ICES Journal of Marine Science, 54: 148–151. 1997

Short communication

Use of carbon rather than dry weight to assess the DNA content and nutritional condition index of sole larvae

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Bergeron, J.-P., Person-Le Ruyet, J., and Koutsikopoulos, C. 1997. Use of carbon rather than dry weight to assess the DNA content and nutritional condition index of sole larvae. – ICES Journal of Marine Science, 54: 148–151.

A new biochemical index has been proposed, based on the DNA amount in the larva related to dry weight, to estimate the nutritional condition of marine fish larvae. Starvation induces a strong and fast increase of this index, which can be assessed at the individual level. Weighing very small quantities of dry matter is difficult. It is suggested that carbon content should be used, which can be quantified by accurate standard techniques. Results of a preliminary calibration experiment on sole larvae fed in captivity at three temperatures show a strong correlation between dry weight and carbon. DNA/C values are not the same for the early development stages (symmetrical larva) or the later ones (metamorphosing larva), both levels being quite independent of the rearing temperatures despite great differences in growth rates. DNA/C should provide a convenient and accurate tool for assessing the nutritional condition of field-caught specimens.

Key words: carbon, dry weight, DNA, nutritional condition, fish larva, Dover sole.

Received 26 October 1995; accepted 29 February 1996.

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Introduction

The role played by starvation in the natural field mortality of the early life stages of marine fishes remains a fundamental concern in fisheries oceanography (Heath, 1992). Much work has been devoted to developing suitable methods for assessing nutritional condition of fish larvae at the time of capture (Ferron and Leggett, 1994). Recently, a new index has been proposed, based on the DNA amount in the larva related to dry weight (DNA/DW), and tested with sole (*Solea solea* L.) larvae reared in the laboratory (Bergeron *et al.*, 1991). Three main features were demonstrated: control fed larvae give two fairly stable levels characteristic of the early symmetrical development stages on one hand and of the metamorphosing stages on the other hand, these levels are the same for two temperature conditions that induce very different growth rates, and starvation is obviously revealed by a fast and sharp increase of values of this index.

The sensitivity of the analytical procedure used for DNA measurement allows an assessment at the individual level, but reliable weighing of such small quantities of dry matter is difficult depending especially on the ambient humidity. Therefore, we suggest the use of carbon content, which can be quantified by accurate standard techniques (Anger and Hirche, 1990), as a base for the expression of DNA concentration in a fish larva (DNA/C). Temperature is a basic factor likely to affect both growth rates and biochemical composition (Blaxter, 1992), and so a preliminary calibration experiment has been carried out in the laboratory on sole larvae reared under three temperature conditions with

1054–3139/97/010148+04 $25.00/0/jm960161

Table 1. Statistics of the relationship between dry weight and carbon in sole larvae fitted by linear regression ($C = a \cdot DW$).

	13°C	16°C	19°C	Pooled data
d.f.	45	32	24	103
a	0.37	0.37	0.35	0.37
r^2	0.92	0.92	0.94	0.92
Std error of Y est.	0.051	0.055	0.048	0.052
Std error of a	0.011	0.012	0.012	0.007

two main purposes, first to investigate the feasibility of substituting carbon for dry weight and, second, to check that temperature has no noticeable influence on the values of the index assessed in fed larvae.

Materials and methods

The experiment took place in the IFREMER Centre de Brest, Department "Ressources Aquacoles", in a laboratory especially devoted to the rearing of fish larvae (see Person-Le Ruyet *et al.*, 1981, for material and technical details) and where a small stock of sole adults was kept in captivity in the conditions described by Girin (1978). The eggs used for the experiment were collected from a single naturally spawned batch and incubated according to Devauchelle *et al.* (1986). Immediately after hatching, the larvae were distributed into six rearing 60 l tanks which were maintained at three temperatures (2 tanks for each), 13, 16 and 19°C, during the whole experiment (temperatures varying within ranges not exceeding 0.5°C). The larvae were fed daily with nauplii of *Artemia*, ranging from 20 nauplii larva^{-1} after opening of the mouth to 200 larva^{-1} at the end of metamorphosis. A constant renewal (approx 15% h^{-1}) of the milieu was maintained by supplying filtered sea water treated by ultraviolet rays, salinity was 34–35. Rearing lasted 24, 18 and 14 d for the complete larval development at 13, 16 and 19°C respectively.

Biochemical analyses were performed on samples taken daily from each batch comprising 50 larvae in the early days of rearing and 35 larvae at the end of larval development. Before analyses, the animals were ground in ice-cold distilled water (4°C) with a Polytron homogenizer (25–30 s, 25 000 rpm). An aliquot was placed in a tin capsule, dried to constant weight at 50°C, weighed for dry matter and introduced into a Carlo Erba elemental analyzer CHN/OS 1106 for total carbon measurement. Another aliquot was used for DNA determination by the fluorometric method initially suggested by Le Pecq and Paoletti (1966), modified by Karsten and Wollenberger (1972, 1977) and tested by Bergeron and Boulhic (1994).

The relationship between dry weight and carbon was estimated using linear regression for each of the different rearing temperatures. The comparison of the slopes of the three regression lines was performed by analysis of covariance (ANCOVA). Previous work (Bergeron *et al.*, 1991) demonstrated that there were two main levels of relative DNA content units characteristic of (1) the early larval stages and (2) the metamorphosing stages. Both periods are linked by a short transitional one (14–16 d post-hatching for 13°C batches, 11 and 12 d post-hatching for 16°C, 9 d post-hatching for 19°C), during which intermediate values were measured. Differences in DNA/C levels for these two main development periods and the three temperature conditions were compared in a two-way analysis of variance (ANOVA).

Results

Carbon–dry weight relationship

In order to test the feasibility of substituting carbon for dry weight, the relationships between carbon units and dry weight were investigated during the whole larval development. A simple linear regression model indicated a strong correlation between the two variables (Table 1). The slope of the relationship for the 19°C treatment appeared smaller, but the difference was not statistically significant (ANCOVA, $F(2,98)=2.14$, $p>0.05$). Therefore a mean conversion factor of 0.37 can be used to convert carbon to dry weight for sole larvae.

Changes in DNA/C during larval development

The major event of metamorphosis occurs during the larval development of sole, when the animal becomes asymmetrical. As previously shown (Bergeron *et al.*, 1991), the earliest stage of this process, characterized by the flexion of the notochord, is an important threshold allowing us to divide larval ontogeny into two periods. During the first period the larva is strictly symmetrical ("preflexion larva", according to Kendall *et al.*, 1984); during the second period major morphological and anatomical changes occur up to the end of metamorphosis ("flexion larva"). As expected from the previous experiments (Bergeron *et al.*, 1991) and the relationship established above, the DNA/C values vary depending on these two periods (Fig. 1). Intermediate values occur within a narrow range during a short transitional period (Table 2).

Despite strong differences in growth rates (cf. duration of the experiments in "Materials and methods" section), the DNA/C variations are independent of temperature conditions (Fig. 1, Table 3). The two-way analysis of variance clearly shows that the only significant effect in determining the DNA/C value is the ontogenetic period (Table 3). No interactions were observed between ontogenetic period and temperature. The upper limit of 95% confidence for the mean DNA/C ratio (Table 4) is approximately 80 for

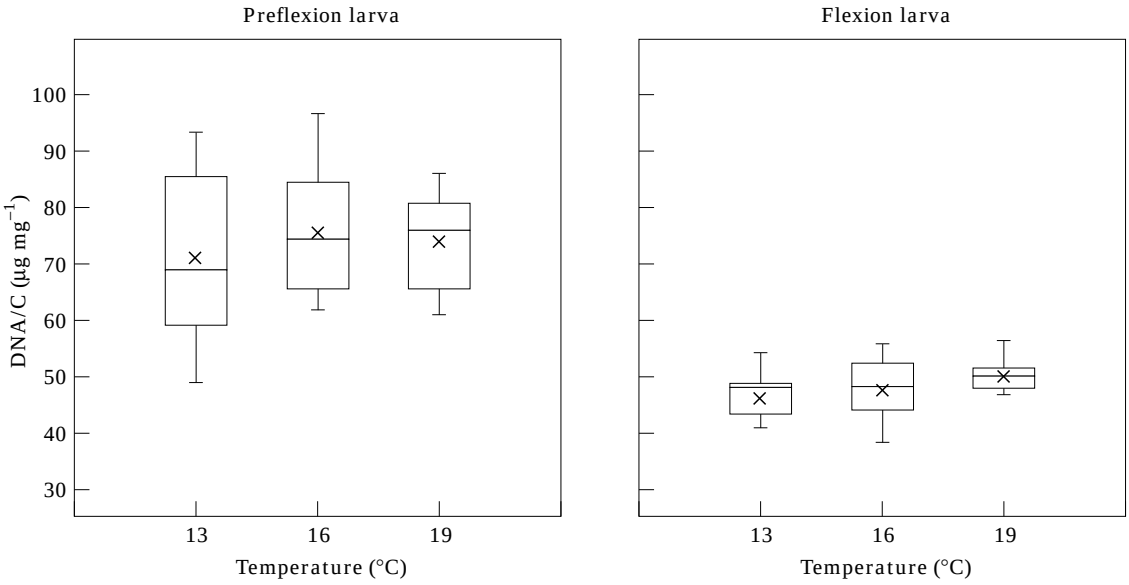

Figure 1. DNA/C ratio (in µg mg^{-1}) of sole larvae for both main development periods and the three temperature conditions. The boxes represent the range containing 50% of the data. The horizontal bar corresponds to the median value, while the cross shows the mean value. The vertical bars indicate the upper and lower values of the sample.

Table 2. DNA/C values (in µg mg^{-1}) measured in sole larvae during the transitional period between the "preflexion larva" and "flexion larva" stages (the period is in days post-hatching).

Temp.	Period days	n	Mean	S.D.
13°C	14–16	6	58.0	4.8
16°C	11–12	4	55.7	2.5
19°C	9	2	57.8	2.8

Table 3. Two-way analysis of variance (ANOVA) of factors affecting the DNA/C (µg mg^{-1}) ratio of sole larvae. "Period" refers to both main development stages described in the text as "preflexion' and "flexion".

Source of variance	d.f.	Mean square	F-ratio	Significance level
Period	1	11 687.5	121.595	$<10^{-4}$
Temperature	2	76.0	0.791	0.457
Period × temperature	2	30.2	0.314	0.731
Residual	75	96.1	—	—

preflexion larval stages which corresponds fairly well to maximum values, just below 30, observed in measuring DNA/DW (Bergeron *et al.*, 1991). For later development stages, a similar comparison gives a slight modification, around 20 rather than 15 µg mg^{-1}, as previously suggested.

Table 4. Least squares means for DNA/C (in µg mg^{-1}) of sole larvae at both main development periods under the three temperature conditions.

		No.	Mean	95% confidence for mean	
Preflexion larva	13°C	22	71.2	67.1	75.4
	16°C	16	75.5	70.6	80.4
	19°C	9	73.7	67.1	80.1
Flexion larva	13°C	16	46.3	41.4	51.2
	16°C	10	47.3	41.1	53.5
	19°C	8	49.8	42.9	56.7
Pooled data	Preflexion larva	47	73.5	70.4	76.5
	Flexion larva	34	47.8	44.3	51.3

Conclusion

Carbon can be used as a substitute for dry weight in expressing the relative DNA content of larval sole, making it easier to define standard conditions for determining both terms of the DNA/C ratio from a single larva. This methodological improvement of the tool previously suggested by Bergeron *et al.* (1991) should now be used for assessing the nutritional condition of early life stages of fishes in the field. Initial experiments are now in progress.

In conclusion, two points should be noted. First, the present results confirm that temperature has no apparent effect on the relative DNA content of sole larvae despite significant differences in growth rates. Second, this experiment clearly indicates that a dramatic

change in this relative DNA content occurs during larval development. This drop is independent of growth rate, lasting no more than 10% of the whole larval period (Table 2), and characterizes the ultimate stage preceding the metamorphosis period (''flexion larva''). From this stage onwards, the larva experiences major morphological and anatomical reorganization, which is preceded by a cellular enlargement as indicated by a decrease of DNA/C.

Acknowledgements

The authors would like to thank the technical assistance of J. C. Alexandre and N. Retière for rearing tasks and biochemical analyses respectively. They are also very grateful to two anonymous reviewers for their constructive comments and help in improving the form of the manuscript.

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