Whether depositing fat or losing weight, fish maintain a balance

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In fish, the relative amount of tissues of different densities changes significantly over short periods throughout the year, depending on the availability of food, nutrition and their developmental status, such as sexual maturation. If a land-living animal accumulates fat it influences not only its general state of health, but also markedly increases its energy expenditure for locomotion owing to the force of gravity. On a body submerged in water, this force, which acts on the centre of gravity (COG), is counter-balanced by a lifting force that is negligible in air and which acts on the centre of buoyancy (COB). Any difference in the longitudinal positions of the two centres will therefore result in pitching moments that must be counteracted by body or fin movements. The displacement of the COG away from the COB is a result of tissues of different density (e.g. bones and fat) not being distributed homogeneously along the body axis. Moreover, the proportions of tissues of different densities change significantly with feeding status. It is still unknown whether these changes produce a displacement of the COG and thus affect the hydrostatic stability of fish. Analysis of computed tomography and magnetic resonance imaging images of Atlantic herring, Atlantic salmon and Atlantic mackerel reveals that the COG is fairly constant in each species, although we recorded major interspecies differences in the relative amount of fat, muscle and bone. We conclude that the distribution of different tissues along the body axis is very closely adjusted to the swimming mode of the fish by keeping the COG constant, independent of the body fat status, and that fish can cope with large variations in energy intake without jeopardizing their COG and thus their swimming performance.

Keywords: buoyancy; hydrostatic balance; tissue density; fat allocation; teleosts; swimming

1. INTRODUCTION

The forces acting on a resting submerged body that produces no hydrodynamic forces are lift and gravitation. Gravitational forces act on the centre of gravity (COG), whereas lift acts on the centre of buoyancy (COB). In a body of uniform density these two centres essentially coincide. In fish, however, they may be separate because fish tissues are not of uniform density (Weihs 1993; Webb 2006). The position of the COB will always be at the centroid of the volume of the fish, because the displaced water is of uniform density. However, the position of the COG depends on the distribution of tissues of different densities along the body; for example, heavy tissues such as bone ($\rho \approx 2.0 \text{ g ml}^{-1}$) may be concentrated in the head region, whereas special low-density organs such as swim bladders are located further posteriorly. Moreover, the relative amount of tissues of different densities depends on the feeding status of a fish (Varpe *et al.* 2005); the fraction of fat ($\rho \approx 0.9 \text{ g ml}^{-1}$) is highly variable and may thus influence the position of the COG. Several studies have shown how seasonal changes in the distribution of body reserves have an impact on the control of buoyancy and static lift,

particularly in some Antarctic species (Eastman 1993; Wells 2005 and citations therein), but the effects on pitching of the positions of body tissues and lipid deposits along the axis remain to be explored. We hypothesize that the distribution of different tissues along the body axis is regulated to keep the COG constant, independent of body fat status, and that fish can cope with seasonal changes in nutritional status/body condition without jeopardizing their COG and thus swimming performance. Because swimming mode and buoyancy regulation differ among species, we have examined the swimming modes of species ranging from subcarangiform to thunniform.

For a land animal, the main opposing forces that have to be overcome are lift and gravity. The general view is that these forces are relatively unimportant in water, and that the major forces become thrust and drag. However, pelagic fish have to take into account static lift as well as the pitch generated by the differences in the COG and COB when swimming both at cruising speed and during burst swimming. The magnitude of these forces is small compared with thrust and drag, but because any imbalances need to be continuously adjusted they will seriously affect the overall energy budget of the fish. In this respect we can compare the situation with aviation, in which an aircraft is trimmed in order to reduce the need to use force on the controls during flight.

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Data on the COG in fish are scarce (see Webb & Weihs 1994 for an overview), and so far no studies have taken into account the contribution of tissues of different density to the COG, owing to the lack of suitable techniques for distinguishing tissues. The introduction of computed tomography (CT) and magnetic resonance imaging (MRI) has solved this particular problem, and we can now quantify the distribution of different fish tissues along the body and thus determine the volume fractions and COGs of bony tissue, muscle tissue and fat, separately. We performed measurements using CT and MRI on three species of fish: Atlantic herring (*Clupea harengus* L., 1758), Atlantic salmon (*Salmo salar* L., 1758) and Atlantic mackerel (*Scomber scombrus* L., 1758). Using individuals of all three species with different nutritional status enabled us to test whether the position of the COG was conserved within each species when tissue composition varied.

2. MATERIAL AND METHODS

(a) Fish material

Atlantic mackerel (*S. scombrus*) were caught commercially off the island of Askøy, and Atlantic herring (*C. harengus*) outside Haugesund, both on the west coast of Norway. Atlantic salmon (*S. salar*) originated from a feeding experiment at the Institute of Marine Research facilities in Matre, Norway. The fish were kept cold on ice until physical measurements and CT and MRI imaging were carried out at the Department of Radiology, Haukeland University Hospital, Bergen. The fish (mackerel and herring) were then frozen at -80°C before dissection and lipid analysis (gas chromatography) at the Department of Chemistry, University of Bergen.

(b) Image analysis

The fish were sequentially imaged using a Light Speed Ultra CT (General Electric, Milwaukee, WI, USA) to estimate the volume and length of whole fish, bones and swim bladder. The CT images were reconstructed using bone, soft tissue and combined algorithms. Typically, 300 axial slices were reconstructed with a resolution of $0.2 \times 0.2 \times 1.25 \text{ mm}^3$.

Fat and muscle volumes were estimated by MRI imaging using a single channel, standard head coil on a whole-body clinical Signa Excite 3T Tesla scanner system (General Electric). Three-point fat–water separation imaging (IDEAL, 3D-FSPGR, field of view 380 mm, echo time TE = 3.372 ms, image matrix 256×256 , bandwidth 31.25 Hz, flip angle 10° , 40 slices with no gap between neighbouring slices, slice thickness 1.5 mm) provided by the scanner manufacturer was used to acquire and quantify the water and fat content in each imaged volume element (voxel; Reeder *et al.* 2005, 2007). This enabled heterogeneities in the main magnetic field to be compensated for (Dixon 1984; Glover & Schneider 1991), and near-isotropic imaging, with image voxel size of $1.5 \times 1.5 \times 1.5 \text{ mm}^3$. Additional T2 weighted fast spin echo pulse sequence imaging was included for completeness (Brix *et al.* 2009).

(c) Data analysis

Regions of interest (ROIs) were defined for all images for volume determinations of the whole fish, bones, swim bladder and muscles, using the nordicICE software (NordicImagingLab, Bergen, Norway). In addition to the slice-by-slice coronal views obtained directly from MRI, the fat image data were re-sliced in an axial representation and

Figure 1. Variability in tissue volume fraction (%) and centre of gravity (COG, % length from the snout) for bones, fat and muscles. COG data for the swim bladder (SB) is included. Black square box, bones; white square box, fat; gray square box, muscles; checked square box, SB. (Note: mackerel do not possess a swim bladder.)

averaged across 5×5 pixels for colour coding and direct read-out of the percentage of fat in the voxels using Matlab (The MathWorks, Natick, MA, USA). All the imaging data were transferred to Excel worksheets for final calculations of lengths, volumes, volume fractions and centre of masses for bone, fat, muscles and swim bladder (salmon and herring).

Body and tissue weights were calculated from the estimated volumes and densities, which were obtained by the upthrust method. According to this method, buoyancy mass m_b was calculated from $m_b = m_o[1 - (\rho_{sw}/\rho_o)]$, where m_o is the mass of the object, ρ_{sw} the density of seawater and ρ_o the density of the object.

(d) Statistical analysis

Statistical analyses for comparing means were carried out using GraphPad InStat v 3.06 for Windows. Non-parametric tests (Mann–Whitney U-test; Kruskal–Wallis non-parametric analysis of variance) were used because parts of the data had a non-Gaussian distribution (CI = 95%).

3. RESULTS AND DISCUSSION

Figure 1 shows that the COGs for bones, fat, muscles and swim bladder (only present in herring and salmon) vary very little, despite very wide differences in volume fractions, especially of muscles and fat tissues. Table 1 shows the COGs and volume fractions of bones, muscles and fat for mackerel and herring in different seasons and with different feeding backgrounds, in addition to the overall means of the same parameters for all three species. In June the fat fractions in mackerel were as low as 3.8–7.9 vol%. Less than two months later, in a period when food is generally abundant (Varpe *et al.* 2005), the fat fraction had risen to 52.1 vol%, corresponding to a change in buoyancy mass for fat from -1.50 to -20.23 g and the mackerel had become virtually

Table 1. Major features of the fish: volume fraction (%), centre of gravity (COG, %), buoyancy mass (BM, g) and torque ($\text{Nm} \times 1000$) of the main tissues as well as COG of the swim bladder of herring and salmon. The mackerel and herring were sampled in different periods of the year (upper rows). The salmon were collected as pairs (male and female) from an experiment with four different feeding strategies and presented as a total average value for the whole experiment together with the total averages for the two other species, in the lower rows. The values are presented as means (χ), standard deviations (σ) and numbers (ν).

Species & Period	bone				muscle				fat				SB COG (%)	lift torque Nm × 1000																			
	vol. (%)		BM (g)		torque Nm × 1000		vol. (%)		COG (%)		BM (g)				torque Nm × 1000																		
	χ	σ	ν		χ	σ	ν		χ	σ	ν		χ	σ	ν		χ	σ	ν		χ	σ	ν		χ	σ	ν		χ	σ	ν		
Mackerel 6 June	4.66	0.84	8	20.54	21.56	5.48	8	17.85	87.47	52.29	9.17	-0.53	7.87	49.35	-3.42	0.23	18.15																
	5.03	0.53	15	21.36	20.15	2.19	15	-15.46	91.13	51.74	8.30	-0.38	3.84	45.58	-1.50	0.12	15.72																
	3.73	0.40	15	21.10	15.25	1.66	15	1.55	44.19	53.34	4.13	-0.37	52.09	52.82	-20.23	1.45	10.37																
Mackerel 17 June	5.23	0.69	17	24.23	12.80	2.94	17	1.17	67.26	47.63	4.23	-0.64	27.77	56.31	-7.15	0.98	5.91																
	4.77	0.99	17	24.94	6.04	2.16	17	1.41	6.09	5.90	0.74	0.22	6.22	1.11	1.31	0.23																	
	4.44	0.81	38	21.10	18.51	3.99	38	4.56	71.83	52.49	6.84	-0.41	25.84	55.30	-9.30	0.67	14.12																
Mackerel (all)	5.92	1.49	34	24.70	9.42	4.27	34	2.09	71.37	56.28	3.25	-0.44	23.78	49.70	-4.84	0.54	4.15																
	4.44	0.81	38	21.10	18.51	3.99	38	4.56	71.83	52.49	6.84	-0.41	25.84	55.30	-9.30	0.67	14.12																
	5.92	1.49	34	24.70	9.42	4.27	34	2.09	71.37	56.28	3.25	-0.44	23.78	49.70	-4.84	0.54	4.15																
Herring (all)	5.92	1.49	34	24.70	9.42	4.27	34	2.09	71.37	56.28	3.25	-0.44	23.78	49.70	-4.84	0.54	4.15																
	4.44	0.81	38	21.10	18.51	3.99	38	4.56	71.83	52.49	6.84	-0.41	25.84	55.30	-9.30	0.67	14.12																
	5.92	1.49	34	24.70	9.42	4.27	34	2.09	71.37	56.28	3.25	-0.44	23.78	49.70	-4.84	0.54	4.15																
Salmon (all)	5.14	0.61	8	30.10	168.41	34.79	8	-199.44	65.40	43.96	58.89	-19.29	29.91	39.22	-114.73	72.72	146.00																
	4.44	0.81	38	21.10	18.51	3.99	38	4.56	71.83	52.49	6.84	-0.41	25.84	55.30	-9.30	0.67	14.12																
	5.92	1.49	34	24.70	9.42	4.27	34	2.09	71.37	56.28	3.25	-0.44	23.78	49.70	-4.84	0.54	4.15

Figure 2. Analysis of torque for mackerel and herring. Torque ($r \times F$) in $\text{Nm} \times 1000$ calculated for mackerel (grey) and herring (white). The lift is calculated from the torques of the three tissues: bone, muscle and fat. The variability in the lift is thus related to the deviations in torque for the tissues. Error bars represent $\pm$ s.d.

neutrally buoyant. Although the uncertainty in determining the COGs of fat at such low fraction levels increases, Table 1 clearly shows that the COGs of fat varied very little throughout the season (6 June, $49.4\% \pm 2.7$; 17 June, $45.6\% \pm 4.5$; 30 September, $52.8\% \pm 1.5$), with the largest deviations occurring during the last month or two before the feeding season commenced fully in the period July–September (6 June–30 September, $p < 0.0042$, $p < 0.0001$ when comparing June and September). This feature thus greatly reduces the impact of this extra fat load on the balance properties of the mackerel, as is illustrated by the minimal changes in torque centred on half fork length (FL/2; figure 2). It is also the case that, in Atlantic herring, fat allocation related to the building up of the gonads in February seems not to have any serious impact on stability.

Like salmon, but in contrast to mackerel, herring have a swim bladder that aids buoyancy and also reduces the 'lift torque' needed for stability, owing to the location of its COG in the anterior part of the fish (figure 3). Even though the herring and mackerel were more or less the same size, the torque related to the bones was larger in mackerel (figure 2). This striking feature is due to the fact that the density of mackerel bone is higher than that of herring (O. Brix 2006, unpublished data) and may actually be related to its thunniform swimming mode, which benefits from posterior to anterior mass displacement. From the calculated torque for mackerel (table 1), we can further calculate the mean overall COG to be 28.2 per cent from snout to fork (28.7%, 29.2% and 26.5% on 6 June, 17 June and 30 September, respectively). The mean COG (fat) was 49.2 per cent (49.4%, 45.6% and 52.8% on 6 June, 17 June and 30 September, respectively). A consequence of the different positions of the overall COG is that mackerel will also tilt even if neutrally buoyant in September, and must generate lift by swimming if horizontal balance and level are to be maintained, irrespective of body fat composition (figures 2 and 3).

Figure 1 makes it clear that the overall COGs can be directly related to swimming mode. The COG of bone in salmon, which have a sub-carangiform swimming

mode and thus undulate a larger proportion of the body than the other two species, is located at 30.1 per cent of the distance from the snout to the tail. This differs from the carangiform herring (24.7%; $p < 0.0001$) and the thunniform mackerel (21.1%; $p < 0.0001$). It is generally accepted, and is consistent with physics, that yaw movements during undulatory locomotion should be minimized in order to reduce drag. This is best achieved by anteriorly located COGs (for review see Webb 1984; Blake 2004; for experimental data see Donley *et al.* 2004). With respect to this it is particularly important to maintain the COG in an anterior location, regardless of seasonal changes in the amount of fat or nutritional state. This fact is well reflected by the data presented here.

There were large differences in fat allocation strategies for the three species (figure 3). Salmon and herring primarily deposit fat dorsally, and particularly in the abdomen. Mackerel deposit fat throughout their muscular tissue, with the highest concentration in the central area of the body, and there are very low concentrations in the tail region or ventrally in the anterior end. In all three species we found very high concentrations of subcutaneous fat throughout the season and also around the length of the backbone. This feature demonstrates that high priority is allocated to maintaining and safeguarding the levels of fat that are associated with the sensory and motor neurons in the nervous system and particularly to ensure swimming activity.

4. CONCLUDING REMARKS

We have demonstrated that the total and tissue-specific COGs remain fairly constant in spite of large seasonal variations in muscle and fat content in particular. Furthermore, the COGs can be directly related to the swimming modes of the fish, thus confirming current theories regarding mass displacement (Evans & Claiborne 2006). Such constancy of COGs is of vital importance to the ability of fish of a given design to reduce energy expenditure. The importance of sensing spatial balance may increase with decreasing size and must be vital for the survival of fish larvae commencing first feeding. This may explain the pronounced appearance of the cochlear apparatus in these larvae. However, depositing tissue, especially fat, with such high accuracy, as this paper has shown, probably requires the sensory inputs to be linked to a sophisticated logistics system for spatial allocation of fat. Because lipid deposits can be used for both metabolic and buoyancy purposes (see review by Eastman 1993), further studies should aim to identify the mechanisms involved in regulating the dynamic deposition and use of fats stored in different tissues for both purposes. A possible mechanism was proposed in a cold-adapted polar fish of the nototheniid family, in which the substrate specificity of triacylglycerol lipases appeared to preferentially recruit unsaturated fatty acids for metabolic use, thus enabling saturated lipids to remain in the deposits and help to maintain static lift (Lund & Sidell 1992).

In the process of spatial allocation and recruitment of fats we believe cytokines such as leptin may play a key role, and future experiments will focus on identifying the mechanisms involved in communication between

Figure 3. The COGs of the tissues and swim bladder (SB) of herring and the fork length (FL) are shown on the superimposed MRI images of the fish.

Figure 4. Spatial distribution of fat. We intended to show mid-sagittal sections but practical constraints made it impossible to keep the vertebral column straight during scans. Because of their size, the salmon images had to be merged from two scans.

different peripheral tissues and the central nervous system centres that control energy homeostasis and growth.

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