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Lipid and elemental composition of sprat (*Sprattus sprattus*) larvae at mixed and stratified sites in the German Bight of the North Sea

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Larvae of sprat (*Sprattus sprattus*) from mixed and stratified water column sites in the German Bight of the North Sea were assessed for nutritional condition by analysis of total carbon and nitrogen and the lipid fractions triacylglycerol (TAG), cholesterol, and polar lipids. There was no indication of any significant variation in condition index related to diel sampling periodicity, based either on percentage carbon composition or TAG/cholesterol ratio. Inclusion of growth data from otolith ring counts with the carbon and lipid results identified larvae at 9–10 mm in length as being in relatively poor condition. There was some evidence from carbon analysis to suggest that larvae in poor condition were more prevalent at the stratified site than the mixed; however, microzooplankton analysis showed mean food abundance for larvae was slightly higher at the mixed site. The results from the condition analyses and the validity of the methods is discussed in relation to studies of survival of fish larvae.

Key words: *Sprattus sprattus* larvae, nutritional condition, growth, lipids, carbon, otoliths.

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Introduction

As part of investigations of the causes of recruitment variability, various analyses of nutritional condition of fish larvae have been studied (e.g. RNA:DNA – Clemmesen, 1987; histological – Theilacker, 1986; morphometric – Frank and McRuer, 1989; lipids – Håkanson, 1989b; elemental composition – Ehrlich, 1974a). The implicit hypothesis under test has most frequently been that food availability is a determining factor for larval survival (Lasker, 1978). Analyses of larval condition, as a measure of incipient starvation, are then used in empirical correlations with indices of plankton abundance and hydrographic events. Development of techniques for ageing larvae within a season using otolith daily growth rings (e.g. Ré, 1984) has allowed an extension of such studies to intra-seasonal events (Methot, 1983). Additionally, the variation in the width of daily growth increments from otoliths of field-captured specimens also gives estimates of larval condition and can be correlated with the timing of particular ontogenetic and environmental events (Ré, 1986).

The present studies were undertaken as a preliminary part of a European Sardine Anchovy Recruitment Project (SARP) based on sprat (*Sprattus sprattus*), sardine (*Sardina pilchardus*), and anchovy (*Engraulis encrasicolus*). The immediate aims were to investigate diel changes in lipid and elemental (carbon and nitrogen) composition of sprat larvae in the German Bight of the North Sea and to compare results obtained at mixed and stratified sites.

Sprat (*Sprattus sprattus*) larvae, in common with many other fish larvae, are visual feeders, having fuller guts during the day than at night (Conway *et al.*, 1991). Since digestive processes and energy acquisition are thus constrained to a diurnal rhythmicity, it is appropriate to investigate condition indices for any similar variation. In terms of assessing the nutritional status of larvae for survival studies, the most suitable indices will be those based on longer-term changes responding to the feeding history of the larvae.

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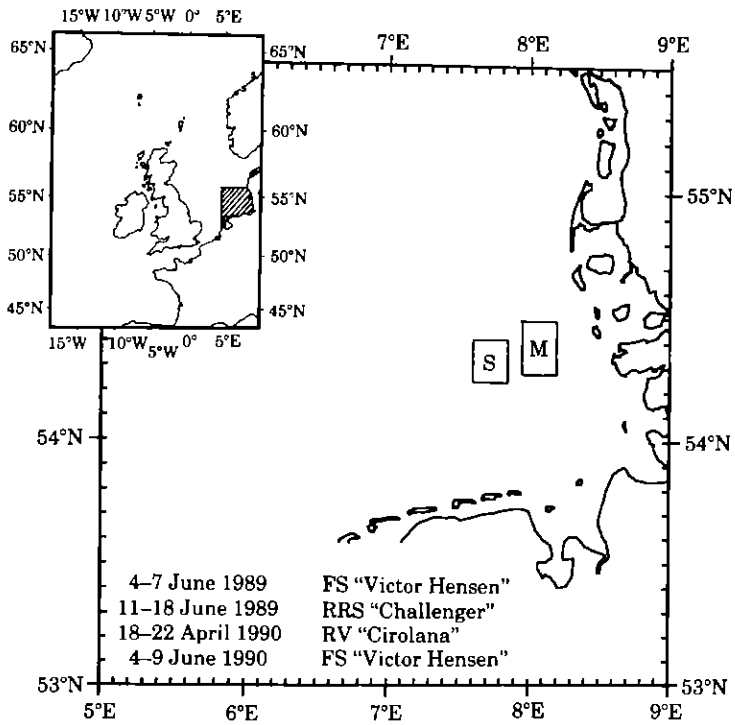

Figure 1. Sampling area (entire main chart) and cruise dates for larvae taken for lipid and elemental analysis. The mixed (M) stratified (S) sites for the June 1990 cruise are indicated by the outlined boxes.

Methods

Sprat larvae were sampled throughout the German Bight on cruises in 1989 and 1990 (Fig. 1). Specimens for carbon and nitrogen analysis were taken on all cruises, whereas those for lipid analysis were taken only on the June 1990 cruise. On that cruise sampling was restricted to two contrasting sites (mixed and stratified), together with a few additional samples at a preliminary aborted stratified site. Selection of these sites was based on the results of UNDULATOR (Aiken, 1981) tows over a grid in the boxed areas shown in Figure 1.

The UNDULATOR consists of a small (~1-m length) hydrodynamic towed body with a servo-controlled diving fin enabling it to follow a saw-tooth dive-and-climb profile while towed behind the survey vessel. Mounted inside the UNDULATOR is a solid-state data logger that records sensor signals at intervals of 5 s, including measurements of temperature, conductivity, and pressure. These results can then be processed to give temperature and salinity depth/distance contour plots along the sampled transects and hence three-dimensional representations of stratification as $\Delta\sigma_t$ from the surface to the bottom (in the present studies ~25-m depth at the stratified site and ~18-m at the mixed). Following selection of a site for each 24-h sampling sequence a sub-surface drogued Argos buoy was released to enable sampling to follow the same water mass.

On all cruises sampling for larvae was by means of relatively slow (~3 knots towing speed) double oblique hauls (surface to near bottom) using a 50-cm high-speed tow net (Beverton and Tungate, 1967) fitted with 200-μm mesh aperture filtering net. Microzooplankton samples were taken on the same hauls by a fine mesh sampler (53-μm mesh aperture) mounted on the main sampler frame.

On completion of a haul sprat larvae were sorted from the catch, measured to the nearest 0.1 mm under binocular microscope and preserved within 15 min either over desiccant (for elemental analysis), or in liquid nitrogen (for lipids). Microzooplankton samples were preserved in 4% formaldehyde solution for subsequent microscope analysis. Since sprat larvae feed predominantly on eggs, nauplii, and copepodite stages of copepods (Conway *et al.*, 1991), identification of the microplankton was to these groups; there were no significant numbers of other microzooplankton in the same size range.

Analysis of individual larvae for total carbon and nitrogen was carried out using a Carlo-Erba Elemental Analyser model 1106; specimens were first weighed to ± 1 μg using a Cahn Electrobalance.

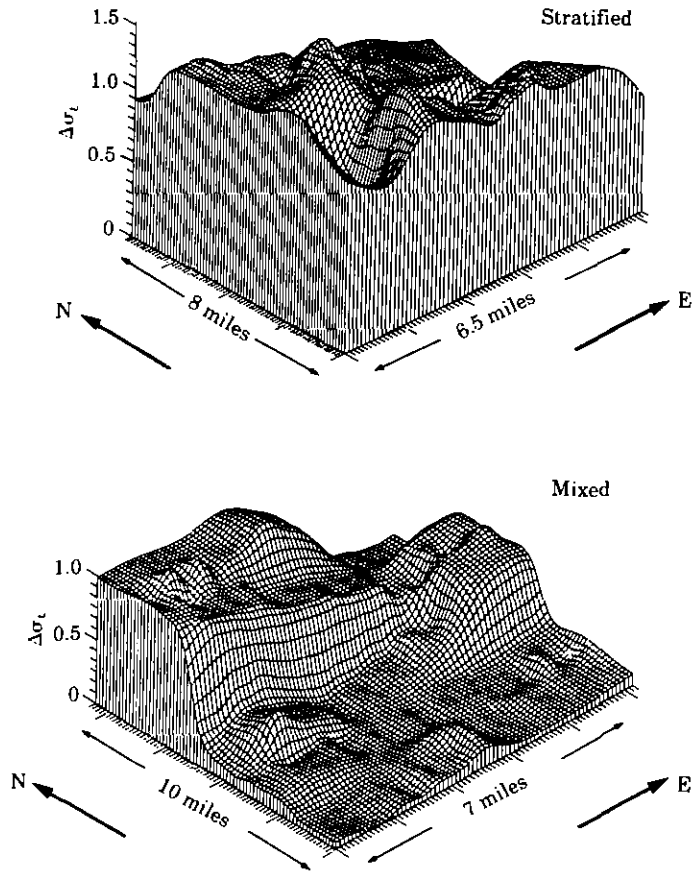

Figure 2. Three-dimensional representation of the density field (surface-to-bottom density difference) at the mixed and stratified sampling sites on the June 1990 cruise.

Following extraction in chloroform/methanol (2 × 24-h) lipid analysis was carried out on individual larvae by thin-layer chromatography using flame ionization detection in an Iatroscan TH-10 analyser as detailed by Håkanson (1989a). Duplicate samples for each larva were analysed to determine triacylglycerol (TAG), cholesterol, and polar lipid content.

Sprat larvae were aged using daily growth ring increments in sagittal otoliths obtained from the defatted carcasses of specimens used for lipid analysis. Microgrowth increments were counted using a compound optical microscope at a magnification of 1000 ×. Both sagittae were examined with the mean count for the pair being used for the data analysis. Measurements of increment widths, using a calibrated ocular micrometer, were used to estimate individual growth rate, assuming that otolith and somatic growth are related (see Ré and Gonçalves, 1993). Growth rates were averaged over the last three days to allow for some delay in the response of otolith growth to body growth. Average daily growth from increment widths and the widths of the last three rings were also determined. Individual otolith growth rates were estimated as the average widths of daily growth units over the last three days. Daily growth hereafter refers to daily otolith growth.

Results

Hydrography and microzooplanton at the 1990 mixed and stratified sites

A comparison of the surface to near-bottom density difference ($\Delta\sigma_t$ as a measure of stratification) at the two 1990 sampling sites is shown in Figure 2. Over much of the area at the stratified site temperature and salinity changed progressively down the water column from around 12.8°C and salinity of 33.1 at the surface to around 11.2°C and salinity of 34.1 at 20-m depth (bottom at ~25 m). This gave $\Delta\sigma_t$ values in the range 0.35–1.23 for consecutive sampling positions at the stratified site (Fig. 3). At the mixed site, in the southern half of the area, the water column was fully mixed with minimal change in vertical profiles of either temperature or salinity (water depth ~ 18 m); in the north of the area the sampling region extended across an inshore frontal zone into more stratified water similar to conditions at

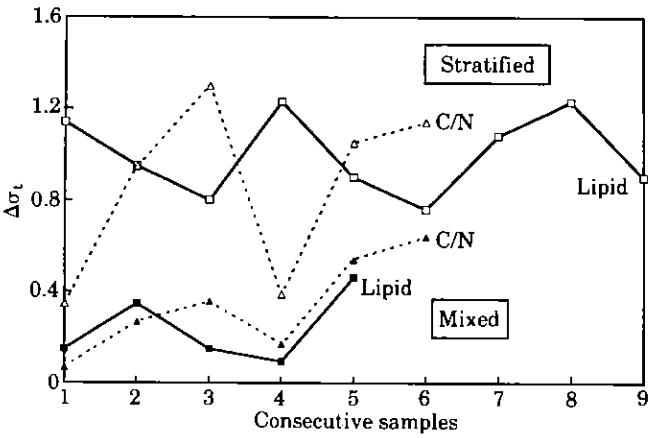

Figure 3. Surface-to-bottom density difference at the mixed (filled symbols) and stratified sites (open symbols) on the June 1990 cruise (see Fig. 1) for consecutive samples taken for lipid (square symbols) and elemental analysis (triangles).

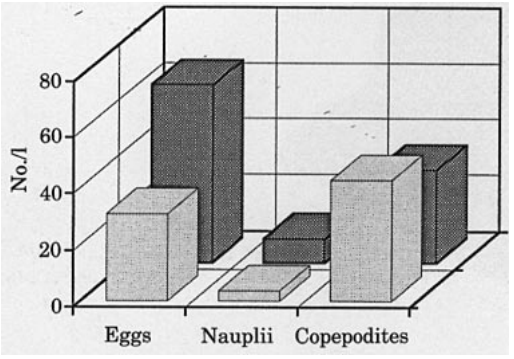

Figure 4. Microplankton abundance from oblique net tows taken at the mixed (light shading) and stratified sites (dark shading) on the June 1990 cruise (see Fig. 1).

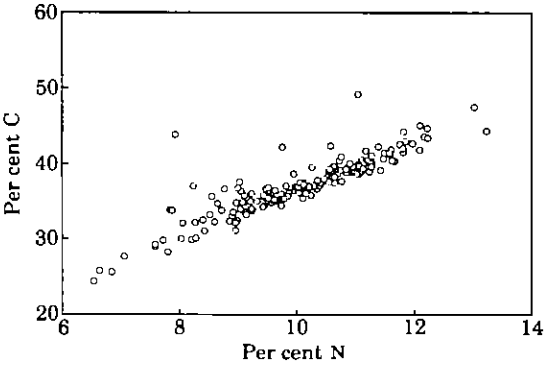

Figure 5. Relationship between carbon and nitrogen as percentage dry weight for sprat larvae taken in 1989 and 1990.

the stratified site. Most sampling at the mixed site was in the southern area where there were low stratification values (Fig. 3; $\Delta\sigma_t$ of 0.07–0.37), but later in the sampling sequence when the drogued buoy drifted into the more stratified region, higher levels of stratification were recorded (Fig. 3; $\Delta\sigma_t$ of 0.46–0.64).

Overall microzooplankton abundance (as combined numbers of copepod eggs, nauplii, and copepodites) was 36% higher at the stratified site than at the mixed (104.7 organisms l^{-1} at the stratified, cf. 76.9 l^{-1} at the mixed). Much of this difference was due to the significantly higher level of copepod eggs at the stratified site (63.2 l^{-1}) than at the mixed (30.6 l^{-1}; Fig. 4).

Carbon and nitrogen

Carbon/nitrogen ratio, as a gross estimate of lipid content, is a useful measure of the physiological status of individual zooplankters, e.g. maturity stage (Williams and Robins, 1982). For assessments of the nutritional condition of fish larvae it is generally less sensitive than percentage of dry weight for each element separately (e.g. Ehrlich, 1974b, and see table 6 of May, 1971). In the present study of sprat larvae there was a parallel variation in carbon and nitrogen (Fig. 5) and similar conclusions regarding condition of larvae were obtained from assessments of either element individually. In the following account changes in carbon are presented since this is generally a more sensitive index of condition than nitrogen (Ehrlich, 1974b).

Considering all specimens from the German Bight in 1989 and 1990 there was no significant variation in carbon content related to day/night sampling periodicity (Fig. 6). However, results from the mixed and stratified stations in the June 1990 sampling sequence showed a small group of larvae in the 9–10-mm length group at the stratified site with relatively low carbon values, suggesting relatively poor nutritional condition (Fig. 7).

Lipids

Cholesterol, as a cell membrane constituent, should be a functionally more relevant measure of body mass than weight or length. Figure 8 shows the linear relationship

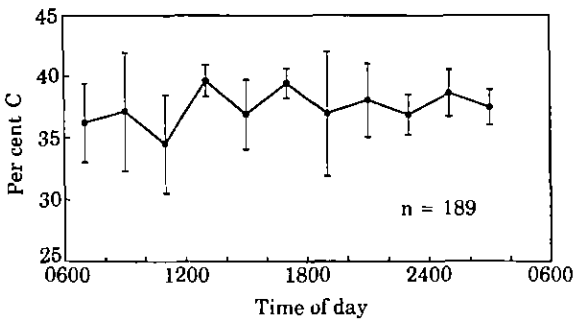

Figure 6. Percentage carbon composition against time of capture for sprat larvae taken in 1989 and 1990. Values with ± 1 standard deviation are plotted by 2-hourly time intervals.

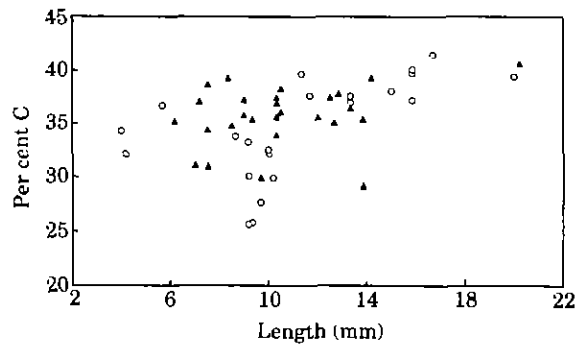

Figure 7. Percentage carbon composition by length for sprat larvae taken at the mixed (triangles) and stratified sites (circles) on the June 1990 cruise (see Fig. 1).

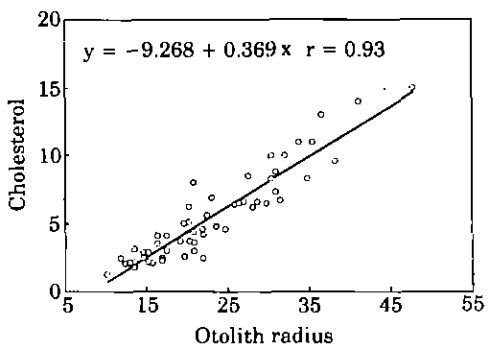

Figure 8. Relationship between cholesterol (µg per larva) and otolith radius for sprat larvae taken on the June 1990 cruise (see Fig. 1).

between cholesterol and otolith radius (as a measure of larval size).

One measure of nutritional condition of fish larvae is the triacylglycerol (TAG)/cholesterol ratio, representing proximate energy reserves against body mass (Håkanson, 1989a). During starvation TAG is used up more rapidly while cholesterol remains essentially unchanged. Thus, the ratio of TAG to cholesterol is sensitive to short-term changes in food supply for the

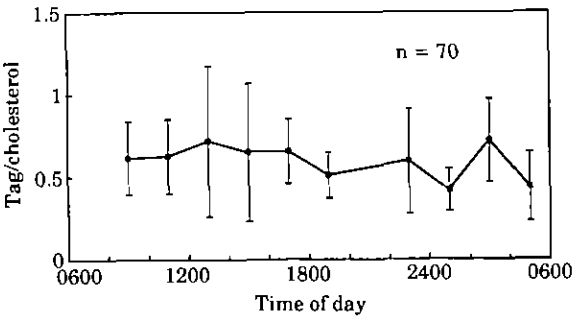

Figure 9. The triacylglycerol (TAG) to cholesterol ratio against time of capture for sprat larvae taken at the mixed and stratified sites on the June 1990 cruise (see Fig. 1).

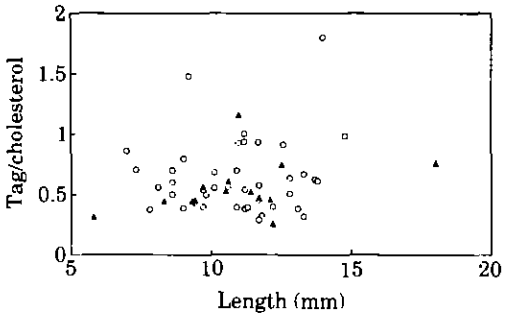

Figure 10. The triacylglycerol (TAG) to cholesterol ratio by length for all larvae taken at the mixed (triangles) and stratified sites (circles) on the June 1990 cruise (see Fig. 1).

larvae. Ratios of <0.2 indicate severely starved larvae which are not likely to recover (Håkanson, 1989a). This ratio was determined for all specimens taken in June 1990 and this more labile measurement did not reveal any significant variation in relation to day/night sampling periodicity (Fig. 9).

The TAG/cholesterol ratio shows few of the larvae actually starving to death (Fig. 10). However, this ratio should only be used to identify severely starving larvae. To determine the more long-term nutritional condition of larvae, absolute lipid levels should be used. Larvae that manage to survive on a low food ration will continue to increase in length but will have lowered cholesterol as well as TAG levels (Håkanson, 1993). Plots of individual lipid components against body length showed a group of 8–10-mm-sized larvae with low TAG and cholesterol levels, but less clearly for polar lipids, at both the mixed and the stratified sites (Fig. 11). Although these larvae are not directly starving to death, they are not able to attain a normal lipid content and would be more susceptible to future starvation.

The 9-mm group of larvae gave similar results when regression were made of TAG and cholesterol against otolith growth rate (Fig. 12). Faster-growing larvae were

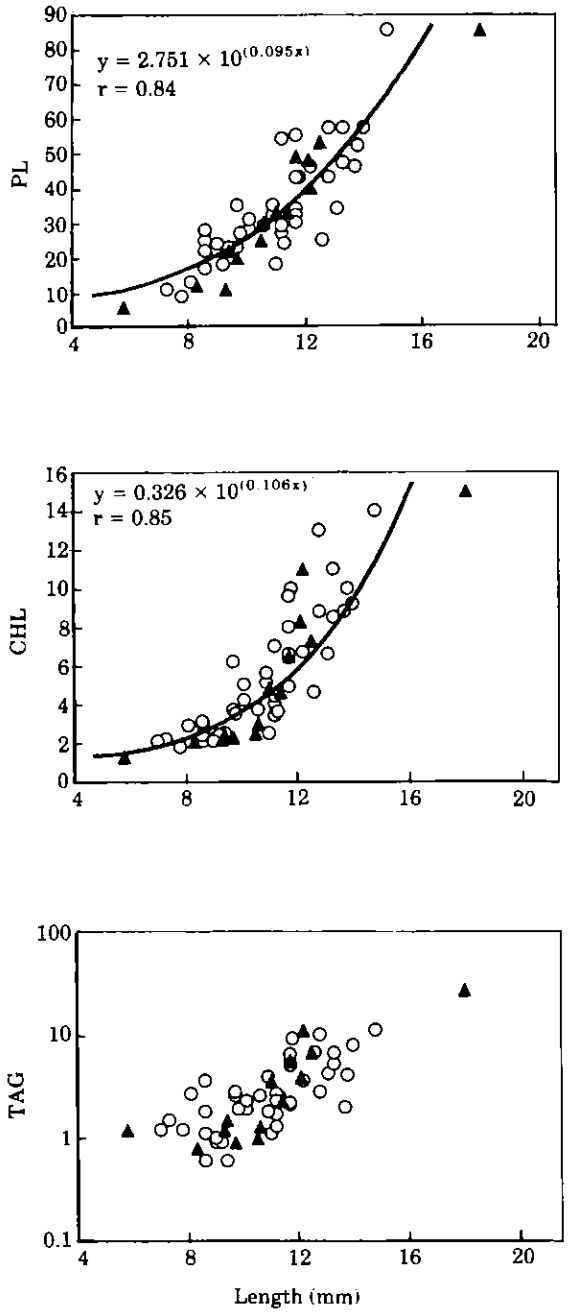

Figure 11. Triacylglycerol (TAG; log scale), cholesterol (CHL), and polar lipids (PL), all as μg per larva, against length for sprat larvae taken at the mixed (triangles) and stratified sites (circles) on the June 1990 cruise (see Fig. 1).

consistently higher in TAG and cholesterol for all sizes except those in the 9–10-mm length group. For these larvae the faster growing specimens were penalized for some reason, as reflected by the inverse relationship with TAG and cholesterol.

Discussion

Assessments of larval condition, as an index of survival potential, are most usefully based on processes operating on longer time-scales than the diurnal feeding rhythmicity of fish larvae. Both elemental and lipid composition have previously been used to gauge larval condition (e.g. Ehrlich, 1974b; Håkanson, 1989b, 1993; Suthers *et al.*, 1992). The absence of any marked diel changes in carbon composition or lipid fractions as measured in the present study (Figs 6, 9) support the validity of their use for condition studies.

Results from both carbon and TAG showed some larvae at about 9 mm in length to be in poor condition (Figs 7, 11). Different larvae were used for each analysis, so that essentially independent evidence is provided for the difference in the 9-mm length group of larvae. Furthermore, the otolith growth rate measurements were also in accord with the findings for carbon and lipids, for some reason the 9-mm larvae were both growing slowly (as measured over the long-term from mean growth rate; Fig. 12) and had low cholesterol levels as well as low energy reserves of TAG; although the 9-mm larvae were not actually starving to death, as shown by the TAG/cholesterol ratios (Fig. 10). From the current assessment of the data it is not possible to distinguish whether the 9-mm group of larvae were suffering from a relatively long-term deficit or whether their poor condition is a symptom of more short-term deprivation. Further data on otolith daily growth rings can give information on this point. However, in order to determine reliable daily otolith increment widths on small larvae (e.g. <10-mm sprat larvae) it is necessary to use electron microscopy or similar scanning techniques: light microscopy, as employed in the current study, cannot measure the widths of the earliest rings with sufficient precision.

Food preference of 9–10-mm sprat larvae is not significantly different between adjacent size classes (Conway *et al.*, 1991) so that inadequate food availability is an unlikely cause of poor condition for that size group alone. Although there was a difference in food abundance between the mixed and stratified sites, it was not marked (Fig. 4), and larvae in relatively poor condition were found at both sites. Within the limitations of using integrated values down the water-column as a measure of food abundance (see Frank and McRuer, 1989, and Coombs *et al.*, 1992), there was no relationship between larval condition and available food.

Condition analyses based on lipids or elemental composition both offer effective methods for SARP-style studies of larval survival in relation to food availability. Both techniques have been validated under experimental conditions and have been successfully applied to field situations. There are notable improvements in discriminating ability if more than one technique can be used, in

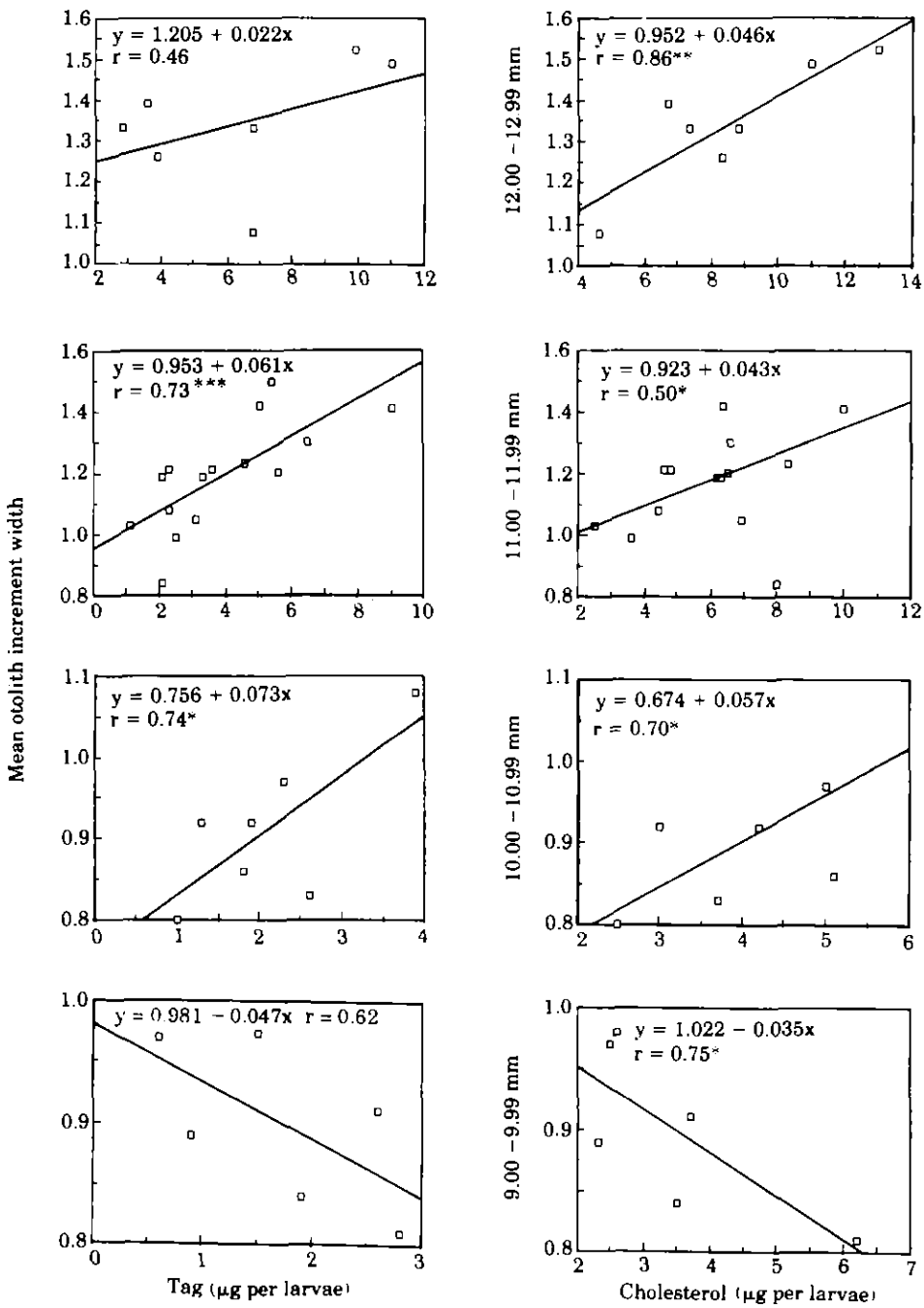

Figure 12. Relationship between otolith growth rate (as mean otolith increment width) and triacylglycerol (TAG) and cholesterol content of sprat larvae taken on the June 1990 cruise (see Fig. 1) plotted by length categories of larvae. Significance levels of 1%, 5%, and 10% are indicated by three, two, and one star, respectively.

particular for lipid analysis where otoliths are also available for growth studies. The value of daily growth ring data in such studies to provide a historical record of growth conditions makes this technique particularly suitable for the older larvae in which the growth rings can be distinguished.

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