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# OPTIMIZING BIOLOGICAL PARAMETERIZATION IN THE EGG ESCAPEMENT MODEL OF THE MARKET SQUID, *(DORYTEUTHIS OPALESCENS)*, POPULATION OFF CALIFORNIA

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U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
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ABSTRACT

Over the last two decades, the market squid (*Doryteuthis opalescens*) fishery has been one of the most productive and valuable fisheries off California. In 2005, the California Department of Fish and Wildlife (CDFW) established a management plan including fishery controls rules with a seasonal catch limitation and a weekend closure of the fishery. In the absence of adequate information to establish a maximum sustainable yield (MSY), the egg escapement method was identified as the best available tool to provide a proxy for maximum sustainable yield/optimum yield (MSY/OY). However, recent published studies identified laboratory processing time as a critical area of concern for the successful application of the egg escapement method. The original laboratory and field protocols required squid mantle tissue to be dried for 14 days at 56°C and female squid gonads to be preserved in 10% neutral buffered formalin and weighed at a later date. These methods led to a significant lag between the time of sample collection and the availability of data needed to apply the egg escapement model. In this study, experiments were conducted at the Southwest Fisheries Science Center (SWFSC) and at CDFW to provide data to re-evaluate the laboratory protocols for drying female squid mantle tissues and to determine the relationship between fresh and preserved gonad weights. Results of these experiments demonstrated that slight modifications to the existing protocols significantly decreased laboratory processing time. Drying mantle tissue at 60°C, 64°C, 68°C, 72°C, or 76°C for 1, 2, 3, or 4 days achieved similar results as in drying the mantles for 14 days at 56˚C. For female squid gonads, the regression model, $W_p = 1.8980 \times W_f - 0.5186$, predicted the relationship between formalin preserved gonad weight ($W_{p)}$ and fresh gonad weight ($W_f$) and explained 97% of the variability in the data. This model will allow new data (i.e. fresh gonad weights from 1.606 to 8.344 grams) to be combined with historical data (i.e., preserved gonad weights) and to be incorporated into the existing egg escapement model. Additionally, obtaining fresh gonad weights will save time and money since there is no longer a need to purchase or dispose of formalin, a hazardous chemical preservative. However, it should be noted that the prediction intervals for converted gonad weight is roughly 1.2 grams. Depending on the weight of the ovary, this uncertainty could impact the resulting egg escapement. As such, additional samples need to be collected to determine if the variability in converted gonad weight is acceptable or if the egg escapement model should be updated to allow for the accommodation of fresh, rather than formalin preserved, gonad weights.

# INTRODUCTION

The market squid (*Doryteuthis opalescens*) is a short-lived semelparous species occurring off the west coast of North America from Mexico to Canada (Butler *et al.* 1999, Macewicz *et al.* 2004). This species plays a vital role in the California Current ecosystem and is prey to numerous fishes, sea birds, and marine mammals (Fields 1965, Lowry and Carretta 1999, Koslow and Allen 2011).

California's market squid fishery began in the late 1800s but it wasn't until the 1980s that the fishery expanded as a result of increased worldwide demand (Vojkovich 1998). Since the 1990s, the market squid fishery has been one of the largest commercial fisheries for the state of California in terms of both revenue and tons landed (Vojkovich 1998, Porzio 2013). In 2005, the Market Squid Fishery Management Plan implemented by the California Department of Fish and Wildlife (CDFW) established fishery control rules including a seasonal catch limitation of 118,000 short tons (107,047 t) and a two-day weekend closure (CDFG 2005). However, due to limited data, no biomass estimate exists for market squid. As a result, the egg escapement method was identified as a tool to provide a proxy for maximum sustainable yield/ optimum yield (MSY/OY).

Based on traditional eggs-per-recruit theories (Beverton and Holt 1957; Sissenwine and Shepherd 1987; Gabriel *et al.* 1989), the egg escapement method uses potential fecundity, daily mortality, and daily rate of egg deposition for adult female market squid captured on the spawning grounds to evaluate the population's response to fishing pressure by estimating the proportional escapement of eggs from the fishery (Macewicz *et. al*. 2004, Maxwell *et al*. 2005, Dorval *et. al*. 2013). To examine these parameters and to estimate residual fecundity (standing stock of oocytes and ova), mature female market squid are collected from the fishery via the CDFW port sampling program. For each female squid collected, gonads (ovary and oviduct), mantle punch (a circular disc of mantle tissue), and statoliths are removed. Dorsal mantle length, weight, sex, and maturity are recorded for all squid.

Following the original protocols established by Macewicz *et al.* (2004), mantle punches from each female squid are frozen and later dried in a natural convection oven for 14 days at 56˚C. In addition, gonads are preserved in 10% buffered formalin and weighed at a later date (Macewicz *et al.* 2004). Once these parameters are measured in the laboratory, residual fecundity is estimated using an equation combining female gonad weight (in grams) and dry mantle weight per surface area (mantle condition index ($mg/mm^2$)). Whether this method is optimal or cost effective has not been established. However, despite the potential to be an effective near "real time" monitoring tool, the application of the egg escapement model has been impaired, in part, due to the amount of time required to process samples in the laboratory (Dorval *et al.* 2013). This study seeks to facilitate the application of the egg escapement method by establishing comparable laboratory protocols that considerably reduce laboratory processing time and expedite data availability.

## MATERIALS AND METHODS

Experiments were conducted at the Southwest Fisheries Science Center (SWFSC) and at CDFW. For market squid mantles punches, increases in temperature or the use of a lyophilizer (freeze dryer) decreases drying time. Thus, the primary goal was to identify the combination of temperature and number of days drying that removes a similar level of moisture as drying for 14 days at 56°C (see Dorval *et al*. 2013). Updating the protocols in this manner will allow for the continued use of condition index data previously estimated using the original methods.

For gonads, the objective was to determine whether a conversion factor could be derived to translate fresh gonad weights into preserved gonads weights to expedite processing time, reduce cost, and allow for the incorporation of new gonad data into the existing egg escapement model.

### Mantle punch experiment

#### *Experimental design*

This experiment consisted of six trials each using two natural convection laboratory ovens (Jeio Tech model ON-12G) and a lyophilizer (VirTis model Benchtop K (6KBTES)). One natural convection oven (oven I) was used to dry mantle punches at 56˚C for 14 days during each of the six trials. The temperature of the second oven (oven II), varied depending on the trial: 60˚C (trial 5660), 64˚C (trial 5664), 68˚C (trial 5668), 72˚C (trial 5672), and 76˚C (trial 5676). For each trial, there were six treatments. A treatment was defined as a drying method, a fixed temperature and day combination (i.e. 1, 2, 3, 4, and 14 days). Finally, for each trial, the lyophilizer was used to dry mantle tissue for one day at -57˚C (Table 1A).

#### *Sample collection and laboratory processing*

In January 2014, CDFW randomly collected 150 market squid from commercial fishing vessels for use in this experiment (Table 1A). Whole squid were thawed and processed at the beginning of each trial. Dorsal mantle lengths were measured to the nearest millimeter (mm) and body weights were measured to the nearest 0.1 gram (g). Squid were then dissected ventrally and sex was recorded.

Mantle tissue was cleaned by removing the outer dermis and innermost membrane from the tissue. Using a size 11 cork borer (3/4 inch or 19.050 mm outer diameter, Humboldt Mfg. Co.), six mantle tissue punches were obtained from each squid (Figure 1).

A trial consisted of 30 randomly selected squid. Six mantle punches were obtained from each squid (Figure 1). One randomly selected mantle punch was assigned to each of the six treatments resulting in a total of 30 mantle punches per treatment and a total of 180 mantle punches per trial (Table 1B). Mantle punches were then placed into labeled and pre-weighed aluminum weigh pans. Wet weights (in grams), defined as pan weight plus wet mantle punch weight, were recorded. Weigh pans were then placed, by treatment group, into their respective oven or lyophilizer. Upon removal from the oven or the lyophilizer, mantle punches were re-weighed in the pan. Dry weights of each mantle punch were calculated by subtracting the weight of the pan from the total weight (dry mantle punch plus pan).

*Data analysis of mantle punches*

For each trial, the six treatments were compared using a randomized block design, with individual squid as the block. Therefore, each of the six treatment groups were replicated only once in each block. As individual squid were of different sizes (Table 2), blocking was important to remove the effects of squid size on mantle dry weight, when determining statistical differences among treatments. Further, when there was a significant difference among treatments, a Tukey's pairwise comparison test was conducted to determine which treatments were different from each other. The percent moisture removed from wet mantles in all treatment groups was normally distributed and their variances were similar. Finally, all statistical tests presented here were performed using SAS 9.4 and were based on the percent moisture removed from the wet mantles, i.e. the proportion of moisture removed from the mantle after drying.

Female Gonad Preservation Experiment

*Sampling and experimental design*

During the 2014 California market squid commercial fishing season, CDFW collected whole female squid from randomly selected boats following sampling protocols described by Kong *et al.* 2003. These squid were placed in 20 mm size bins based on dorsal mantle length. Size bins were as follows: bin 1: 80-100 mm; bin 2: 101-120 mm; bin 3: 121-140 mm; bin 4: 141-160 mm; and bin 5: 161-180 mm. The objective was to obtain 20 female squid from each size bin. Gonads (ovary, oviduct, and oocytes/eggs) were removed and weighed fresh to the nearest gram. Each gonad was then placed in an 8 ounce jar filled with 10% buffered formalin solution. After two weeks of preservation, the gonads were removed and blotted dry. All loose eggs were collected with a sieve and blotted dry. The gonad structure and loose eggs (if any) were then weighed to the nearest gram.

*Data analysis of gonads*

Due to small sample sizes per size bins, data were pooled for statistical analyses. A linear regression was used to determine a conversion factor where preserved weight was predicted based on a known fresh gonad weight. Uncertainty was examined by calculating prediction intervals around the regression line. These statistics were computed using Microsoft Excel 2010.

## RESULTS

Mantle Tissue Sample Experiment

Each trial compared the amount of moisture removed for 14 days of drying at 56˚C in oven I to that removed during 1, 2, 3, and 4 drying days at either 60˚C, 64˚C, 68˚C, 72˚C or 76˚C in oven II, and during one day of drying at -57˚C in the lyophilizer. Across all trials, each of the six treatments were significantly different (Tables 3A, 4A, 5A, 6A, and 7A) and the pairwise comparison showed that the lyophilizer removed less moisture (77.78%-81.15%) than was removed by the natural convection oven treatments (78.78%-82.36%). Results specific to each trial are presented below.

*Trial LP5660*

The lyophilizer removed significantly less moisture, (80%), than the other treatments which ranged from 81.14% to 81.69% (Table 3B and Figure 2). Further, drying the mantles at 56˚C for 14 days and at 60˚C for 3 and 4 days led to similar percent moisture removal. Similarly, there was no significant difference when mantles were dried at 56˚C for 14 days and at 60˚C for 1, 2, or 3 days. These results indicated that drying mantles at 60˚C for 1, 2, 3, or 4 days are highly likely to lead to similar results as drying them for 14 days at 56˚C.

*Trial LP5664*

The lyophilizer removed significantly less moisture, (81.15%), than the other treatments. Drying the mantles at 56˚C for 14 days and at 64˚C for 1 to 4 days did not lead to a significant difference in the level of moisture removed from the mantles (Table 4B, Figure 2). The amount of moisture removed by these treatments ranged from 81.69% after one day to 82.11% after four days of drying at 64˚C. Therefore any of these four treatments could be used to achieve similar results as achieved by drying the mantles for 14 days at 56 ˚C, i.e. 81.80%.

*Trial LP5668*

The lyophilizer removed significantly less moisture, (80.9 %), than the other treatments. Drying the mantles at 56˚C for 14 days and at 68˚C for 1 to 4 days did not lead to significant difference in the level of moisture removed from the mantles (Table 5B, Figure 2). The amount of moisture removed by these treatments ranged from 82.06 % after one day to 82.36% after four days of drying at 68˚C. Therefore, any of these four treatments could be used to achieve similar results as in drying the mantles for 14 days at 56˚C, i.e. 82.24%. Also, contrary to the previous trials, no treatments showed outliers in this trial.

*Trial LP5672*

The lyophilizer removed significantly less moisture, (77.78%), than the other treatments which ranged from 78.78 % at day 1 to 79.85% at day 4. Further, drying the mantles at 56˚C for 14 days and at 72˚C for 2, 3, and 4 days led to similar percent moisture removal (Table 6B, Figure 2). Similarly, there was no significant difference when mantles were dried at 56˚C for 14 days and at 72˚C for 1 or 2 days. These results indicated that drying mantles at 72˚C for 1, 2, 3, or 4 days are likely to lead to similar results as drying them for 14 days at 56˚C. Note that this trial considerably removed less amount of moisture than all other trials, because the mantles came from squid which were, on average, smaller in size than squid used in other trials (Table 2, Figure 2).

*Trial LP5676*

The lyophilizer removed significantly less moisture, (80.91 %), than the other treatments. Drying the mantles at 56˚C for 14 days and at 76˚C for 1 to 4 days did not lead to a significant difference in the level of moisture removed from the mantles (Table 7B, Figure 2). The amount of moisture removed by these treatments ranged from 81.94 % after one day to 82.30% after four days of

drying at 76˚C. Therefore, any of these four treatments could be used to achieve similar results as in drying the mantles for 14 days at 56˚C, i.e. 82.06%.

Female Gonad Preservation Experiment

During the 2014 sampling period, CDFW was unable to obtain 20 female squid gonads from each of the size bins (Table 8).

The regression plot illustrates a strong positive linear relationship between fresh and preserved gonad weight regardless of size. Fresh gonad weight ranged from a minimum of 1.606 grams to a maximum of 8.344 grams. As fresh weight increased, preserved weight increased. Prediction intervals were approximately 1.2 grams, regardless of size (Figure 3, Tables 9-10). Based on these results, formalin preserved weight can be estimated, using the following equation:

$$W_p = 1.8980 \times W_f - 0.5186,$$

Where $W_p$ represents the weight of the female market squid gonad preserved in 10% neutral buffered formalin (g) and $W_f$ represents fresh gonad weight (g).

## DISCUSSION

This study demonstrated that market squid female gonad and mantle punch samples can be processed more efficiently with relatively minor adjustments to the current laboratory methods. It is anticipated that the use of the proposed methods in this study will improve the efficiency of laboratory processing of biological data and thus will have a significant impact on the application of the egg escapement model to monitor the market squid stock off California.

Mantle Punch Experiment

Results suggest that increasing oven temperature by 4°C to 20°C removes a similar level of moisture as compared to the original protocol and reduces drying time from 14 days to one day, thereby, hastening data availability for the application of the egg escapement model. Although it is recommended that CDFW select only one temperature and drying day option to serve as the new protocol, Tables 3-7 provide a suite of options that can be considered. Percent moisture removed by the natural convection ovens in this experiment are consistent with findings from previous studies which have shown that squid mantle tissue contains approximately 74 to 83.2% moisture content, depending on the species (Ke *et al*. 1979, Berntsen 1987, Perez 1994, and Chu *et al*. 1995). Berntsen (1987) found that market squid mantle tissue contains approximately 83.2% +/-0.27 moisture content which is consistent with the levels of the percent moisture removed by the natural convection ovens during this experiment.

The lyophilizer was selected for comparison since it preserves tissue without altering protein activity or RNAs, thus providing the potential for future research projects using these tissue samples (Wu *et al*. 2012). However, results from this experiment demonstrated that the lyophilizer removed significantly less moisture than the range of natural convection oven temperatures and therefore may not be the best approach for ensuring continued use of previous mantle condition index time series data used in Dorval *et al*. (2013). Since, these authors used a natural convection oven to estimate the mantle condition index by quarter and by fishing region,

new mantle condition data can be added to existing data to provide an updated time series to better understand the regional, seasonal and internal variability in stock productivity and exploitation.

Female Gonad Preservation Experiment

Since 2010, CDFW has been measuring fresh gonad weights to increase laboratory processing efficiency, reduce cost, and to facilitate timely data collection and analysis. However, the egg escapement model only accommodates preserved gonad weights. Therefore, the primary objective of this experiment was to determine the relationship between fresh and preserved gonad weights so that new data could be incorporated into the existing model. The regression model derived in this experiment explained 97% of the variability in the data, and thus it can be used to convert gonad fresh weight to preserved weight. However, the prediction intervals around the regression line were, approximately, 1.2 grams which could potentially be problematic in the egg escapement model since fresh gonad weight in this study ranged from 1.606 to 8.344 grams.

During the sampling period for this study, market squid were not available for all of the anticipated size bins; thus, sampling additional seasons would be beneficial in filling in this gap as fluctuations in DML has been noted by both season and catch location (Hixon 1983, Protasio *et al.* 2014).. Also, it has been well documented that the process of preservation can alter the accuracy of the measurements obtained from biological specimens (Parker 1963, Mills *et al.* 1982, Fleming and Ng 1987, Andriguetto and Haimovici 1988, DiStephano *et al*. 1994, Shields and Carlson 1996, Ramon and Bartoo 1997, Puigcerver 1999, Srinivasan *et al.* 2002, Frimpong and Henebry 2012). Therefore, it would be beneficial to re-weigh the gonad samples after 2, 4, and 6 weeks of preservation in 10% neutral buffered formalin to determine if length of preservation time affects gonad weight. Increasing the sample size across all size bins and examining the effect of preservation time on gonad weight may help determine whether size or the effect of formalin on the gonad tissue is responsible for the variability in the data. If an increased sample size does not reduce the variability seen in the prediction intervals, then it may be more beneficial to update the input parameters of the egg escapement model to accommodate fresh gonad weights.

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TABLES

Table 1. **(A)** Lyophilizer and oven temperatures (°C) per trial are presented. Thirty whole squid were used for each trial for a total of 150 squid for the entire experiment. **(B)** Treatment groups per trial are presented by drying method and time. One mantle punch from each of the 30 squid was included in each of the six treatments, per trial. For each trial, the treatments were compared using a randomized block design, with individual squid as the block (see Figure 1).

**(A)**

| | Lyophilizer (°C) | Oven I (°C) | Oven II (°C) | Number of whole squid |
|---|---|---|---|---|
| 5660 | -57 | 56 | 60 | 30 |
| 5664 | -57 | 56 | 64 | 30 |
| 5668 | -57 | 56 | 68 | 30 |
| 5672 | -57 | 56 | 72 | 30 |
| 5676 | -57 | 56 | 76 | 30 |
| | | ***TOTAL WHOLE SQUID (all Trials combined)*** | | ***150*** |

**(B)**

| **TREATMENT GROUPS** | | |
|---|---|---|
| **Drying Method** | **Drying Time (in days)** | **Number of Mantle Punches** |
| Lyophilizer (LP) | 1 | 30 |
| Natural Convection (Oven II) | 1 | 30 |
| Natural Convection (Oven II) | 2 | 30 |
| Natural Convection (Oven II) | 3 | 30 |
| Natural Convection (Oven II) | 4 | 30 |
| Natural Convection (Oven I) | 14 | 30 |
| ***TOTAL MANTLE PUNCHES (per Trial)*** | | ***180*** |

Table 2. Mean market squid dorsal mantle length (mm) and mean body weight (g) with standard deviations indicated.

| Trial | Mean Dorsal Mantle Length (DML, in mm) | Std Dev DML (mm) | Mean Body Weight (BW, in g) | Std Dev BW (g) |
|---|---|---|---|---|
| LP5660 | 109.07 | 7.57 | 24.97 | 5.05 |
| LP5664 | 109.77 | 6.15 | 24.52 | 4.31 |
| LP5668 | 108.87 | 10.00 | 25.17 | 5.80 |
| LP5672 | 106.53 | 8.90 | 23.73 | 5.36 |
| LP5676 | 111.63 | 6.99 | 25.80 | 5.31 |

Table 3. Results of ANOVA based on a randomized block design (**A**) and the Tukey's pairwise comparison test (**B**) for trial LP5660. Means with the same letter are not significantly different. Gray shading represents the treatment closest to that of oven I (56°C for 14 days).

**(A)**

| Source | DF | Type I SS | Mean Square | F Value | P |
|---|---|---|---|---|---|
| Squid-block | 29 | 0.01636599 | 0.00056434 | 22.35 | 0.0001 |
| Treatment | 5 | 0.00334029 | 0.00066806 | 26.46 | 0.0001 |

**(B)**

| Tukey Grouping | | Mean | N | Treatment |
|---|---|---|---|---|
| | A | 0.816819 | 30 | 4 |
| B | A | 0.814449 | 30 | 14 |
| B | A | 0.813734 | 30 | 3 |
| B | | 0.811593 | 30 | 1 |
| B | | 0.811395 | 30 | 2 |
| | C | 0.803128 | 30 | LP-1 |

Table 4. Results of ANOVA based on a randomized block design (**A**) and the Tukey's pairwise comparison test (**B**) for trial LP5664. Means with the same letter are not significantly different. Gray shading represents the treatment closest to that of oven I (56°C for 14 days).

**(A)**

| Source | DF | Type I SS | Mean Square | F Value | P |
|---|---|---|---|---|---|
| Squid-block | 29 | 0.01483327 | 0.00051149 | 14.31 | 0.0001 |
| Treatment | 5 | 0.00209278 | 0.00041856 | 11.71 | 0.0001 |

**(B)**

| Tukey Grouping | Mean | N | Treatment |
|---|---|---|---|
| A | 0.821126 | 30 | 4 |
| A | 0.820947 | 30 | 2 |
| A | 0.82088 | 30 | 3 |
| A | 0.81795 | 30 | 14 |
| A | 0.816891 | 30 | 1 |
| B | 0.811515 | 30 | LP-1 |

Table 5. Results of ANOVA based on a randomized block design (**A**) and the Tukey's pairwise comparison test (**B**) for trial LP5668. Means with the same letter are not significantly different. Gray shading represents the treatment closest to that of oven I (56°C for 14 days).

**(A)**

| Source | DF | Type I SS | Mean Square | F Value | P |
|---|---|---|---|---|---|
| Squid-block | 29 | 0.01462623 | 0.00050435 | 20.64 | 0.0001 |
| Treatment | 5 | 0.00450185 | 0.00090037 | 36.84 | 0.0001 |

**(B)**

| Tukey Grouping | Mean | N | Treatment |
|---|---|---|---|
| A | 0.823625 | 30 | 4 |
| A | 0.823222 | 30 | 3 |
| A | 0.822385 | 30 | 14 |
| A | 0.820613 | 30 | 1 |
| A | 0.820314 | 30 | 2 |
| B | 0.809023 | 30 | LP-1 |

Table 6. Results of ANOVA based on a randomized block design (**A**) and the Tukey's pairwise comparison test (**B**) for trial LP5672. Means with the same letter are not significantly different. Gray shading represents the treatment closest to that of oven I (56°C for 14 days).

**(A)**

| Source | DF | Type I SS | Mean Square | F Value | P |
|---|---|---|---|---|---|
| Squid-block | 29 | 0.03406436 | 0.00117463 | 12.02 | 0.0001 |
| Treatment | 5 | 0.00911517 | 0.00182303 | 18.66 | 0.0001 |

**(B)**

| Tukey Grouping | | Mean | N | Treatment |
|---|---|---|---|---|
| | A | 0.798461 | 30 | 4 |
| | A | 0.797855 | 30 | 3 |
| B | A | 0.794838 | 30 | 2 |
| B | A | 0.792152 | 30 | 14 |
| B | | 0.787768 | 30 | 1 |
| | C | 0.777734 | 30 | LP-1 |

Table 7. Results of ANOVA based on a randomized block design (**A**) and the Tukey's pairwise comparison test (**B**) for trial LP5676. Means with the same letter are not significantly different. Gray shading represents the treatment closest to that of oven I (56°C for 14 days).

**(A)**

| Source | DF | Type I SS | Mean Square | F Value | P |
|---|---|---|---|---|---|
| Squid-block | 29 | 0.01854328 | 0.00063942 | 19.35 | 0.0001 |
| Treatment | 5 | 0.00411123 | 0.00082225 | 24.88 | 0.0001 |

**(B)**

| Tukey Grouping | Mean | N | Treatment |
|---|---|---|---|
| A | 0.823001 | 30 | 4 |
| A | 0.822985 | 30 | 3 |
| A | 0.82151 | 30 | 2 |
| A | 0.820602 | 30 | 14 |
| A | 0.819437 | 30 | 1 |
| B | 0.809137 | 30 | LP-1 |

Table 8. Market squid sample sizes (N) by size bin.

| Size bin (DML, in mm) | Sample size (N) |
|---|---|
| 80-100 | 5 |
| 101-120 | 20 |
| 121-140 | 20 |
| 141-160 | 14 |
| 161-180 | 0 |
| ***TOTAL (all size bins)*** | ***59*** |

Table 9. Summary statistics for fresh gonad weight.

**SUMMARY OUTPUT**

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.98461893 |
| R Square | 0.96947443 |
| Adjusted R Square | 0.96893889 |
| Standard Error | 0.5970022 |
| Observations | 59 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 645.2073087 | 645.207 | 1810.29 | 6.94251E-45 |
| Residual | 57 | 20.31546287 | 0.35641 | | |
| Total | 58 | 665.5227715 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | -0.5185766 | 0.215047437 | -2.4115 | 0.01913 | -0.94920166 | -0.0879515 | -0.9492017 | -0.0879515 |
| X Variable 1 | 1.89801436 | 0.044609339 | 42.5475 | 6.9E-45 | 1.808685696 | 1.98734302 | 1.8086857 | 1.98734302 |

Table 10. Lower and upper prediction intervals are presented for a range of fresh gonad weights ($x_0$) and their corresponding predicted preserved weights (g).

| $x_0$ (g) | $y_{est}$ (g) | Lower prediction interval | Upper prediction interval |
|---|---|---|---|
| 1.606 | 2.530 | 1.297 | 3.762 |
| 2.500 | 4.226 | 3.008 | 5.445 |
| 3.000 | 5.175 | 3.962 | 6.388 |
| 3.500 | 6.124 | 4.916 | 7.333 |
| 4.000 | 7.073 | 5.867 | 8.280 |
| 4.500 | 8.022 | 6.817 | 9.228 |
| 5.000 | 8.971 | 7.765 | 10.178 |
| 5.500 | 9.920 | 8.711 | 11.129 |
| 6.000 | 10.869 | 9.656 | 12.082 |
| 6.500 | 11.818 | 10.600 | 13.037 |
| 7.000 | 12.767 | 11.541 | 13.994 |
| 7.500 | 13.716 | 12.481 | 14.951 |
| 8.000 | 14.665 | 13.420 | 15.911 |
| 8.300 | 15.235 | 13.982 | 16.487 |
| 8.450 | 15.520 | 14.263 | 16.776 |

FIGURES

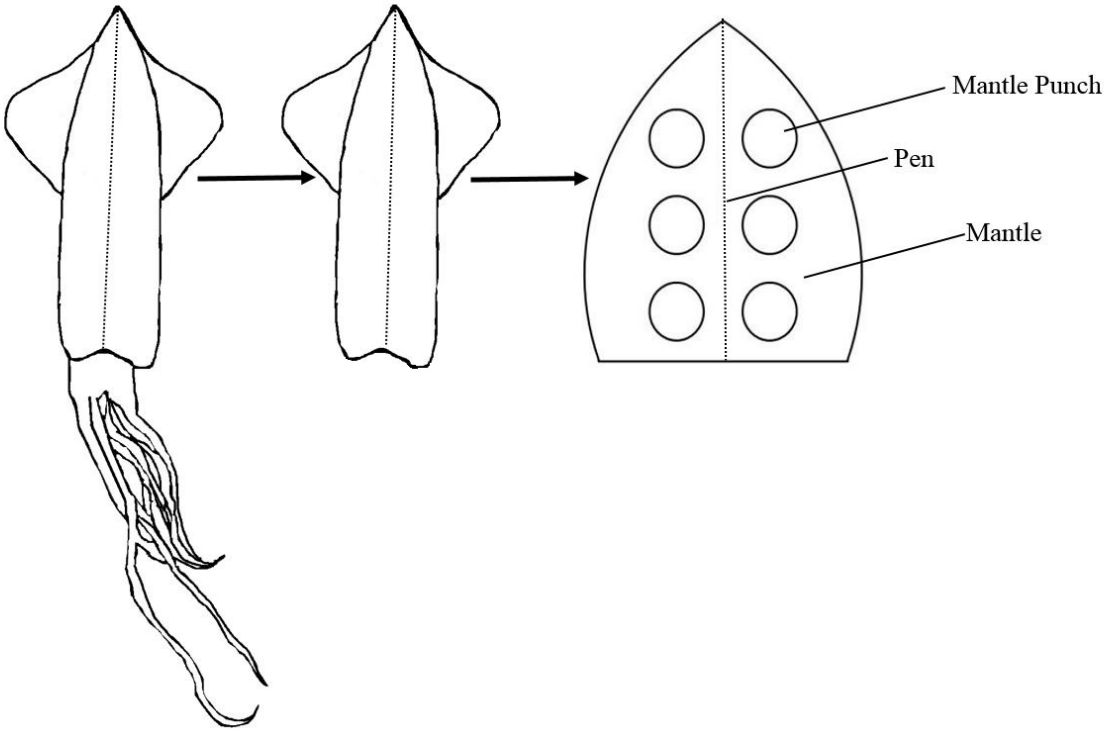


Figure 1. Schematic diagram showing the process for obtaining mantle punches from individual squid. First, the mantle is removed from a whole squid and cut open so that it lies flat. Next, the mantle tissue is cleaned and the outer dermis is removed. A cork borer (19.050 mm outer diameter) is used to punch six mantle discs per squid. Then, each mantle punch was randomly assigned to one of the six treatment groups, hence each squid was treated as a block in this study (see Table 1).

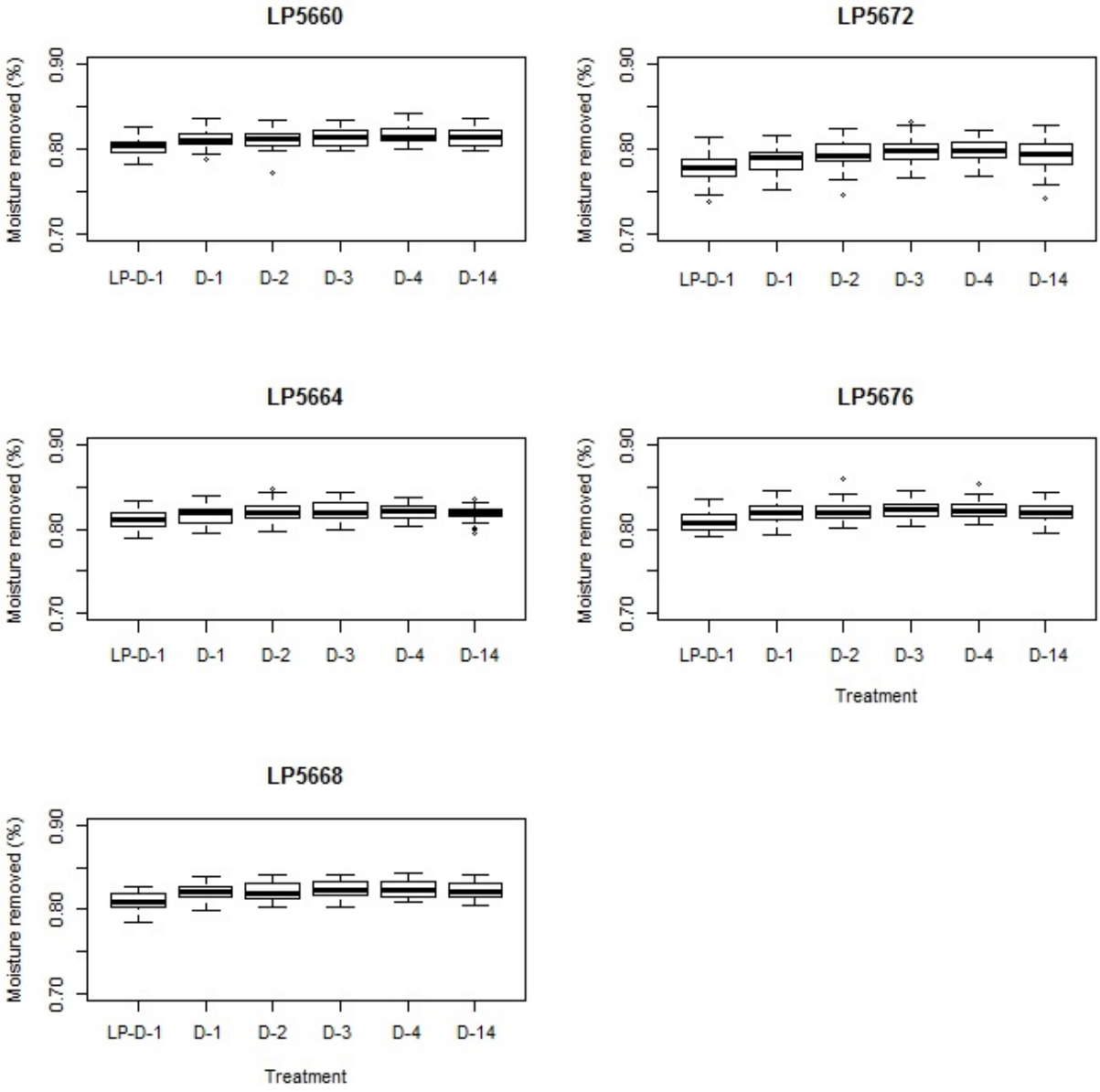


Figure 2. Box plots showing percent moisture removed from the mantle tissue per treatment and trial. Each box represents the interquartile range of values, with the bold horizontal line indicating the median percent moisture removed per treatment. The vertical lines capped by horizontal lines indicate values that are within a distance of 1.5 times the interquartile range from the upper and lower quartiles. Extreme values that are within 3 times the interquartile range are represented by dots.

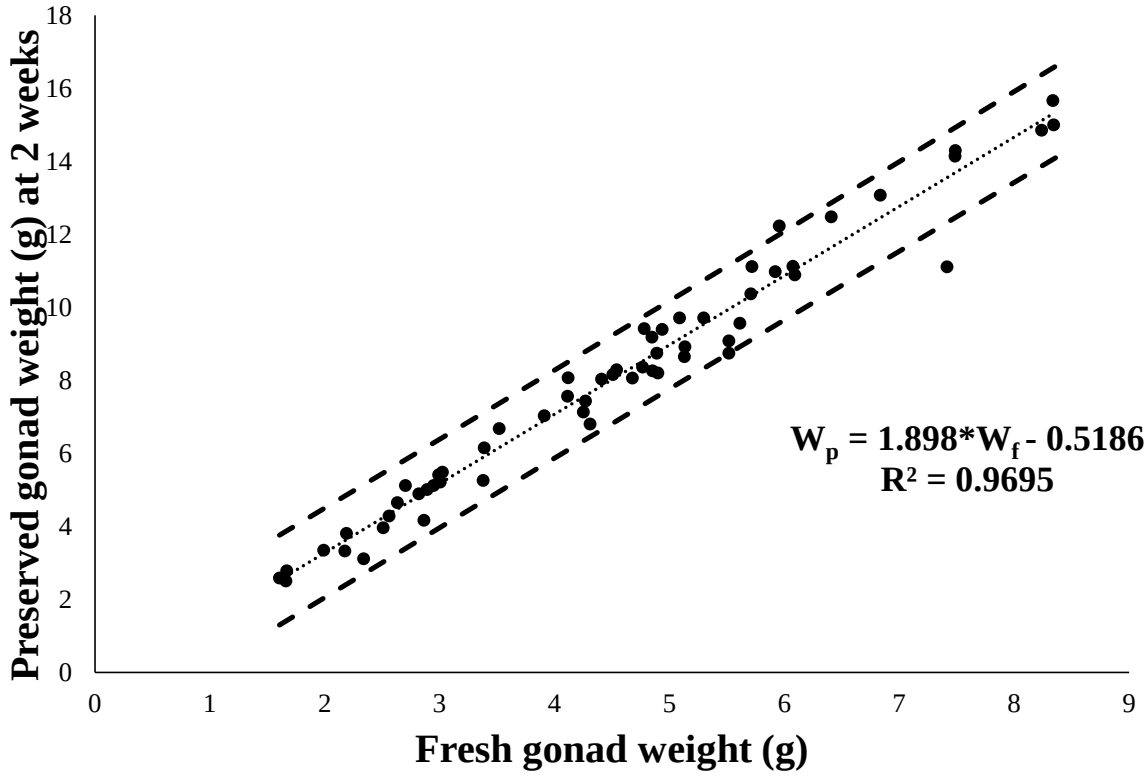


Figure 3. Relationship between fresh gonad weight (all size bins combined), ($W_f$, g) and preserved gonad weight ($W_p$, g) after two weeks of preservation in 10% neutral buffered formalin. The dashed lines represent 95% prediction intervals.