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RESAMPLING TECHNIQUES USED IN CALCULATING ESTIMATORS AND CONFIDENCE INTERVALS OF GASTRIC EVACUATION RATES.

by

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ABSTRACT

A nonlinear model was fitted by least-squares methods to wet weight and ash-free dry weight data from gastric evacuation experiments conducted on Atlantic cod (*Gadus morhua* L.). The regression model contained parameters for estimating the slope and shape of the digestion curve. Stomach contents samples were collected using a modified gastric lavage method that determined the amount of particulate material that passed through the collection screens.

Computer-intensive, bootstrap and jackknife techniques were used to resample the data to estimate variance in evacuation rates. Joint 95% confidence intervals were constructed for the model parameters in order to compare experimental effects such as prey type, meal size, and sample constituents. Results from different studies could also be compared by using these techniques.

INTRODUCTION

There has been a considerable amount of discussion what is the exact model that describes food evacuation in fishes. Several functions, ranging from linear to curvilinear, have been routinely fitted to gastric evacuation data: linear (Jones, 1974; Bagge, 1977; Bromley, 1991), exponential (Tyler, 1970; Elliott and Persson, 1978; Elliott, 1991), and square root (Jobling, 1981). Temming and Andersen (1992) suggested using a primary model that defines the depletion rate in stomach contents as dependent upon the amount of food present in the stomach.

$$\frac{dS}{dt} = -R \cdot S^{B} \qquad (1)$$

Where S = stomach contents.
t = time.
$-R$ = rate of depletion (slope of curve).
B = degree of curvilinearity (shape of curve).

Temming and Andersen's model incorporates all of these models by not pre-defining the shape of the curve but retaining it as a parameter:

- $B = 0.00$: linear model.
- $B = 0.50$: square-root model.
- $B = 0.67$: volume dependent model.
- $B = 1.00$: exponential model.

Integrating equation (1) over a specific time gives the following:

$$S_t = [\, S_0 - R \bullet (1 - B) \bullet t \,]^{\frac{1}{1-B}} \qquad (2)$$

Where S_0 = original meal size at time (0).
S_t = stomach contents at time (t).
t = post-prandial time.

Recently computer-intensive statistical techniques for estimating variance, namely the bootstrap (Efron, 1982, 1987) and jackknife (Miller, 1974; Duncan, 1978; Fox, et al., 1980), have become more prevalent in biological studies. Jackknife and bootstrap methods make no assumptions about the distributions of variables and in problems where it is difficult to measure variance directly, these techniques have been helpful. Meyer, et al. (1986) have used resampling methods to compare growth rates in cladoceran populations by determining the variance and reducing the bias in their estimates.

In this study, jackknife and bootstrap techniques were used in conjunction with the primary model to estimate rates and variances of gastric evacuation in Atlantic cod. The experimental factors investigated were prey type, meal size, and stomach content sample constituents.

METHODS

DATA

Gastric evacuation data were collected from experiments conducted on Atlantic cod at the University of Rhode Island, Graduate School of Oceanography Aquarium facilities during the summer of 1992. Atlantic cod ($\bar{x}$ = 1665 g; range = 972 – 3072 g; N = 45) were caught south of Cape Cod, Massachusetts, USA and were maintained at 10 ± 0.5°C in two 7000-L tanks (3 m in diameter). During experiments, individually tagged cod were voluntarily fed known meals of prawn (*Pandalus* sp.) or herring (*Clupea harengus*) consisting of 1, 2, or 3 prey items (Table 1). Three to five cod were sampled approximately every 2 hours for 24 hours. Stomach contents were collected on baskets with 500-μm nylon-mesh screen by a modified gastric lavage technique (Robertson, 1945; dos Santos, 1990).

Unlike other studies that have used gastric lavage, the filtrate water was collected to determine the amount of particulate material that passed through the screens. Filtrate water volume was measured and three sub-samples of water were filtered through glass-fiber filters (type A/E, Gelman, Inc.) capable of extracting 95% of material greater than 1 µm. Total particulate material was measured from these three sub-samples and extrapolated to the entire water sample. Various components of the stomach content samples were measured: wet weight >500 µm (WET), ash-free dry weight >500 µm (AFD), and total ash-free dry weight with particulate material <500 µm (TAFD).

STATISTICAL ANALYSES

Experimental data were cut off after 2 data points fell below 10% of the original meal size (Elashoff, 1982) and the resulting sets included 21 – 33 data points for each series (Table 1).

An IBM PC equipped with Statistical Analysis Systems (SAS, version 6.03) software for the PC was used to fit the model by non-linear least squares' methods that did not use derivatives (NLIN procedure, secant method, SAS, 1988).

For the jackknife samples each successive data point was removed and the parameter estimates were recalculated, resulting in N recalculations, each completed with N – 1 data points. Because –R and B were highly correlated and non-normally distributed, the estimates had to be transformed (Miller, 1974). The slope parameter, –R, was transformed by the exponential function transformed, while the shape parameter, B, was square-root transformed. The resulting N number of pairs of parameter estimates were multiplied by (N – 1) and subtracted from N times the transformed original estimate from the full sample:

$$\tilde{\Theta}_i = N\hat{\Theta} - (N-1)\hat{\Theta}_{-i} \quad (3)$$

Where i = (1, 2, 3, ... N) data point number.

$\tilde{\Theta}_i$ = i^{th} pseudo-value, transformed pair of parameter estimates, (B, -R).

$\hat{\Theta}$ = full sample estimates, transformed.

$\hat{\Theta}_{-i}$ = estimate with i^{th} data point removed, transformed.

This essentially reduces the bias (Efron, 1982) and creates "pseudo-values" whose average and variance was then further studied.

The jackknife estimator is the average of the pseudo-values,

$$\hat{\Theta}_J = \frac{1}{N}\sum_{i=1}^{N}\tilde{\Theta}_i \quad (4)$$

and the 95% joint confidence regions (Duncan, 1978) are defined by:

$$\{ \Theta : (\hat{\Theta}_J - \Theta)^T \mathbb{S}^{-1} (\hat{\Theta}_J - \Theta) \leq \frac{p}{N-p} F_{1-\alpha}(p, N-p) \} \tag{5}$$

Where $\hat{\Theta}_J$ = Jackknife estimators, transformed pair of parameter estimates.
$\mathbb{S}$ = variance-covariance matrix of transformed pseudo-values.
$F_{1-\alpha}$ = F value for N – p degrees of freedom.
p = 2, number of parameters in model.

For all data sets, bootstrap procedures consisted of randomly sampling N number of original data points (with replacement) N number of times. Each data point may be sampled more than once or not at all. Sampled data points were tallied and weight coefficients were calculated. These steps were repeated to create 250 coefficient vectors. The 250 x N matrix of weights was then merged with each original data set to create 250 bootstrap samples. Nonlinear fitting procedures generated pairs of parameter estimates (B, –R) from the merged bootstrap samples. These estimates were then transformed similar to the jackknife pseudo-values. Univariate statistics were performed on the final set of transformed estimates, now normally distributed.

The bootstrap estimator is the average of the bootstrap values,

$$\hat{\Theta}_B = \frac{1}{250} \sum_{b=1}^{250} \tilde{\Theta}_b \tag{6}$$

Where b = (1, 2, 3, ... 250) sample number.
$\tilde{\Theta}_b$ = bootstrap value, transformed pair of parameter estimates, (B, –R).
$\hat{\Theta}_B$ = Bootstrap estimator, transformed.

Because of the larger number of resampling points, the following equation (Duncan, 1978) was used to create joint 95% confidence intervals from the Means and variances of the bootstrap values.

$$\{ \Theta : (\hat{\Theta}_B - \Theta)^T \mathbb{S}^{-1} (\hat{\Theta}_B - \Theta) \leq \chi^2_{1-\alpha}(p) \} \tag{7}$$

Where $\hat{\Theta}_B$ = Bootstrap estimators, transformed pair of parameter estimates.
$\mathbb{S}$ = variance-covariance matrix of transformed bootstrap values.
$\chi^2_{1-\alpha}$ = chi squared value for p degrees of freedom.
p = 2, number of parameters in model.

Finally, bootstrap estimators were adjusted for bias by using the following equation (Meyer, et al., 1986):

$$\hat{\Theta}_{B(b.a.)} = 2\hat{\Theta} - \hat{\Theta}_B$$

Where $\hat{\Theta}_{B(b.a.)}$ = bias adjusted estimates.
$\hat{\Theta}$ = full sample estimates.
$\hat{\Theta}_B$ = Bootstrap estimator.

RESULTS

Parameter estimates from the jackknife and bootstrap procedures are summarized in Table 2. The jackknife and bootstrap values for the estimates of B and –R were very similar. The shape of herring prey evacuation (10 g) was approximately 1.0 (exponential model), while prawn evacuation varied from –0.181 to 0.815 depending upon what constituent was measured. Slopes ranged from –0.136 to –0.043 for the jackknife and –0.431 to –0.049 for the bootstrap.

The different herring meal sizes (10, 20, and 30 g) represented 0.7%, 1.2%, and 1.7% wet weight BW%, respectively (Table 1). The slope parameter was approximately the same for the different meals (TAFD weight basis, Table 2), while there was a trend for decreasing shape parameter with increasing meal size. The larger meal was less curvilinear (exponential) and possibly more volume dependent (B = 0.67).

Equations (5) and (7) delineate ellipses in the context of transformed parameters, but the 95% confidence regions become unsymmetrical and stretched shapes when B and –R are untransformed (Figures 1 and 2). Because of this, a comparison of regions is more descriptive than a comparison based upon statistics. All confidence regions were tilted in the same direction with higher shape parameters favored with more positive slopes. 95% confidence regions for wet weight samples extended well beyond valid values of –R and B, positive values and negative values, respectively, while AFD and TAFD confidence regions for both shrimp and herring prey were almost coincident.

DISCUSSION

Jackknife and bootstrap estimators were approximately the same. It appears that the jackknife and bootstrap methods worked equally well in defining gastric evacuation rates. There may be an advantage to the jackknife, because they the same N are required for both methods but the jackknife is less computer intensive.

Stomach contents weighed on a wet weight basis do not accurately estimate gastric evacuation (Hopkins and Larson, 1990). When compared to AFD weight, wet weight overestimates the slope while underestimating the shape parameter (prawn prey, figure 1A, 2A). When shrimp prey are digested, the percentage of water in the stomach contents increases, presumably to hydrolyse or break down chitin in the carapace and exoskeleton. The added water weight smooths a curvilinear function to make it appear more linear. If the amount of water is large enough, a lag phase or delay in the beginning of evacuation may result. The differences are not as dramatic for herring (Figures 1B, 2B).

On the other hand, the AFD weight of stomach contents collected on mesh screens does not accurately estimate gastric evacuation rate as compared to total AFD weight contents including material <500 µm (Figures 1A, 2A, and Table 2). A considerable amount of particulate material is consistently being lost in gastric lavage filtrate water. On the average this material represents only 5% of the original meal, but can reach up to 25%. The amount of material <500 µm is not constant over the whole digestive process. In the early stages of digestion this fraction does not contribute very much but by the end can significantly influence the evacuation curve. This would make a seemingly linear process more curvilinear, as seen with prawn prey (figures 1A, 2A). For herring prey the effects are not as great, although –R is overestimated and B is roughly the same.

Experimental procedures in previous gastric evacuation studies have not been standardized. Different components of stomach content samples have been used to estimate evacuation rates. Stomach contents retrieved by gastric lavage are usually screened to remove excess water, but the mesh size of the screens has varied from 200 µm to 1 mm. The fraction of material that is collected on the screens can represent different proportions of the original meal when comparing separate studies.

CONSUMPTION RATES

Previous consumption estimates were calculated with shape parameters of 0.27 – 0.47. If the gastric evacuation process is more exponential ($B \approx 1.0$), then the true consumption rates for cod populations are 3 – 5 times higher than those from Temming and Andersen.

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Table 1. Summary of conditions for Atlantic cod gastric evacuation experiments.

Temp.	Predator size. (±CI)	Prey type.	Meal size, wet wt. (±CI)	BW% wet wt. basis. (±CI)	N.
10°C	1928 ± 185 g	Prawn	10.1 ± 0.3 g	0.56% ± 0.07%	22
	1638 ± 164 g	Herring	10.8 ± 0.5 g	0.72% ± 0.08%	33
	1789 ± 208 g	Herring	20.1 ± 0.8 g	1.21% ± 0.14%	25
	1992 ± 247 g	Herring	30.4 ± 1.1 g	1.67% ± 0.25%	21

Table 2. Summary of results. Estimated slope and shape parameters from bias adjusted bootstrap and jackknife methods.

Prey type.	Meal size.	Sample constituents.	Parameters (B , –R) Jackknife estimate.	Bootstrap estimate.
Prawn	10 g	WET	(0.004 , –0.136)	(–0.181 , –0.431)
Prawn	10 g	AFD	(0.690 , –0.114)	(0.682 , –0.113)
Prawn	10 g	TAFD	(0.846 , –0.087)	(0.815 , –0.088)
Herring	10 g	WET	(1.073 , –0.043)	(1.069 , –0.055)
Herring	10 g	AFD	(1.216 , –0.089)	(1.258 , –0.090)
Herring	10 g	TAFD	(1.235 , –0.070)	(1.237 , –0.071)
Herring	20 g	TAFD	(1.425 , –0.045)	(1.180 , –0.049)
Herring	30 g	TAFD	(0.698 , –0.059)	(0.610 , –0.072)

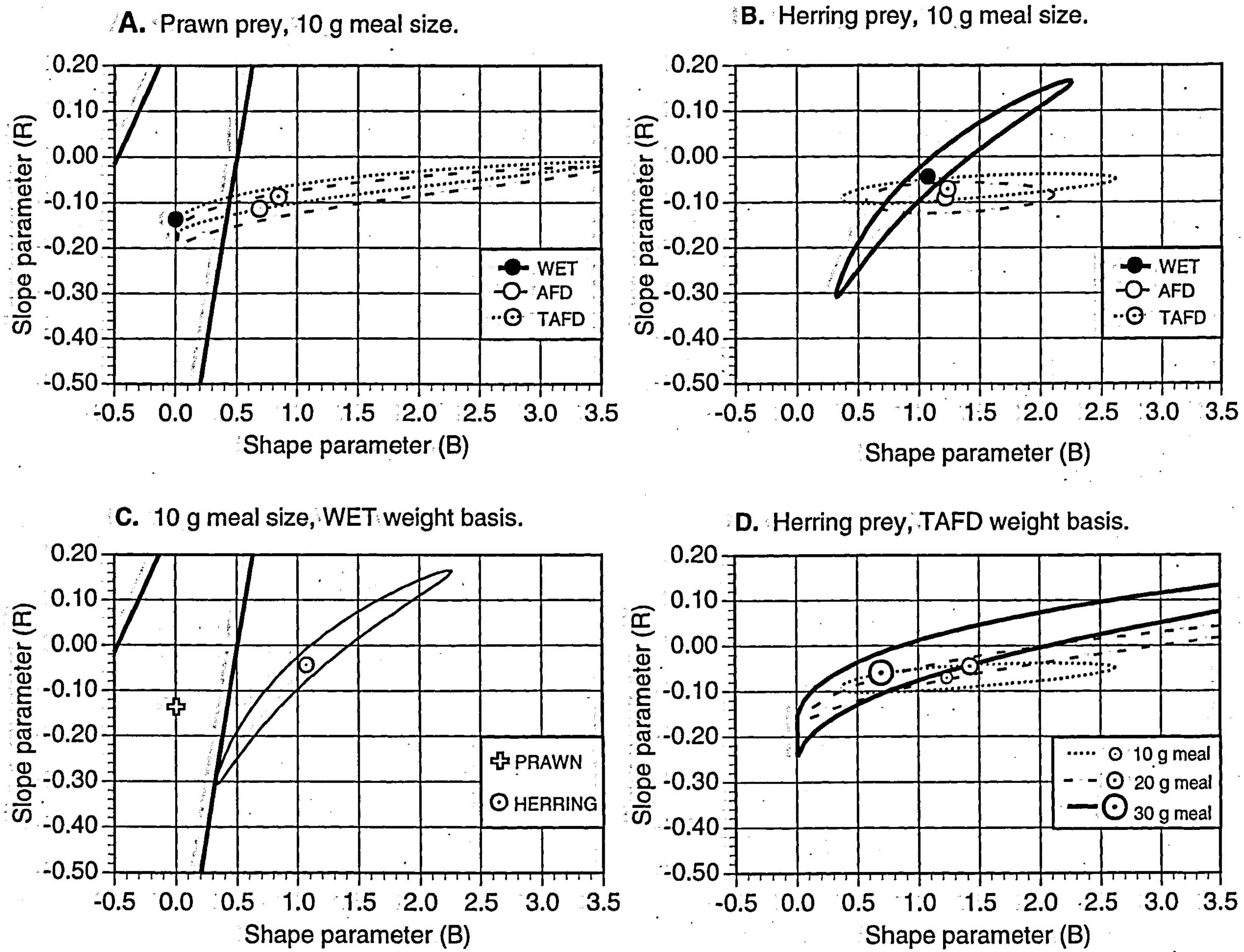

Figure 1. Jackknife estimators and joint 95% confidence intervals for gastric evacuation rates in Atlantic cod. A) Comparison of sample consituents, prawn prey; B) Comparison of sample consituents, herring prey; C) Comparison of prawn and herring prey on a WET weight basis; D) Comparison of meal size, herring prey on a TAFD weight basis.

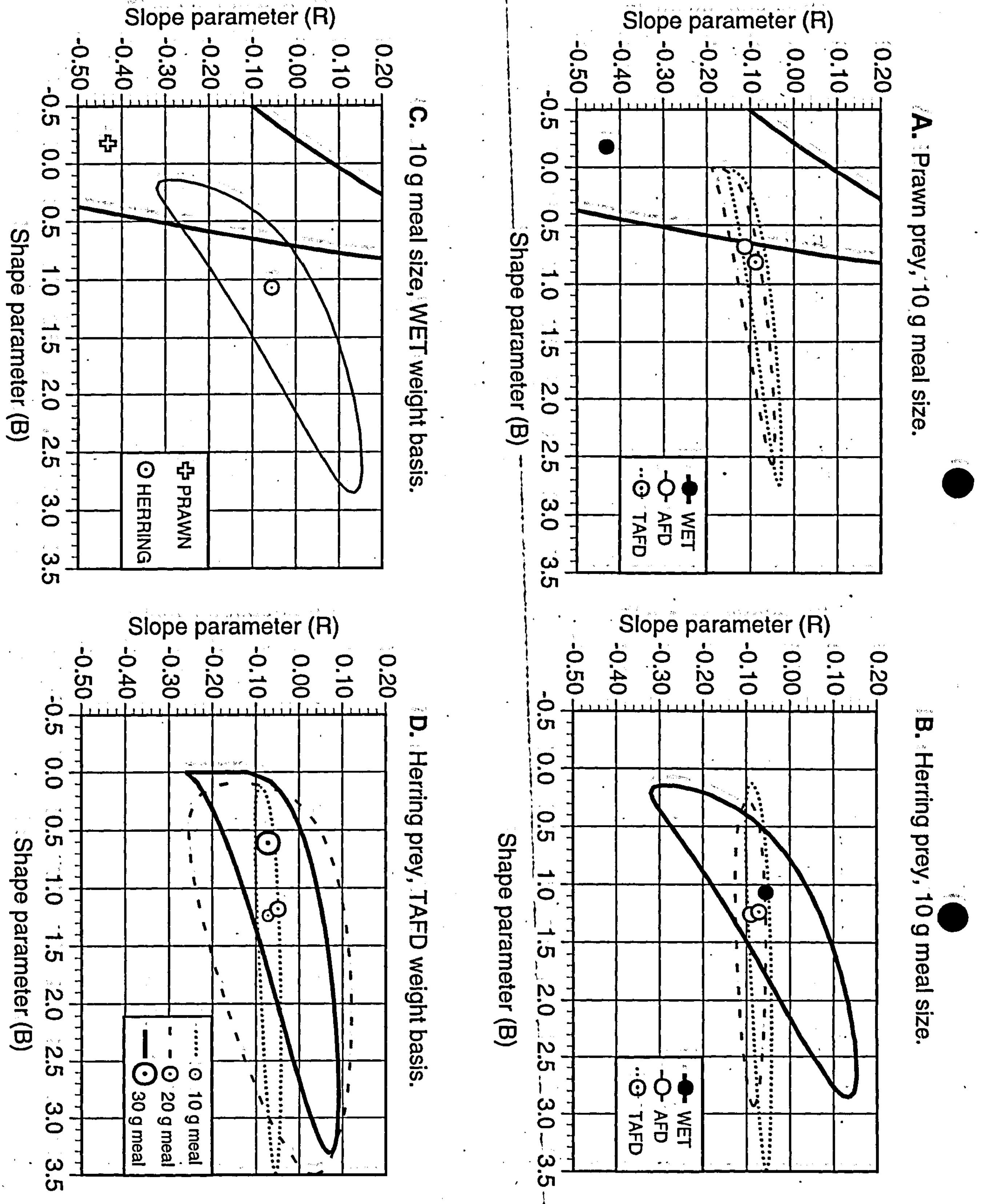

Figure 2. Bootstrap estimators and joint 95% confidence intervals for gastric evacuation rates in Atlantic cod. A) Comparison of sample consituents, prawn prey; B) Comparison of sample consituents, herring prey; C) Comparison of prawn and herring prey on a WET weight basis; D) Comparison of meal size, herring prey on a TAFD weight basis.