of the experiment. We compared proportionate change in prevalence of any infection symptom (any symptom scored as an infected individual) between the 3 sampling treatments and between the 2 species with a 2-way ANOVA.

### Effectiveness of BIOPOLE versus fin-clipping for genetic analyses

The number of samples collected per unit effort (SPUE; mean no. of ind. collected d<sup>-1</sup>) was calculated for both the BIOPOLE and hook-and-line fin-clipping collection methods in order to directly compare the effectiveness of each gear type. Sampling days were defined as a single vessel outing with a crew typically

consisting of 4 divers or 4 fishers. Divers typically conducted 3 dives on a sampling day, with each dive lasting 60–80 min, while fishers typically fished for 6–7 h. Both methods required a total of 40 person-hours per sampling day and were conducted independent of each other (i.e. fishing and diving were not conducted simultaneously from the same vessel). A total of 56 replicate sampling days were conducted using BIOPOLE and 71 using hook-and-line fishing. In cases of barotrauma for fishes captured using hook-and-line fishing, a descending device was used to return fish to depth.

SPUE was calculated for each sampling method for both the total catch (i.e. all species pooled) and for a single target species (*S. atrovirens*). One-way ANOVAs were used to determine if differences in the mean number of samples differed by gear type and across replicate sampling days.

To compare the utility of sampling methods for generating genotype data, genomic DNA was extracted from dried tissue samples using DNeasy 96 Blood and Tissue kits on a BioRobot 3000 (Qiagen) with an elution volume of 200 µl. Extracted DNA was stored at 4°C. DNA samples were prepared using the Genotyping-in-Thousands by Sequencing (GT-seq; Campbell et al. 2015) protocol and sequenced on an Illumina MiSeq instrument. Genotype data were processed and filtered as described by Baetscher et al. (2018).

The concentration of extracted DNA per sample can vary dramatically, sometimes by orders of magnitude, depending on the amount and type of tissue used. However, genotyping protocols that include a PCR step are often resilient to discrepancies in starting DNA concentration. Because our genotyping method included PCR, we defined effective sample collection as those samples that produced a successful genotype (less than 10% missing data), regardless of the amount of input DNA. To test differences in genotyping success rate between BIOPOLE and hook-and-line fishing, we compared the number of samples collected with each gear type to the number of samples successfully genotyped. In addition, to determine whether there was a threshold concentration of extracted DNA necessary to produce a successful genotype, we quantified a subset of samples with a Qubit 2.0 Fluorometer (ThermoFisher Scientific) and Qubit dsDNA BR (broad range) Assay Kit according to the manufacturers' protocols, using 1 µl of extracted DNA in 199 µl assay solution. This assay can detect 2–1000 ng of DNA. Concentrations were recorded for 40 *S. atrovirens* that produced successful genotypes (20 sampled using BIOPOLE and 20 with hook-and-line).

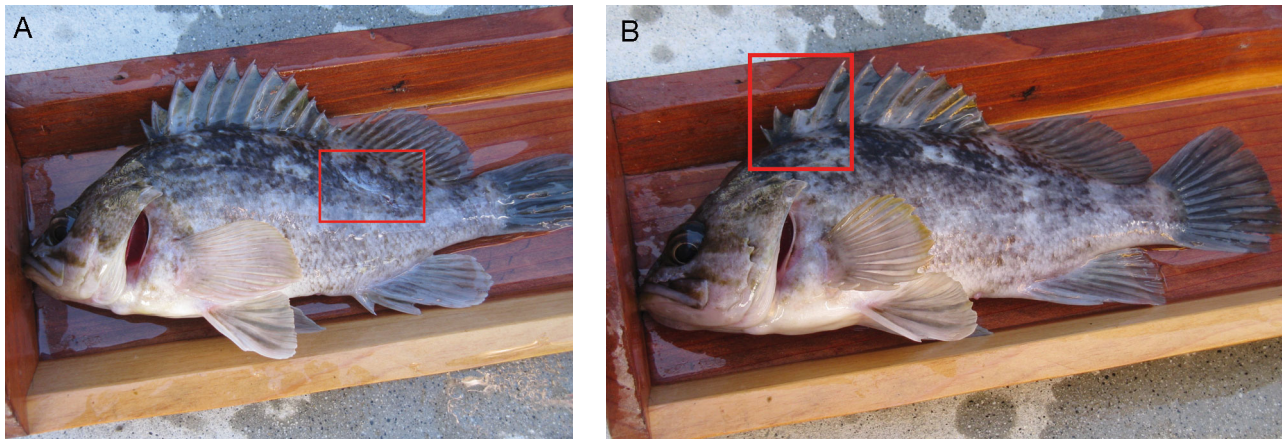


Fig. 4. Kelp rockfish 14 d following (A) biopsy collection via BIOPOLE and (B) fin-clipping

## RESULTS

### *In situ* sample collection via BIOPOLE

The focal species of our sampling efforts was kelp rockfish *Sebastes atrovirens*, which comprised 1368 of the 1591 total samples. Sample retention rate was not recorded because nearly all probes that contacted fish resulted in the extraction of tissue. Of all samples collected via BIOPOLE, only 1 mortality was observed, which resulted from a misfire. All other fish were released unharmed and resumed normal behavior following biopsy. In contrast, visible signs of barotrauma were recorded in 43 of the 1330 fish collected using hook-and-line fishing.

### Sub-lethal effects of BIOPOLE and fin-clipping

In the lab experiment, Fulton's condition factor ( $K$ ) showed no significant difference in the overall condition of fish within or across species, and between gear types ( $F_5 = 1.11$ ,  $p = 0.36$ ). Additionally, the reduced mixed model showed no difference in the mean change of fish weight, girth, or length as a function of treatment or species ( $F_3 = 0.75$ ,  $p = 0.59$ ). After 14 d, all lesions had healed without visual signs of infection (Fig. 4). Scale regeneration did not occur for individuals sampled using BIOPOLE. However, the lesions that resulted from BIOPOLE appeared minimal compared to fin-clipped individuals. All 66 fish resumed normal behavior post-treatment and were successfully released back to the reefs where they were captured following lab trials.

### SPUE for BIOPOLE versus hook-and-line fishing

SPUE for target species was different between gear types ( $F_1 = 21.49$ ,  $p < 0.0001$ ; Fig. 5). Post hoc comparisons using the Tukey HSD test indicated that the mean  $\pm$  SD SPUE score for the BIOPOLE gear type ( $21.37 \pm 22.96$  samples  $d^{-1}$ ) was greater than the hook-and-line gear type ( $8.19 \pm 6.49$  samples  $d^{-1}$ ;  $p < 0.05$ ). Although target species catch rates were significantly higher for BIOPOLE, there was no difference in the pooled total catch rates between gear types ( $F_1 = 3.21$ ,  $p = 0.07$ ).

### Effectiveness of BIOPOLE versus fin-clipping for genetic analyses

Of the 1368 *S. atrovirens* collected by BIOPOLE, 88.7% provided usable genotype data, while 96.6%

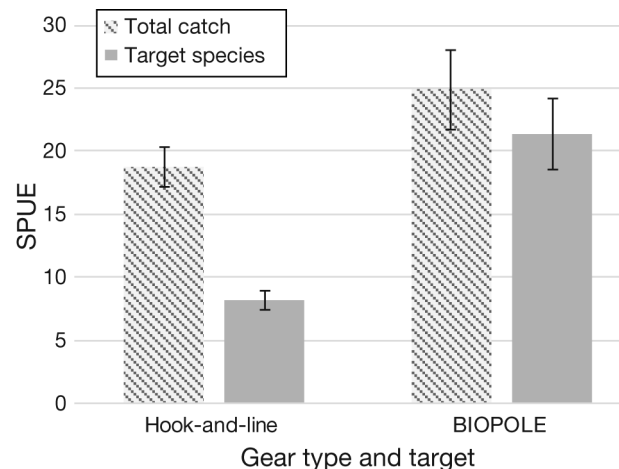


Fig. 5. Samples per unit effort (SPUE; mean no. of ind. collected  $d^{-1}$  [ $\pm 1$  SE]) for the target species (*Sebastes atrovirens*) and pooled total catch (i.e. all species) between gear types

Table 2. DNA concentrations, sampling days, and number of samples collected for each gear type, excluding 5 BIOPOLE DNA samples that fell below the detection threshold (<2 ng). Successful: samples from the target species that resulted in successful genotype

Gear type	—DNA concentration ( $\mu\text{g ml}^{-1}$ )—			Sampling days (n)	Samples (n)		
	Minimum	Maximum	Mean		Total	Total from target sp.	Successful
BIOPOLE	2.16	38.80	11.53	64	1591	1368	1213
Hook-and-line	28.80	113	62.50	71	1330	582	562

of the 582 target species collected by hook-and-line generated successful genotypes.

All 40 of the samples tested for DNA concentration produced high-quality genotypes. However, 5 of the 20 samples collected by BIOPOLE were below the minimum detectable concentration (<2 ng). For the remaining 35 samples, DNA concentration ranged from 3–14  $\mu\text{g ml}^{-1}$  for tissue collected via BIOPOLE (mean  $\pm$  SD:  $11.53 \pm 11.70 \mu\text{g ml}^{-1}$ ) and from 46–71  $\mu\text{g ml}^{-1}$  for the 20 fin-clipped individuals captured from hook-and-line ( $62.50 \pm 21.31 \mu\text{g ml}^{-1}$ ; Table 2).

## DISCUSSION

The efficiency of the underwater BIOPOLE for obtaining tissue samples of marine teleost fishes provided a significant advantage over traditional fin-clipping with no substantial decrease in effectiveness, especially when target species was of concern. This technique allowed for the collection of tissue samples without the adverse lethal and sub-lethal (e.g. barotrauma, hook punctures) effects associated with other gear types that require bringing fish to the surface (e.g. hook-and-line fishing). Although only 3% of fish sampled by hook-and-line in this study exhibited barotrauma, previous research has indicated reduced survival (68% survival rate) in cases of mild to severe barotrauma for fishes captured using hook-and-line, and that surface holding time is inversely related to short-term survival (Jarvis & Lowe 2008).

The SPUE of the BIOPOLE was nearly 3-fold greater than hook-and-line fishing for a given target species, and provided a cost-effective method for extracting tissue from target species without bycatch. The true advantage of the BIOPOLE lies in minimizing time wasted while sampling. Time spent in unproductive areas or collecting non-target species was virtually eliminated when divers could visually identify individuals. In many cases, the number of target species sampled during a 1 h dive exceeded the number collected from a full day of hook-and-line

fishing. Despite this, the benefits of BIOPOLE may be impacted by species behavior (authors' pers. obs.). For example, kelp rockfish *Sebastes atrovirens* were often suspended mid-water and would occasionally approach divers, providing an easy target, whereas black-and-yellow rockfish *S. chrysomelas* were cryptic and difficult to probe when they were concealed in crevices or overhangs.

Although the proportion of samples collected by BIOPOLE that provided successful genotype data was slightly less than that for hook-and-line fishing (88.7 and 96.6%, respectively), divers were able to more effectively target species of interest, and therefore collect many more samples using the biopsy technique. However, initial DNA concentration required for successful genotyping and other potential applications (e.g. RNA transcriptomics, stable isotope or fatty acid analysis) is dependent on the laboratory protocol, extraction technique, and, in the case of population genetics, type of genetic marker (e.g. microsatellites or single-nucleotide polymorphisms) used for a particular study (Campbell & Narum 2009). In our case, the 40 *S. atrovirens* DNA extractions that provided high-quality genotypes using an amplicon-sequencing method (Campbell et al. 2015) displayed substantial differences in mean DNA concentration using the BIOPOLE ( $11.53 \mu\text{g ml}^{-1}$ ) compared to those obtained from fin clips collected via hook-and-line ( $62.5 \mu\text{g ml}^{-1}$ ). PCR-based methods, such as the protocols frequently employed in genetic studies, are resilient to low starting concentrations and variable DNA inputs across samples (Campbell & Narum 2009).

Small tissue samples (e.g. a single scale with connective tissue), like those obtained using the BIOPOLE, provide sufficient DNA for many population genetic and pedigree analyses. However, the tissue type and quantity required for a particular molecular or biochemical analysis can vary substantially, and experimental design must take this into consideration. For the data presented here, even the 5 BIOPOLE DNA samples that fell below the concentration detection threshold (<2 ng) produced high-quality

genotypes using a sequencing protocol that is resilient to small amounts of input tissue. These genotyping results further highlight the utility of BIOPOLE for studies where little tissue is required, which minimizes lethal and sub-lethal effects on study species.

Fishes sampled using BIOPOLE rapidly swam away and resumed normal behavior, with small superficial lesions at the probe location. Extended monitoring of probed fishes in the laboratory revealed that lesions did not result in infection or abnormal behavior. Because sample collections with BIOPOLE generally leave little evidence of sampling relative to fin clipping, it is important that operators using BIOPOLE take care not to resample the same individuals. We avoided this by sampling different locations on sequential days of sampling. Also, standard genetic analyses for matching genotypes can remove redundant data from re-sampled individuals with little additional effort.

The utility of BIOPOLE for underwater sampling presents significant advantages over other capture methods when target species are difficult to locate, or when restrictions prohibit the taking of particular species. Indeed, minimally invasive sampling techniques are becoming increasingly important for populations of organisms at risk in order to mitigate capture-related stress. The BIOPOLE can be fired from multiple power levels based on target distance, size, and stress tolerance, which makes it applicable to a wide range of study subjects and environments. All fish in this study were resilient to sampling via BIOPOLE, but care should be taken to understand the behavior and stress tolerance of target organisms.

In this study, we developed and experimentally evaluated the performance of a non-lethal biopsy pole (BIOPOLE) for extracting DNA from tissue samples in marine fishes. The utility and effectiveness (i.e. capture rate and DNA integrity) of BIOPOLE outweighed traditional hook-and-line fishing for obtaining tissue samples from target species. Non-lethal sampling offers benefits to both study animals and researchers. The application of BIOPOLE provides researchers with an efficient and less invasive technique for obtaining dermal tissue from marine fishes, where suitable conditions permit the use of SCUBA.

*Acknowledgements.* This project was supported by the National Science Foundation under Grant No. OCE-1260693 and by the California Department of Fish and Wildlife. All experiments were undertaken with approval from the UCSC Institutional Animal Care and Use Committee. We thank Ellen Campbell and Cassie Columbus for

genetics laboratory assistance. The views expressed herein do not necessarily reflect the views of any of those individuals or organizations.

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