

## Acoustic ontogeny of a teleost

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The influence of growth on the intensity and variability of acoustic echoes from individuals and groups of a teleosts was quantified using Donaldson trout (rainbow–steelhead hybrid) *Oncorhynchus mykiss*. Fish growth was linear in total length ( $L_T$ ) and quadratic in mass. Dorsal swimbladder area increased exponentially with  $L_T$ . Allometric growth ratio (*i.e.*  $k$ ) values of swimbladder length linearly increased with  $L_T$ . Average swimbladder volumes occupied 3–6% of fish body volume and increased exponentially with  $L_T$ . The aspect angle that resulted in the maximum average acoustic intensity from the group shifted from 80 to 86° through the experimental period. Mean echo intensities increased at both 38 and 120 kHz as mean  $L_T$  increased. Predicted echo intensities at 38 kHz exceeded that at 120 kHz at  $L_T < 150$  mm but were less than that predicted at 120 kHz at  $L_T > 280$  mm. Generalized additive mixed models using  $L_T$ , swimbladder angles and lateral elongation ratios of fish bodies were better predictors of echo intensities than  $L_T$  alone.

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Key words: backscatter model; Donaldson trout; swimbladder; target strength.

### INTRODUCTION

The growth history and development of individuals, known as ontogeny, are non-linear and indeterminate over the life span of a fish (Barton, 1996). If ontogeny of the swimbladder and body are non-linear (*i.e.* allometric), then age-dependent changes in shapes and sizes of body components are important when estimating fish lengths and densities from acoustic data. McClatchie *et al.* (1996, 2003) identified the need to examine how allometric growth in swimbladder length, cross-section and overall fish length affect echo intensities (*i.e.* target strengths) of swimbladdered fishes. Swimbladders are especially important since they reflect a minimum of 90% of the acoustic energy from individual fishes (Haslett, 1962; Foote, 1980a) and vary in shape and size within (Foote, 1985) and among (Whitehead & Blaxter, 1964) fish species. Conversion of reflected energy (*i.e.* backscatter) to fish length is based on species-specific, empirical relationships between backscatter from isolated targets and known fish lengths. Backscatter and fish length data can be measured directly in controlled

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conditions (McCartney & Stubbs, 1971; Nakken & Olsen, 1977) or indirectly by combining field backscatter measurements from individual fish (*i.e. in situ*) with coincident length samples from directed net hauls (Foote & Traynor, 1988; Cordue *et al.*, 2001).

Accuracy and precision of backscatter to fish length conversions are dependent, in part, on the samples used in the regression equation. Target strength (TS) to fish length relationships may be based on samples that encompass a limited length range (Williamson & Traynor, 1984), include a mixture of several cohorts and are assumed to be normally distributed. Caution must be used when converting TS values to fish lengths from samples that were not used in the formulation of the original regression equation (Horne, 2003). A single regression relationship between TS and fish length may not be appropriate for a population containing both small and large fish. Precision of TS to fish length relationships derived using indirect methods (*i.e. in situ* measurements) may be further reduced when multimodal TS distributions are matched to unimodal length frequency distributions (Williamson & Traynor, 1984). In this case, it is not known if the net was selectively catching large fish or if single targets were reflecting multiple echoes.

Potential ontogenetic differences among life-history stages and among individuals suggest the need to quantify changes in swimbladder and body shapes. From an acoustic perspective, changes in swimbladder size and shape may increase variability in backscatter from groups of animals, may change TS to fish length relationships over specified length ranges and may ultimately decrease accuracy of acoustic-based abundance estimates. This study examines effects of growth on the acoustic characteristics of individuals within a group of teleosts.

## MATERIALS AND METHODS

Thirty-five Donaldson trout *Oncorhynchus mykiss* (Walbaum), a rainbow–steelhead hybrid known for their rapid growth, were obtained from and maintained at the University of Washington, School of Aquatic and Fishery Sciences Fish Hatchery in Seattle, Washington, U.S.A., from October 2001 to May 2002. Fish were kept in small raceways until overcrowding necessitated a change to larger holding tanks. To stimulate growth during winter, heated water was added to holding tanks from January to the end of February 2002. Lake Washington water was used from March until the end of the experiment. Fish were regularly fed to repletion using Moore-Clark Nutra Fry (1.2 mm to 4.0 mm; Vancouver, British Columbia, Canada) and when larger, Pedigree Salmon Brood (5.0–12 mm; Moore-Clark) fish pellets except for 2 days prior to each radiography session. The cessation of feeding minimized undigested food in the stomach to provide an index of maximum potential backscatter. Feeding was resumed immediately after each measurement session. To ensure identification of individuals during the study, each fish was injected with a passive integrated-transponder (PIT) tag (Unimark, 2 × 12 mm; Boise, ID, U.S.A.). Fish were observed to recovery quickly from tagging. Lower *et al.* (2005) showed that cortisol levels in fishes implanted with PIT tags returned to pre-stress levels within 12 h.

Sixteen rounds of dorsal and lateral radiographs were taken during the 8 month grow-out period. Fish were individually netted and then transferred in small batches from the raceway or holding tank to an anaesthetic bath containing 50–60 ppm clove oil (eugenol) solution. The number of fish anaesthetized in each batch was dependent on the number of fish that could be imaged on a single piece of radiographic film. The fish

were left in the anaesthetic bath until they had reached stage four anaesthesia (*i.e.* total loss of muscle tone and equilibrium, slow but regular opercular pumping and loss of spinal reflexes; Summerfelt & Smith, 1990). Fish were transferred to radiograph film holders or rare-earth cassettes and radiographs were taken in both lateral and dorsal planes (XTEC 90P Laseray plus; Columbia City, IN, U.S.A.). Exposure settings varied among sessions, with intensity (kVp) and time increasing as the fish size increased (Table I). Following the exposures, fish were transferred to individual aerated recovery tanks.

Total length ( $L_T$ ) and mass ( $M$ ) were monitored throughout the experimental grow-out period. Each fish was briefly removed from its recovery tank to measure  $L_T$  and  $M$ , and to scan and record the PIT tag number. The  $L_T$  and  $M$  were tabulated to track changes in individual and group growth and to calculate a  $L_T$  and  $M$  relationship for the group during the experiment.

To track changes in two-dimensional shape, swimbladder and fish body areas were measured using digital images created from dorsal and lateral radiographs. Relative growth was indexed using lengths of major and minor swimbladder and fish-body axes to calculate elongation values (*i.e.* minor and major axis). An elongation value of one indicates a circle. As elongation values decrease, the organ is 'stretched' along its sagittal axis. Rates of change in length were compared using allometric growth ratios. Allometric growth ratios ( $k$ ) are slopes of log-log regressions used to compare the relative growth of any two body components, which may include overall length (Huxley, 1932). If the value of  $k$  equals one, then both components are growing at the same rate. If  $k$  does not equal one, then one component is growing faster than the other. Change in swimbladder development was also monitored by measuring the angle between the

TABLE I. Dates, media type and settings used for *Oncorhynchus mykiss* radiographs with the XTEC Laseray 90P

Date	Media type	Intensity (kVp)	Time (s)	Number of fish
22 October 2001	Redipak	80	2:00	35
29 October 2001	Redipak	80	2:00	34
19 November 2001	Redipak	90	2:00	32
03 December 2001	Redipak	90	2:00	31
17 December 2001	Redipak	90	2:00	31
04 January 2002	Redipak	90	2:00	31
14 January 2002	Redipak	90	2:00	30
28 January 2002	Redipak	90	2:00	30
7 February 2002	Redipak	95	2:00	29
25 February 2002	Redipak	95	2:00	29
11 March 2002	Redipak	95	2:00	28
	Cassette	60	0:12	
1 April 2002	Redipak	95	2:00	28
	Cassette	70	0:18	
15 April 2002	Redipak	95	2:00	28
	Cassette	70	0:18	
29 April 2002	Redipak	95	2:00	27
	Cassette	70	0:18	
15 May 2002	Redipak	95	2:00	26
	Cassette	70	0:18	
28 May 2002	Redipak	95	2:00	24
	Cassette	70	0:18	

medial axis of the swimbladder and the sagittal axis of the fish body using the lateral radiograph from each measurement period.

Changes in three-dimensional shape were monitored using swimbladder and fish body volumetric measurements derived from lateral and dorsal radiographs. Paired, digitized outlines of the swimbladder and fish body from each measurement period were elliptically interpolated at 45° increments to create three-dimensional wireframes with 1 mm resolution. Both the fish body and swimbladder are represented by sets of contiguous 1 mm cylinders. Body component volumes were estimated by summing the area of each contiguous cylinder.

To quantify effects of individual growth trajectories on acoustic backscatter, dorsal and lateral radiographs were used to estimate TS values of each fish at each radiograph session. The TS values were calculated using a Kirchhoff-ray mode (KRM) model parameterized for *O. mykiss* in fresh water (Table II). The body is represented by a set of contiguous fluid-filled cylinders surrounding a set of contiguous gas-filled cylinders that represent the swimbladder. Backscatter from each cylinder in the body and the swimbladder is estimated and then added coherently to estimate total backscatter as a function of fish caudal length ( $L_C$ , m) and acoustic wavelength ( $\lambda$ , m). The acoustic wavelength is a function of the speed of sound in water ( $c$ ,  $\text{ms}^{-1}$ ) and the transmitting frequency ( $f$ , Hz) (*i.e.*  $\lambda = c f^{-1}$ ). Full details of the model can be found in Clay & Horne (1994), Jech *et al.* (1995) and Horne & Jech (1999). The  $L_C$  used in backscatter model predictions were converted to  $L_T$  using a regression developed from the experimental fish:  $L_T = 1.093 L_C + 2.73$  ( $r^2 = 0.9938$ ,  $n = 384$ ). To examine the effect of linearly scaling a fish in all dimensions when estimating TS values over a range of lengths, digital images from radiographs from 15 of 16 measurement periods were used to estimate TS of a single fish over a  $L_T$  range of 120–320 mm at 38 and 120 kHz. The TS values for each fish were also estimated at the average  $L_T$  at each measurement period at 38 and 120 kHz. All mean and s.d. were calculated in the linear domain before being transformed to  $\log_{10}$  TS values.

To examine if contributions to acoustic reflectivity from factors other than  $L_T$  are significant, generalized additive mixed models (GAMM: Lin & Zhang, 1999; Fahrmeir & Lang, 2001; Wood, 2006a) were constructed using the multi-general cross-validation (mgcv, version 1.3-27) package in R (R Development Core Team, 2007) to predict TS at 38 and 120 kHz using individual fish as the group variable. A GAMM is a non-parametric regression that contains multiple measures on a group of individuals but inferences can be made on the population if smooth terms are used as random effects. Covariates considered for inclusion in GAMM models included:  $L_T$ , swimbladder tilt angle, and dorsal and lateral elongation ratios of the fish body. All models were fitted using the Gaussian family and an identity link function. No assumptions of linearity were made for the covariates so each predictor variable was initially represented as a smooth function. A full GAMM was initially fitted for each frequency. Non-significant terms were removed and the model was refitted. An ANOVA was used to compare the fit of the full and reduced models. The best GAMM at each frequency was determined using two criteria: the highest  $r^2$  value and the lowest Akaike information criterion (AIC) value (Akaike, 1974). The final GAMM model was compared to a GAMM TS model using  $L_T$  as the only covariate.

TABLE II. Sound speeds and densities of fish bodies swimbladders and water used in Kirchhoff-ray mode backscatter models

Component	Sound speed ( $\text{m s}^{-1}$ )	Density ( $\text{kg m}^{-3}$ )
Fish body	1570	1080
Swimbladder	340	2.64
Water	1490	1000

## RESULTS

When not being radiographed, fish were held in raceways or tanks at the University of Washington fish hatchery for a total of 218 days from late October 2001 until late May 2002 (Table I). Water temperatures in holding tanks (Fig. 1) paralleled the annual cycle of near-surface temperatures in Lake Washington except for a 2 month period (January to February) when heated water was added to enhance fish growth. Water temperatures rapidly decreased from a high of near 17° C in October to a low of 9° C by mid-December. Water temperature slowly increased and reached 15° C by the following June.

Twenty-four of the 35 fish that were initially PIT tagged survived through 16 rounds of radiographs (Table I). Data from fish that did not survive the 16 rounds of radiographs were excluded from all analyses. PIT tags remained in gut cavities for the duration of the experiment. Both PIT tags and swimbladders were visible in dorsal and lateral radiographs as each fish grew (Fig. 2). To provide a baseline example of individual growth, a random fish (PIT tag: 023 317 030) (Fig. 2) was selected and used as an example for subsequent figures. Swimbladders of all fish remained inflated for the duration of the experiment.

Despite the seasonal cooling and warming of water in holding tanks,  $L_T$  increased at a near constant rate throughout the duration of the experiment. This pattern was consistent for each individual and for the group (Fig. 3). Average  $L_T$  increased almost 1.37 mm day<sup>-1</sup> ( $L_T = 109.64 + 1.37x - 0.001x^2$ , where  $x = \text{day}$ ;  $n = 16$ ,  $r^2 = 0.997$ ). The growth trajectory of the example fish was below the mean of the group. Overall, average  $L_T$  increased 256 mm from the start (107 mm) to the final (363 mm) round of radiographs. Variance in  $L_T$  among individuals increased with the mean  $L_T$  of the group. Growth rates averaged 1.18 mm day<sup>-1</sup> over the 218 day measurement period.

Individual and group  $M$  also increased during the experiment. A polynomial regression fit to the 24 surviving fish (Fig. 4) predicts a daily increase in  $M$  of

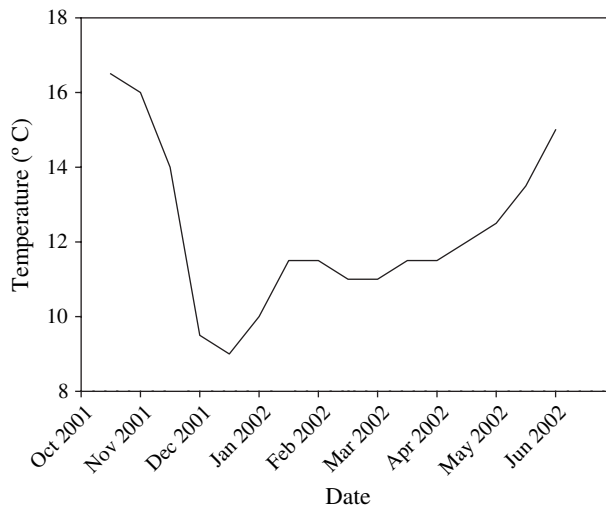


FIG. 1. Water temperature in University of Washington fish hatchery holding tanks during the experiment.

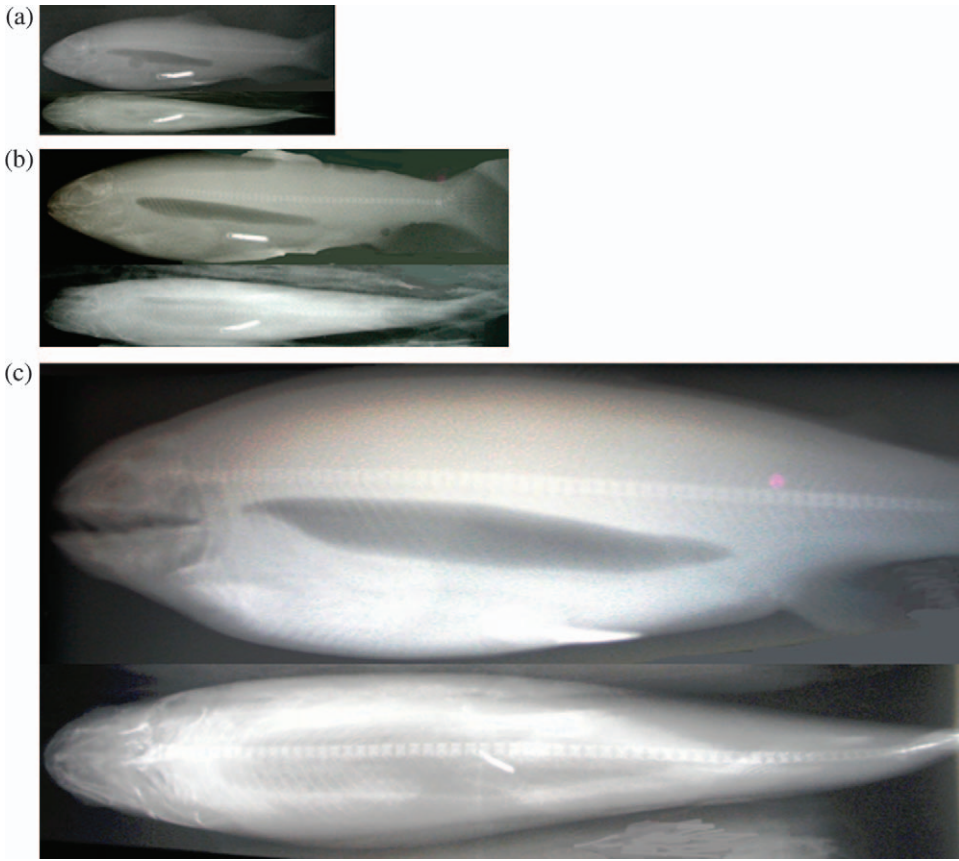


FIG. 2. *Oncorhynchus mykiss* lateral (upper) and dorsal (lower) radiographs taken from the same fish at (a) round 2 (of 16 rounds) of radiographs – 106.5 mm total length ( $L_T$ ), (b) round 5 – 170 mm  $L_T$  and (c) round 15 – 333 mm  $L_T$ . The swimbladder is the dark organ below the spinal column. The passive integrated transponder (PIT) tag is visible below the swimbladder.

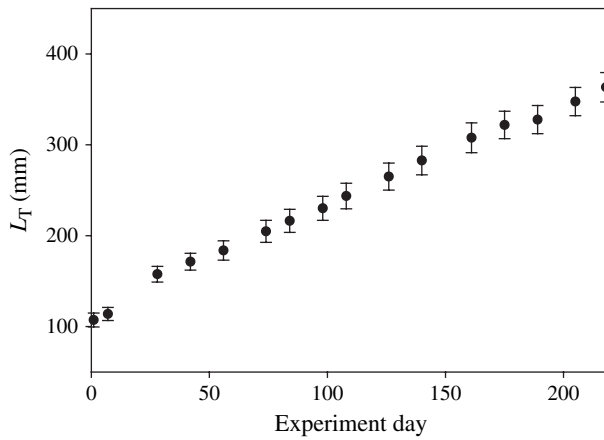


FIG. 3. Mean  $\pm$  s.d. total length ( $L_T$ ) of *Oncorhynchus mykiss* at each measurement period.

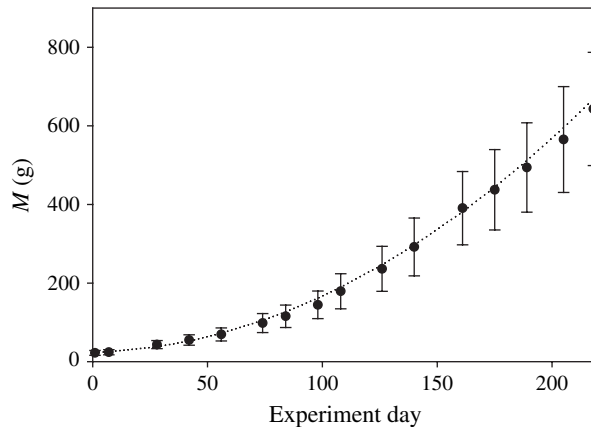


FIG. 4. Mean  $\pm$  s.d. mass ( $M$ ) of *Oncorhynchus mykiss* at each measurement period. The curve was fitted by a polynomial regression equation [shown for the group growth trajectory ( $\cdots$ ):  $y = 23.38 + 0.15x + 0.001x^2$  ( $n = 16$ ,  $r^2 = 0.998$ )].

$0.15 \text{ g g}^{-1} \text{ day}^{-1}$  and an average  $M$  gain of  $2.95 \text{ g day}^{-1}$  over the experimental period. The  $M$  gain in the example fish initially matched that of the group until the day 28 and was less than average during the remainder of the grow out period. Using average  $L_T$  and  $M$  from 16 rounds of radiographs (Fig. 5), the  $\ln$  relationship between mean  $L_T$  and  $M$  indicated that  $M$  scaled approximately with the volume of the fish (*i.e.*  $L_T^3$ ).

Dorsal swimbladder surface areas of each individual and the group increased over time. Areas varied among individuals and became more variable overall as fish grew [Fig. 6(a)]. Dorsal swimbladder area variability as a function of  $L_T$ , measured using coefficients of variation (c.v.), differed by a factor of two and was greatest from experimental days 50 to 140 [Fig. 6(b)]. A quadratic regression was used to characterize changes in mean dorsal swimbladder surface areas of the group over the experimental period [Fig. 6(b)]. Shapes of

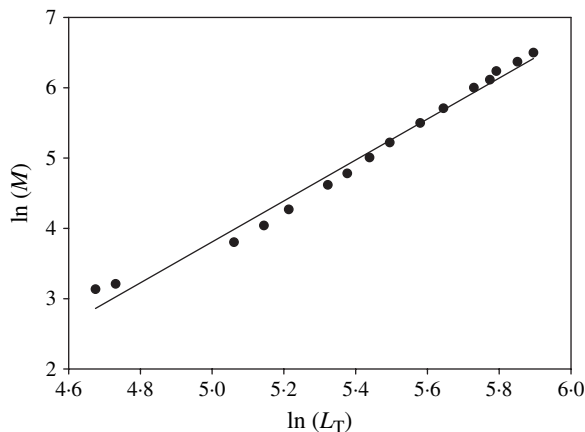


FIG. 5. Mean  $\ln$  total length ( $L_T$ ) and mass relationship for 24 *Oncorhynchus mykiss* over the 218 day experimental period. The curve was fitted by:  $y = -10.75 + 2.91x$  ( $n = 16$ ,  $r^2 = 0.98$ ).

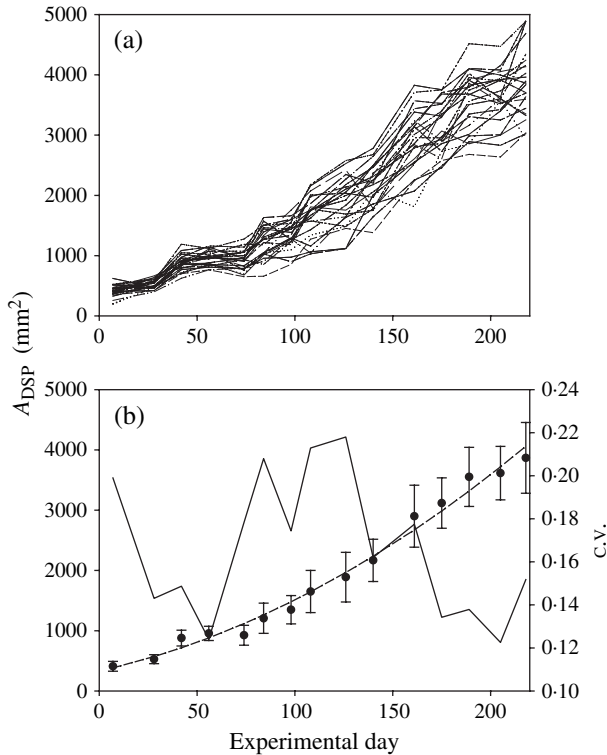


FIG. 6. Dorsal swimbladder surface area ( $A_{DSB}$ ) of (a) 24 *Oncorhynchus mykiss* obtained from radiographs and (b) mean  $\pm$  s.d.  $A_{DSB}$  of the group with the corresponding coefficients of variation (c.v., —) and quadratic regression curve:  $y = 328.360 + 7.330x + 0.045x^2$  ( $n = 16$ , adjusted  $r^2 = 0.984$ ) (----), over the 218 day experimental period.

swimbladders and fish bodies varied among individual *O. mykiss* at each measurement session and through the experimental period. Average swimbladder lateral elongation ratio values were consistently less than but close to dorsal values (Fig. 7). Lateral swimbladder elongation values remained constant after the fifth round of radiographs (*i.e.* experimental day 56). Dorsal swimbladder elongation values tended to decrease as swimbladders grew. A small increase in mean dorsal swimbladder elongation values was observed between measurement rounds 11 and 12. All elongation values were  $<1$ , indicating that growth in length exceeded that in width. In contrast, average fish-body lateral elongation values exceeded dorsal values (Fig. 7). The spread between the dorsal and lateral body ratios was greater than that observed among dorsal and lateral swimbladder ratio values. Dorsal elongation values remained constant across all experimental measurements. The traditional cylindrical fish shape was maintained in fish body and swimbladder dorsal planes with minor axes being *c.*  $<20\%$  the length of major axes throughout the experiment. Average swimbladder length increased with  $L_T$  [Fig. 8(a)]. The allometric growth ratio of swimbladder to  $L_T$  based on all individual fish measurements [Fig. 8(b)] was 1.187 (c.v. = 0.686,  $n = 358$ ,  $r^2 = 0.984$ ,  $P < 0.001$ ), indicating that swimbladders were growing 19% faster on average than  $L_T$ . To characterize the rate of change in swimbladder shape



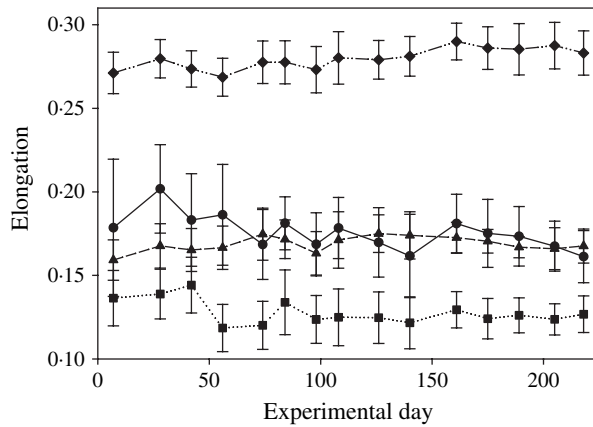


FIG. 7. Mean  $\pm$  s.d. elongation values of 24 *Oncorhynchus mykiss* at each measurement period for the dorsal swimbladder ( $\bullet$ ), lateral swimbladder ( $\blacksquare$ ), dorsal fish body ( $\blacktriangle$ ) and lateral fish body ( $\blacklozenge$ ).

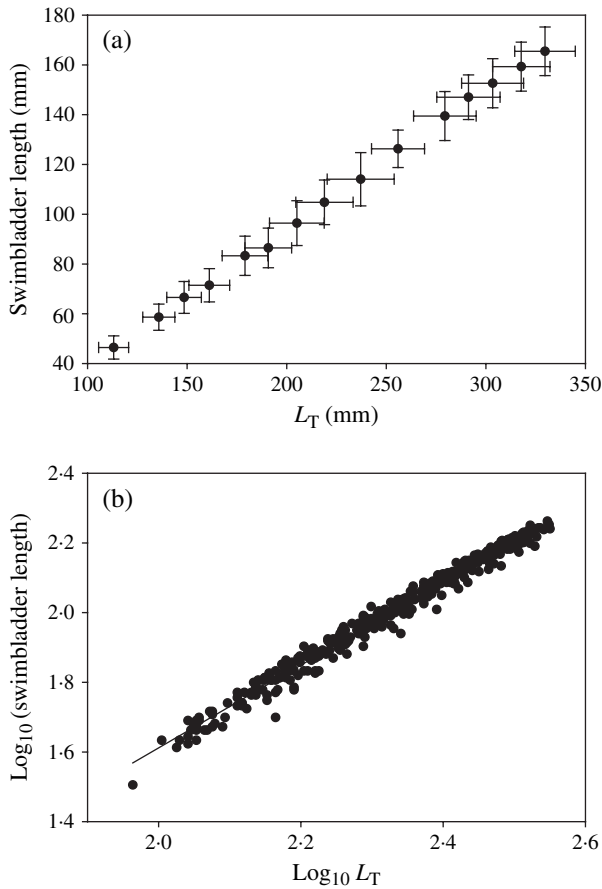


FIG. 8. (a) Group mean  $\pm$  s.d. of *Oncorhynchus mykiss* swimbladder length plotted as a function of fish total length ( $L_T$ ) at each measurement period and (b) allometric growth ratio of swimbladder to fish body,  $k = 1.187$  ( $r^2 = 0.984$ ,  $P < 0.001$ ,  $n = 358$ ).

relative to the rate of change in  $L_T$ , allometric growth ratios were calculated using dorsal and lateral swimbladder elongation ratios relative to  $L_T$ . In both cases,  $L_T$  increased faster than changes in elongation values (lateral swimbladder elongation:  $-0.094$ ,  $r^2 = 0.058$ ,  $P < 0.001$ ,  $n = 358$ ; dorsal swimbladder elongation:  $-0.117$ ,  $r^2 = 0.057$ ,  $P < 0.001$ ,  $n = 358$ ). The negative, low values indicate that swimbladders became more cylindrical as  $L_T$  increased.

Swimbladder angles also changed through the course of the experiment. Swimbladder angles for most fish generally increased at each successive measurement [Fig. 9(a)]. This trend was reflected in the group mean [Fig. 9(b)], with a shift in mean swimbladder angle from  $80$  to  $86^\circ$  between the start and the end of the experiment. A non-significant decrease ( $0.67^\circ$ ) in mean swimbladder angle occurred between the 10th and the 11th round of measurements at experimental day 140 when mean  $L_T$  was 283 mm.

Swimbladder volumes increased exponentially through the experiment [Fig. 10(a)]. Variance among individuals increased in proportion to the mean swimbladder volume [Fig. 10(b)]. Mean volumes increased  $>27$ -fold from

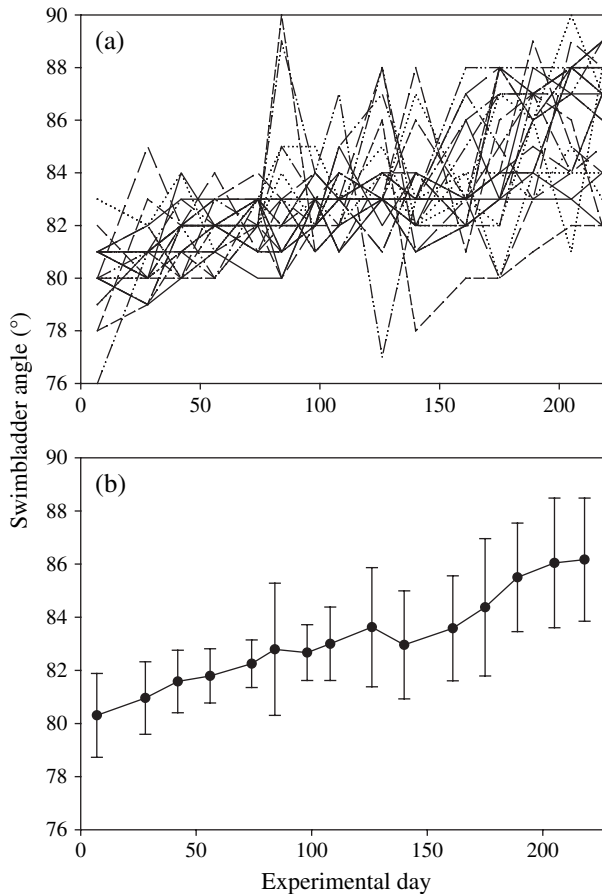


FIG. 9. Angle of swimbladder relative to *Oncorhynchus mykiss* sagittal axis for (a) all fish and (b) the mean  $\pm$  s.d. of the group at each measurement period.

1206 mm<sup>3</sup> at day 7 to 32 971 mm<sup>3</sup> at the end of the experiment (day 218). Despite variation among individuals, a ‘jump’ in mean swimbladder volumes occurred between measurement rounds 11 and 12. Ratios of individual swimbladder to fish body volumes tended to increase but did not exceed 8% over the experimental period [Fig. 11(a)]. Mean ratio values fluctuated between 0.045 and 0.055 with large variability at each measurement period [Fig. 11(b)]. Swimbladder growth was rapid relative to fish-body growth over the first three measurement periods. Consistent with changes in swimbladder volumes, an increase in swimbladder to fish-body volume ratio value was observed between the 11th and the 12th measurement rounds.

Predicted TS of the example fish at any  $L_T$  differed depending on the initial  $L_T$  of the fish used to estimate acoustic reflectivity. If growth of an individual fish was linear in all dimensions, then TS at any  $L_T$  from all measurement rounds were predicted to be equal. To illustrate how ontogeny affects acoustic reflectivity, TS were predicted at 38 [Fig. 12(a)] and 120 kHz [Fig. 12(b)] at a mm resolution for 14 radiograph sessions over a  $L_T$  range of 120–320 mm. For each set of backscatter predictions, fish bodies and swimbladders were proportionately scaled in all dimensions across the  $L_T$  range. The predicted TS at

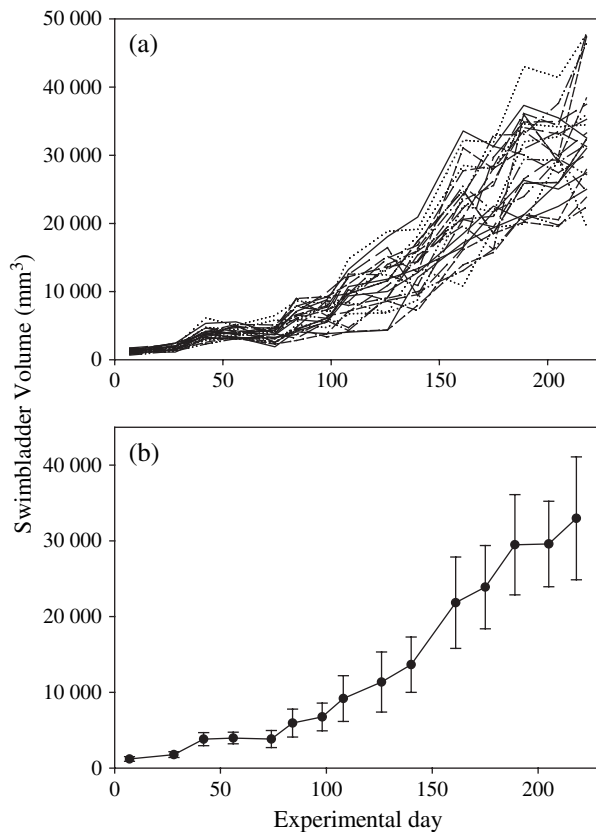


FIG. 10. *Oncorhynchus mykiss* swimbladder volumes of (a) each fish and (b) the mean  $\pm$  s.d. of the group at each measurement period.

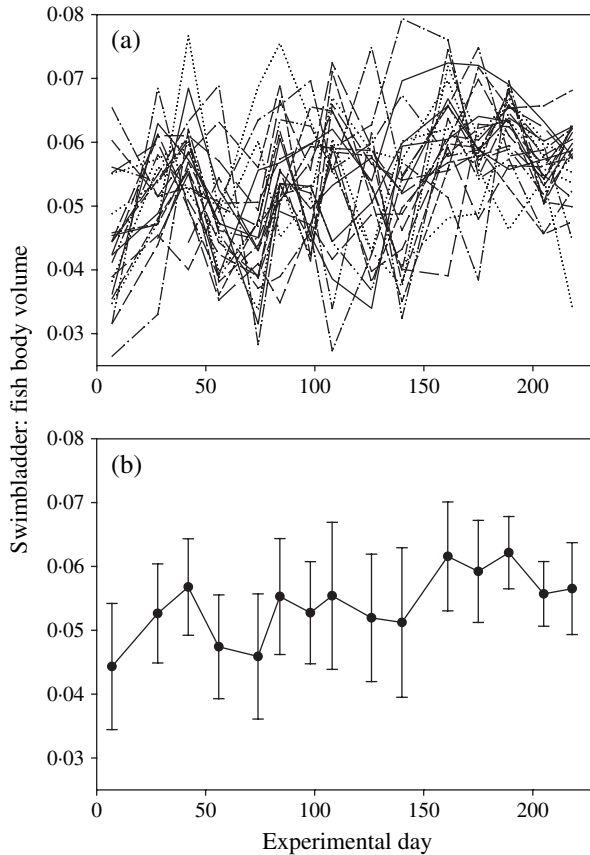


FIG. 11. Swimbladder to fish body volume ratios of *Oncorhynchus mykiss* for (a) each fish and (b) the mean  $\pm$  s.d. of the group at each measurement period.

the observed  $L_T$  from that measurement period are indicated (Fig. 12). As expected, the range of predicted TS at each  $L_T$  was lower at 38 kHz than at 120 kHz. At 38 kHz, the variability of predicted TS increased at longer  $L_T$ . Variability among TS at 120 kHz was larger at any  $L_T$  than at 38 kHz and across the entire  $L_T$  range. Variability in TS using observed fish  $L_T$  was lower between measurement rounds than at any individual measurement round using scaled  $L_T$ . Despite  $k$  values being close to unity,  $L_T$  influenced predicted TS when  $L_T$  were proportionately scaled over a range of lengths.

Mean, predicted acoustic backscatter increased at both 38 and 120 kHz as mean  $L_T$  increased from radiograph rounds 2 to 16 (Fig. 13). With the exception of round 7 ( $t$ -test, d.f. = 46,  $P < 0.05$ ), TS at 38 kHz significantly exceeded those at 120 kHz until radiograph round 11, which was not significant ( $t$ -test, d.f. = 46,  $P > 0.05$ ). TS values at 120 kHz exceeded but were not significantly different from those at 38 kHz from 283 until 363 mm. The change in relative TS amplitudes at 283 mm coincided with increases in swimbladder angle [Fig. 9(b)] and in the ratio of swimbladder to fish body volume [Fig. 11(b)]. Backscatter was more variable among fish within the group at 120 kHz than at 38 kHz.

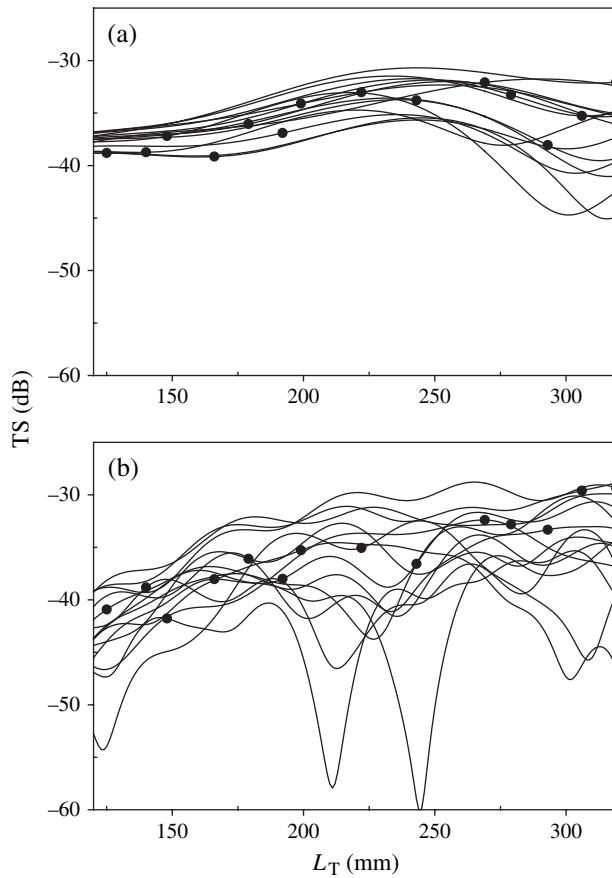


FIG. 12. Predicted target strengths (TS) of the example *Oncorhynchus mykiss* at (a) 38 and (b) 120 kHz over a total length ( $L_T$ ) range of 120–320 mm using the radiographs from each measurement period. ● the predicted TS of the fish at each measurement period.

To be consistent with empirical TS ( $y$ ) to  $L_T$  conversion equations, regressions were fitted to the 38 and 120 kHz predicted backscatter curves. When slopes were fixed at 20 (Foote, 1980a), regression intercept values differed between frequencies by *c.* 2.5 dB:  $-62.6$  at 38 kHz ( $r^2 = 0.15$ ,  $n = 15$ ) and  $-65$  at 120 kHz ( $r^2 = 0.82$ ,  $n = 15$ ). When slopes were allowed to ‘float’, regression equation fits improved at both frequencies:  $y = 10.75 \log_{10} L_T - 49.80$  ( $r^2 = 0.58$  at 38 kHz,  $n = 15$ );  $y = 25.26 \log_{10} L_T - 72.26$  ( $r^2 = 0.86$  at 120 kHz,  $n = 15$ ).

All covariates except dorsal fish body elongation ratio were significant in final 38 (adjusted  $r^2 = 0.64$ ,  $n = 358$ ) and 120 (adjusted  $r^2 = 0.37$ ,  $n = 358$ ) kHz GAMMs (Table III). Intercept values of final models were  $-36.44$  at 38 and  $-39.47$  at 120 kHz (both *t*-test,  $P < 0.001$ ). In the 120 kHz multiple covariate model, swimbladder angle and lateral fish-body elongation ratio were fitted as linear factors. An additional multi-covariate GAMM model at 120 kHz fitting swimbladder angle and lateral body-elongation ratio as linear terms, did not improve the fit of the model ( $r^2 = 0.37$ ,  $n = 358$ ). Plots of smooths showed that all variables at 120 kHz had TS values that increased with an increase in

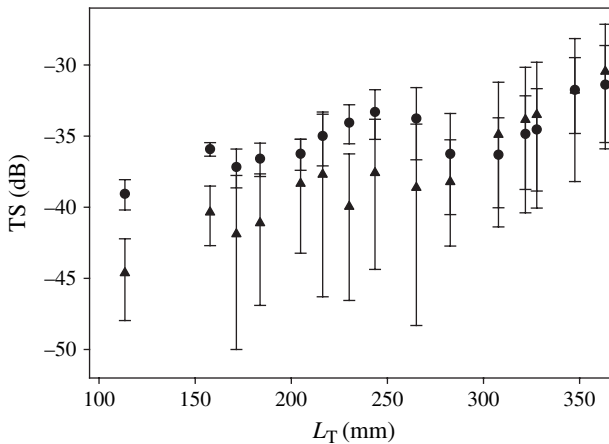


FIG. 13. Predicted mean  $\pm$  s.d. target strength (TS) of 24 *Oncorhynchus mykiss* at 38 (●) and 120 kHz (▲) as a function of total fish length ( $L_T$ ).

each variable. This pattern was also observed and was stronger at 38 kHz for swimbladder angle. At 38 kHz, the effect of  $L_T$  varied about the mean TS over the range of  $L_T$  included in the regression. Lateral fish body elongation ratio only differed from the mean  $>0.30$  at 38 kHz. ANOVA comparisons of multiple covariate to  $L_T$  only GAMM models showed that multiple-term models were a better fit to the data at 38 (AIC = 1591.37,  $P < 0.001$ ) and 120 (AIC = 2106.62,  $P < 0.001$ ) kHz.

## DISCUSSION

The combination of terms acoustic and ontogeny is not normally associated with reflection of sound from fishes. Acoustic ontogeny is traditionally used to characterize hearing development and sound production in aquatic vertebrates such as fishes (Wysocki & Ladich, 2001; Amorim & Hawkins, 2005), seals (Charrier *et al.*, 2001) and dolphins (McCowan & Reiss, 1995). In this study,

TABLE III. Approximate significance of smooth terms in final generalized additive mixed models (GAMM) of predicted target strength (TS) as a function of fish total length ( $L_T$ ), swimbladder angle ( $\theta$ ), and fish-body lateral (ellatbody) and dorsal (eldordbody) elongation ratios at 38 and 120 kHz

Variable	edf		$F$		$P$	
	38	120	38	120	38	120
$L_T$	6.83	3.47	12.20	4.29	0	0
$\theta$	7.29	1.00	24.12	28.49	0	0
Ellatbody	5.26	1.00	4.70	6.98	0	0.001
Eldordbody	NS	NS				

edf, effective d.f. (Wood, 2006a).

NS, non-significant ( $P > 0.05$ ).

acoustic ontogeny is used to characterize morphological changes that influence the intensity of backscattered sound. Morphological changes potentially include alteration of material properties and changes in size or shape of structures that reflect sound. The definition of material properties in this study is limited to density ( $\rho$ ) and the speed of sound ( $c$ ) of scattering organs, and whose product defines the acoustic impedance (*i.e.*  $z = \rho c$ ). The proportion of sound reflected at the interface of any two structures depends on the difference between the two acoustic impedances. The larger the difference in acoustic impedances, the more reflected sound. The largest acoustic impedance difference in a fish occurs at the interface of flesh and a gas-filled swimbladder. In addition to obvious differences among tissues, densities of structures can differ during growth of an individual animal (Horne & Jech, 2005).

Interindividual and intraindividual growth differed among *O. mykiss* used in this experiment. Changes in  $L_T$  and  $M$  of *O. mykiss* are faster than in many other salmonid species (Barton, 1996). The  $L_T$  attained during this experiment were equivalent to mean  $L_T$  of 2 year-old fish when compared to seven freshwater salmonid species (Carlander, 1969). Individuals from natural *O. mykiss* populations mature in 4 years and weigh *c.* 0.68 kg. *Oncorhynchus mykiss* (hybrid) mature in 2 years and can weigh as much as 4.5 kg (Donaldson, 1968). Consistent with other fish species, conventional ( $L_T$  and  $M$ ) and acoustically relevant (swimbladder surface area and swimbladder volume) growth metrics increased with  $L_T$ . Over the grow out period, average swimbladder dorsal surface area increased 9.5 times (from 408.7 to 3867.6 mm<sup>2</sup>) with the range of areas at the last radiograph round spanning over 1850 mm<sup>2</sup> (*i.e.* 3036.3 to 4887.1 mm<sup>2</sup>). The corresponding increase in swimbladder volume exceeded 27-fold with the group average increasing from 1206.2 to 32 971.5 mm<sup>3</sup>. By the end of the experiment, swimbladder volumes ranged over a factor of two (22 401.5–47 782.8 mm<sup>3</sup>). Despite average changes in swimbladder surface areas and volumes, the proportion of swimbladder volume as a fraction of body volume remained fairly constant, between 4 and 6%. This fraction is well within the range cited for marine and freshwater fishes (Alexander, 1966; Sand & Hawkins, 1973; Harden Jones & Scholes, 1985).

Differences in shape and size of swimbladders have large potential impacts on the amount of energy reflected from a fish as it grows. The largest change in acoustic ontogeny will occur at first inflation of the swimbladder (McEwen, 1940; Schwarz, 1971). Swimbladder first inflation ranges from 3 to 15 days depending on species and temperature (Doroshev *et al.*, 1981; Bailey & Doroshev, 1995). Haslett (1962) and Foote (1980*a*) have shown that the swimbladder contributes a minimum of 90% to the backscatter from swimbladdered fishes, which includes *O. mykiss*. To illustrate the acoustic contribution of the swimbladder using the example fish at a  $L_T$  of 333 mm, TS values were reduced by 9.3 dB re 1  $\mu$ Pa at 38 kHz and 21.7 dB re 1  $\mu$ Pa at 120 kHz when the swimbladder was removed from model calculations. Any change in swimbladder angle during development will have the second largest effect on acoustic backscatter. A reduction (*i.e.* towards horizontal) in mean tilt angle of 6° within the group was observed between the start and the end of the experiment. Although not a direct comparison if the angle of the swimbladder was reduced by 6° in the example fish, the predicted TS at 38 kHz increases by 8.8 dB re 1  $\mu$ Pa at

a  $L_T$  of 333 mm. The third major factor influencing acoustic backscatter is change in swimbladder surface areas and shapes. Among *O. mykiss*, swimbladder dorsal surface areas increased by the square of  $L_T$ . To provide a comparison, dorsal swimbladder surface areas as a function of  $L_T$  were linearly modelled for walleye pollock *Theragra chalcogramma* (Pallas) (Horne, 2003), although the regression fit would improve if a quadratic function were used within the caudal length range of 100–400 mm. *Oncorhynchus mykiss* mean TS did not scale proportionately to increases in dorsal swimbladder surface area at 38 or 120 kHz, which was used as a proxy for acoustic backscattering cross-section. Changes in swimbladder shape were characterized using elongation ratios (*i.e.* ratios of minor to major axes). Dorsal swimbladder elongation ratio values exceeded lateral values throughout the experimental period and decreased as fish grew. Lateral swimbladder elongation ratio values were less than comparable values from *T. chalcogramma*, Pacific herring *Clupea pallasii* Valenciennes and capelin *Mallotus villosus* (Müller) of similar lengths (Gauthier & Horne, 2004), indicating that *O. mykiss* swimbladders are more elliptical relative to the other species. As suggested by changes in shape, rates of change in swimbladder volumes were not constant. Mean volumes increased exponentially over time with an increasing ratio of swimbladder to fish body volume.

Any developmental change that alters the shape or angle of the swimbladder will change the TS of a fish. At geometric scattering frequencies (*i.e.* when  $L_T$  exceed acoustic wavelengths), swimbladder surface area largely determines the amount of energy reflected by a fish. These conditions led Foote (1980a) to suggest setting the slope of the TS ( $y$ ) to  $L_T$  regression equations to 20:  $y = 10\log_{10}(L_T^2) + a = 20\log_{10}(L_T) + a$ , where  $a$  is the intercept. This approach facilitates comparison among species using regression intercepts, but large residuals from empirical regression equations prompted McClatchie *et al.* (1996, 2003) to discourage the use of 20 as an arbitrary slope value. An arbitrary slope regression may not be appropriate for all species (Gauthier & Horne, 2004), all life-history stages (Horne, 2003) or even within life-history stages (Abe *et al.*, 2004). The fit of the 38 and 120 kHz fixed and floating slopes for *O. mykiss* also illustrate the point. Jørgensen (2003) found that for *M. villosus*, a better relationship existed between swimbladder length and TS than between TS and  $L_T$ . The switch in relative mean backscatter amplitudes at 38 and 120 kHz among *O. mykiss* corresponded to shifts in swimbladder angle and shape. A similar shift in TS among juvenile *T. chalcogramma* is the only other documented example (Abe *et al.*, 2004, 2005) of an allometric ontogenetic effect on acoustic backscatter.

Allometric growth by individual animals will decrease accuracy of TS to  $L_T$  conversions used in acoustic-based abundance estimates. Data used to quantify species-specific relationships between TS and fish length are acquired using *in situ* TS measurements from animals in low-density aggregations (Burczynski *et al.*, 1987), or by measuring backscatter from tethered (Nakken & Olsen, 1977; Horne *et al.*, 2000) or caged (Edwards & Armstrong, 1984) individuals of known lengths. These methods provide snapshots of TS characteristics from groups of animals but do not track development among individuals. Tracking fish ontogeny was facilitated by PIT tagging each animal using a sample from a cohort. Non-linear changes in growth trajectories of fish swimbladders and



bodies have been documented for fish cohorts (Abe *et al.*, 2004) or species (Butler & Pearcy, 1972) but are rarely quantified for individual fishes. Application of TS to fish length regressions across life-history stages or the proportionate scaling of fish bodies and swimbladders without determining allometric growth constants may invalidate TS predictions when used over extended length ranges. Using *T. chalcogramma* as an example, Abe *et al.* (2005) derived a different intercept in the average TS and length relationship among juvenile fish (50 to 200 mm fork length,  $L_F$ ) compared to that used in the regression equation based on adult fish (Foote & Traynor, 1988). Differences in the two equations may result from observed changes in growth ratios (*i.e.*  $k$  values) between swimbladder length and  $L_F$  at  $L_F > 100$  mm. Within juvenile *T. chalcogramma*, the reduction in slope of dorsal swimbladder area to  $L_F$  ratios occurred at  $L_F > 100$  mm (Abe *et al.*, 2004, 2005). Among *O. mykiss*, the growth ratio of swimbladder length to  $L_T$  indicated that growth was nearly isometric over the observed  $L_T$  range, and that proportionate scaling of fish bodies and swimbladders used in backscatter modelling was appropriate. Based on the simple comparison of growth ratios in *T. chalcogramma* and *O. mykiss*, acoustic ontogeny should be considered species specific, as allometric growth may be restricted to early life-history stages.

Ontogeny was linear throughout the  $L_T$  range examined for *O. mykiss* for most acoustically relevant variables. Relevant acoustic variables include swimbladder shape, tilt and dorsal surface area. Swimbladder elongation and  $k$  values were near constant through the experimental period indicating that changes in swimbladder shape were linear. A slight non-linear shift was observed among swimbladder mean tilt angles but this shift was not significant. The only factor exhibiting non-linear increase was mean dorsal swimbladder area. At  $L_T < 200$  mm, swimbladder increases in dorsal surface area were exponential. Resulting TS increased with  $L_T$  but backscattering curves were not linear, or monotonic, at 38 or 120 kHz. Traditional TS and  $L_T$  regression equations fitted better when slopes were allowed to float. The non-linear increase in TS with  $L_T$  is attributed to constructive and destructive interference between the swimbladder and the fish body. A direct relationship between dorsal swimbladder area and TS has not been observed and is not expected to exist for any fish species.

The use of radiographic imaging facilitated additional variables being added to acoustic size to  $L_T$  regression equations. GAMM regression was an appropriate approach as multiple measurements were taken from a group of individuals, with the resulting error term representing a mixture of group and population effects (Wood, 2006b). The use of splines (*i.e.* smooths) when modelling covariates has the advantage that no assumptions are made about relationships among covariates or the response variable. A disadvantage is that this technique is relatively new and that standardized procedures to measure significance are somewhat *ad hoc* and exploratory (Wood, 2006a). Final GAMMs showed that multi-covariate models were better predictors of TS than  $L_T$  alone (*i.e.*  $r^2$  values increased and AIC values were lower at 38 and 120 kHz). If these additional variables were added to TS and  $L_T$  regression equations, then fish body elongation ratio data could be obtained from field measurements but swimbladder angles could not be determined without advanced imaging techniques. The fact that swimbladders can deviate up to  $10^\circ$  posterior

down from horizontal (Blaxter & Batty, 1990; Clay & Horne, 1994) depending on  $L_T$  (Fig. 9) and species, makes inclusion of swimbladder angles impractical for acoustic size to  $L_T$  regressions when based on *in situ* TS measurements. As an alternative, TS and length regression models have been developed using fish-aspect distributions and transducer beam shapes (Foote, 1980*b*; K. Olsen, unpubl. data). This practice is not standard when estimating population abundances using acoustic data, and the number of published tilt-angle distributions is limited. Additional fish tilt and swimbladder angle measurements are needed to quantify intraspecific and interspecific variability in swimbladder orientation and to validate generalization of fish orientation when converting acoustic sizes to fish sizes.

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